

Physiological monitoring of welfare for conservation of  
Arabian oryx, *Oryx leucoryx*

Submitted by

Mansoor Hamed AlJahdhami

to the University of Exeter as a thesis for the degree of Doctor of  
Philosophy in Biological Sciences by Research.

University of Exeter, UK  
College of Life and Environmental Sciences  
Biosciences

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Mansoor H. AlJahdhami



Feeding of a captive herd of Arabian oryx at Jaaluni enclosure, the field center of the Arabian Oryx Sanctuary, Sultanate of Oman. (*Photo: Mansoor AlJahdhami*)

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### ***Abstract***

The endangered Arabian oryx, *Oryx leucoryx* faces a wide range of issues that potentially have adverse effects on their welfare while they are free-ranging in their natural habitat, housed in captivity for conservation breeding or when they are translocated from the wild to captivity or vice versa. Furthermore, the global increase in the number of captive Arabian oryx (currently more than 95 % of the world population of about 8000 individuals), gives rise to particular concern for their welfare and health within captive conditions.

Thorough assessment of the welfare of animals involves physiological and behavioural measures. Methods for assessment of welfare in Arabian oryx have not been established and the present studies aim at establishing physiological tools for assessment of welfare. Therefore, the present studies developed and applied new methods for non-invasive assessment of welfare in the Arabian oryx (using faecal samples), and established reference values for a range of haematological, biochemical and clinical parameters. The potential disturbances in these parameters were investigated after immobilisation and tranquillisation and post- transportation.

Two enzyme immuno-assays (EIA I and II) for faecal glucocorticoid metabolites (FGM) were validated by stimulation and suppression of the hypothalamic-pituitary-adrenal axis through injection of synthetic adrenocorticotrophic hormone (ACTH) and dexamethasone, respectively. These studies established a lag-time of  $14 \pm 1$  h between secretion of glucocorticoids into the blood stream and excretion of the measured FGM. Faecal incubation at 30°C for 3 days showed that EIA I measured more stable faecal glucocorticoid metabolites than EIA II, and has greater potential for application in field conditions. This method was found to be invaluable for measuring stress and hence assessment of welfare status, and its use is recommended in planning welfare improvements. Measurement of FGM successfully detected the stress of road transportation (630 km for 8-10 h), showing an increase 2 days after transport, followed by recovery to basal FGM levels after re-housing for up to 11 days. Releasing oryx to the wild, in Oman, and tracking for 11 days, after transportation 50-70 km from the captive site (Arabian Oryx

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Sanctuary, Jaaluni), caused an increase in FGM to the highest levels seen in these studies, and suggests a high level of stress was experienced after release of oryx.

Published reference values for haematological, biochemical, hormonal and clinical parameters for Arabian oryx are limited, with little information for non-immobilised and non-tranquillised oryx or consideration of possible age and sex differences. Therefore, reference values and inter-percentile ranges (2.5 and 97.5 percentiles) were established for 32 parameters, in separate groups of male and female adult oryx, without using immobilising or tranquillising chemicals during capture. The haematological parameters investigated were white blood cell count and differentiation (%) of cell types (neutrophils, lymphocytes, monocytes, eosinophils, basophils), number of platelets, red blood cell count, haemoglobin concentration and haematocrit, erythrocyte cell volume, erythrocyte haemoglobin content and concentration, serum osmolality and ions (sodium, potassium, chloride, calcium, magnesium and phosphorus). Biochemical parameters investigated were serum urea, glucose, total protein, albumin and plasma lactate concentrations. Clinical parameters investigated were body temperature, heart and respiratory rates. Hormonal parameters measured were cortisol, free-thyroxine, free-triiodothyronine and insulin concentrations. Near basal values for serum cortisol were measured in Arabian oryx sampled within 2 min, while values were significantly higher in oryx sampled within 5-10 min. The reference values established in these studies are considered valuable tools for diagnosis of disease and physiological alterations in male and female Arabian oryx.

To investigate the possible effects of the common practice of immobilisation and tranquillisation on physiological and biochemical status, two restraint chemicals (xylazine and perphenazine enanthate) were evaluated. Xylazine (an immobilising agent) caused changes in many clinical, hormonal, haematological and biochemical parameters; respiratory rate decreased by 74 %, heart rate decreased by 58 %, causing a decrease in red blood cell count, haemoglobin concentration and haematocrit, serum albumin and total protein concentration. Xylazine also induced a decrease in serum insulin, which probably caused the observed increase in serum glucose.

Perphenazine enanthate (a long-acting tranquilliser) was found to have no adverse effects on most parameters, which generally remained in the reference ranges. However, a

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reduction in blood haematocrit and related parameters (red blood cell count and plasma haemoglobin concentration) occurred, 1-3 days after injection. The tranquilliser also plays a role in reducing stress and significantly reduced serum cortisol 2-3 days after injection in oryx held in captivity compared to oryx that received a saline (control) injection. FGM increased significantly one day after injection of perphenazine enanthate and saline, suggesting the animals were initially stressed by the handling and venipuncture, taking into consideration the lag-time from cortisol secretion to appearance of FGM.

The baseline concentration of serum cortisol was used in assessing the stress caused by handling before and after transporting Arabian oryx for 630 km (8-10 h) and the acute effects of handling and injections. Increased serum cortisol was always associated with leukocytosis, neutrophilia and lymphopenia. Serum cortisol of non-transported oryx was reduced by the tranquilliser perphenazine enanthate, but transportation of tranquillised Arabian oryx during hot ambient temperature (maximum 42 °C) resulted in fatigue and prevented reaching a clear conclusion of the role of the tranquilliser in reducing transport stress. Non-tranquillised oryx transported at a maximum of 26-30 °C showed a similar level of stress as implied by the level of faecal glucocorticoid metabolites, but without fatigue. However, the tranquilliser induced calmness in Arabian oryx for up to 7 days, which facilitated capture and handling. Therefore, perphenazine enanthate has a potential to be used in the management practices, such as movement and transport of Arabian oryx.

This thesis discusses the current and future welfare issues that face Arabian oryx in captivity, upon release and in the wild. Additional methods are proposed for thorough assessment and improvement of welfare to complement the methods established by the present studies.

***Special Thanks***

I owe special thanks to my father, mother, brothers and sisters. I particularly thank my mother for the continuous prayers for me. I am very grateful to my wife Naama for encouragement, unlimited support and patience throughout my study. My sincere gratitude is due to my beloved daughter Rayan and son AlMukhtar.

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### *Acknowledgments*

The re-introduction of Arabian oryx to Oman, originally inspired and continuously supported by His Majesty Sultan Qaboos bin Said, is entirely funded by the Government of the Sultanate of Oman. I am grateful to the Diwan of Royal Court for the sponsorship of this study and for this I would like to thank the Minister, His Excellency Sayyid Ali bin Hamood AlBusaidi and the Secretary General, His Excellency Sheikh Dr Talib bin Hilal AlHosni.

I owe a great debt to Sheikh Saud bin Ali Al-Khalili, Chairmen of Al-Taher group, for partially funding the first year of the study before I joined the Diwan of Royal Court as biologist and his willingness to sponsor the whole study.

I owe an enormous debt of gratitude to my supervisor at the University of Exeter, Professor Anne Brown for encouragement, continuous and unlimited support throughout my study. She was always there when I needed any help. I am also greatly indebted to the unfailing support of my local supervisor at the Department of Biology, Sultan Qaboos University, Dr Abdulaziz bin Yahya AlKindi, the former Dean of College of Science. Without his encouragement and support, this study would not have become a reality. I also thank Professor Ibrahim Mahmoud for editorial help with some chapters. I am very grateful to Dr Shehla Amer for the editorial help and valuable comments on the thesis. The assistance of academic and technical staff at the Department of Biology, Sultan Qaboos University is much appreciated. I would like to thank in particular the technicians Abdullah AlShuraiqi, Khamis AlDhafri and Jamila AlBalushi. At the Department of Chemistry, College of Science, Sultan Qaboos University, I thank Salim AlSaidi and Farook Khan for provision of some chemicals and consumables needed for the analysis. I am grateful to Dr Tabisam Khan, the director of Center Analytical and Applied Research Facility (CAARF), at College of Science for friendship and making the facilities of the Center available for preparation of samples during the study. The technical assistance at the Electron Microscopy Unit, Department of Pathology at the College of Medicine is much appreciated. I thank in particular Mr Issa AlAmri, Khamis AlRyiami, Kawther AlAdwai and Mohammed AlKindi for assistance with screening of Arabian oryx blood cells. I would like

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to thank Rashid AlBusaidi from the College of Agriculture and Marine Sciences for providing some blood sampling tubes.

At the Sultan Qaboos University Hospital, I thank Dr Masood Kashoob, the former Director General for all support. At the Department of Haematology, I must thank Dr Salam AlKindi, the head of department, for permitting haematological analyses of oryx blood samples. I thank all the technical staff who assisted me with the analysis of blood samples and in particular Leonard Brown, Hamed AlGhaithi and Abdulrahman Noor Rashid. At the Department of Clinical Biochemistry, I thank Professor Riad Bayoumi for support and Halima, Matar AlMaani and Huda AlSaadi for technical assistance with analysis of hormonal, ions and biochemical parameters.

I am very grateful to Professor Rupert Palme at the College of Veterinary Medicine, Vienna, Austria for allowing me to analyse oryx faecal samples for glucocorticoid metabolites at their laboratory in Vienna. I also thank Professor Eric Möstl for answering my curious questions about the enzyme immuno-assay they developed. I also thank Edith Rassam for the help in analysis of oryx faecal samples. I appreciate the warm welcome and hospitality of Professor Rupert Palme's family while I was in Vienna.

I would like to thank Sheikh Hilal bin Hamed AlKalbani, the Director General of Administrative Development, Diwan of Royal Court, for his sincere support for the study. At the Office for Conservation of the Environment, Diwan of Royal Court, I am greatly indebted to the strong support and encouragement of Sheikh Abdulaziz bin Hamood AlRawahi, the former Director General of the Administration and Finance. I would also thank Khamis Shabin, the former Director of Finance for all his encouragement and support. I would like to thank also Dr Andrew Spalton, the former Adviser for Conservation of the Environment for all the support he provided and for the valuable comments on the thesis. I also thank all staff at the Office of Conservation of the Environment for the support of paperwork during my study. Special thanks are due to Shiekh Hemiar AlAbri, the Director of Administration and Mr Ahmed AlAlawi, the Director of Finance for all the logistic support.

At the Arabian oryx project, the unlimited support and sincere encouragement and hospitality of Lieutenant Colonel Juma AlHikmani, the former Field Manager of the

Arabian Oryx Project was very much appreciated. Many thanks are due to the biologists Yasser AlKharousi, Eng. Salah AlMahdhoury and Haitham AlAmri, for field assistance and provision of information about the project. The assistance of head rangers and rangers of the Harasis tribe with the handling of oryx and tracking of oryx in the desert is much appreciated. I thank in particular Salih Musalam AlHarsusi, the current Field Manager of the Arabian Oryx Project, Hamed Hamid AlHarsusi, Amer Hamed AlHarsusi, Salim Salih AlHarsusi and all rangers who helped me. I also thank the cook Christopher Vas for the nice dishes and sense of humour while I was in Jaaluni.

At the Royal Court Affairs, special thanks are due to Eng. Mahmood bin Bader AlAbri, the Director General of Veterinary Services and his deputy Dr Ayoub Rajab AlBalushi for permission to carry out my studies at the Omani Mammals Breeding Center. I would like to give special thanks to all the veterinarians who helped me while I was conducting the studies on Arabian oryx. I thank in particular, Dr Elias Nikolakopoulos, Dr Faiz AlZadjali, Dr Salim AlMukhaini, Dr Tariq AlZadjali and Dr AlMutasim AlZadjali and all assistant veterinarians who helped me throughout my study. I owe a huge debt of gratitude to Dr Khalid AlRasbi, the director of the Omani Mammals Breeding Center for great support and provision of facilities and animals for the main experiments of the study. I owe a special thanks to all the staff of the center for handling and caring for the animals during all experiments.

I also would like to acknowledge the cooperation of Royal Transport for providing of trucks for transporting three batches of oryx from Muscat to Jaaluni and vehicles for the accompanying veterinarian and assistants. I also thank the Department of Meteorology, Directorate General of Civil Aviation & Meteorology, Ministry of Transport and Communications, for providing climate information for Muscat and Jaaluni.

Many people contributed to this thesis, not by analysis or field assistance, but by encouragement, support and friendship both in Oman and Exeter. At Exeter, I owe a special thanks to my supervisor Professor Anne Brown and her husband Dr Philip Smith for friendship and generous hospitality when I was at Exeter with my family. I also thank Yaqoob AlWaily, Nasser AlRawahi and their families for friendship and warm hospitality when I was in Exeter with my family. I owe a special thank to Dr Abdelmalek

Benattayallah and his family for friendship and generosity and for the regular visits between our families. In Oman, at Sultan Qaboos University, I thank Dr Elsadiq Eltayeb and Professor Abdulrahman Obaid (sadly passed away) for friendship, generosity, wisdom and sense of humour during the coffee break that is greatly missed.

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***List of Abbreviations***

°C	Degrees Celsius
ACTH	Adrenocorticotrophic Hormone
ALT	Alanine Aminotransferase
ANOVA	Analysis of Variance
AOP	Arabian Oryx Project
AOS	Arabian Oryx Sanctuary
AST	Aspartate Aminotransferase
BAV-3	Bovine Adenovirus 3
bpm	Breaths per minute (pulmonary ventilation) or beats per minute (heart rate)
BRSV	Bovine Respiratory Syncytial Virus
BSA	Bovine Serum Albumen
CCCAO	The Coordination Committee for the Conservation of Arabian Oryx
CL	Caseous Lymphadenitis
CMO	Carboxymethyloximes
CRH	Corticotropic Releasing Hormone
DADDOO	1,8-Diamino-3,6-dioxaoctane
DDW	Double Distilled Water
df	Degrees of Freedom
dL	decilitre
DOA	11,17-Dioxoandrostanes
DRC	Diwan of Royal Court
EIA	Enzyme-Immuno-assay
FAWC	Farm Animal Welfare Council
FGM	Faecal Glucocorticoid Metabolites
fL	femto-litre
FMD	Foot And Mouth Disease
FSH	Follicle-Stimulating Hormone
GLM	General Linear Models
h	Hour
HCL	Hydrochloric Acid
HPA	Hypothalamic-Pituitary- Adrenal Axis
HPLC	High Performance Liquid Chromatography
HS	Hemisuccinate,
IFCC	The International Federation of Clinical Chemistry
IUCN	The International Union For Conservation of Nature
LCMS	Liquid Chromatography and Mass Spectrometry
LH	Luteinizing Hormone
M	Molar
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
MCV	Mean Corpuscular Volume
min	Minute
OIE	The World Organisation of Animal Health
OMBC	The Omani Mammals Breeding Center

pg	picogram
POD	Peroxidase
PPR	Peste Des Petits Ruminants
RBC	Red blood cells
RIA	Radioimmuno-assay
SD	Standard Deviation
SEM	Standard Error of the Mean
T <sub>3</sub>	Triiodothyronine
T <sub>4</sub>	Thyroxine
UAE	United Arab Emirates
WBC	White Blood Cells

## Chapter 1

### General introduction

#### 1.1 Arabian oryx

##### 1.1.1 Re-introductions and the current status

Historically, Arabian oryx (*Oryx leucoryx*) roamed throughout Arabia and was reported to exist in Syria (Horwitz et al., 1999), Iraq, Jordan, Palestine, Saudi Arabia, United Arab Emirates, Yemen, Oman (Jones, 1988; Harrison and Bates, 1991) and Egypt (Manlius, 2000). Arabian oryx belongs to the subfamily Hippotraginae of the family Bovidae in the order Artiodactyla (Estes, 1991).

Due to intense hunting, Arabian oryx became extinct in the wild in 1972 (Henderson, 1974). Fortunately, extinction of Arabian oryx was anticipated in the early 1960s, by the Fauna Preservation Society. This society, aided by the World Wildlife Fund organised an expedition to find and capture some of the last remaining Arabian oryx in “Operation Oryx” (Grimwood, 1962, 1967, 1988). The operation was successful in capturing some oryx and with some donations of oryx from private collections of Arabian leaders, a captive breeding programme was established in Phoenix zoo, in the USA, to form what was known as the “World Herd” of Arabian oryx (Turkowski and Mohny, 1971; Homan, 1988). The aim of that programme was to conserve the species and to re-introduce this species to its former range. Since then, a number of re-introductions in different countries have been initiated.

From the captive herd, the first re-introduction of Arabian oryx to the wild was in Oman in 1982 as an initiative of His Majesty Sultan Qaboos bin Said, the Sultan of Oman (Fitter, 1982; Stanley Price, 1986; Daly, 1988; 1989; Spalton, 1991, 1993). Arabian oryx were reintroduced in the central desert of Oman (Jiddat AlHarasis). The re-introduction area was called later (in 1994) the Arabian Oryx Sanctuary (Figure 1.2). The number in the re-

introduced population increased to about 400 individuals in 1996, but since then heavy poaching led to the collapse of the wild population. In 1998 there were about 138 individuals in the wild (Gorman, 1999; Spalton et al., 1999), and a captive breeding programme was re-established to rescue the oryx from poaching in the wild (Figure 1.2) (Spalton et al., 1999). This captive group was also re-established to form a 'stock' in case the wild population is lost. In Oman, although less than 30 male Arabian oryx are left in the wild and no females, poaching is still a threat (Al-Kharousi, 2006). Poaching prevents the release of captive oryx to the wild, which has contributed to the increase of oryx in captivity at the Arabian Oryx Sanctuary, to about 250 individuals (AlMahdhoury, S. and AlAmri, H., Personal Communication). The re-introduction project of Arabian oryx in Oman was known to be one of the most successful re-introductions (Kleiman, 1989; Stanley Price, 1989). Furthermore, the Re-introduction Specialist Group of the Species Survival Commission of IUCN, established in 1988, evolved from the oryx re-introduction project in Oman (Spalton, 2003; Stanley Price and Soorae, 2003). Unfortunately, the poaching problem led to the setback of the re-introduction project. There are current plans to fence the Arabian Oryx Sanctuary, an area of 2,824 square kilometres, with a perimeter length of 230 km, which will protect the Arabian oryx as well as other wildlife in the area, such as Arabian gazelles (*Gazella gazella*) and Nubian ibex (*Capra ibex nubiana*) from poaching.

The majority of captive Arabian oryx in the Arabian Oryx Sanctuary at Jaaluni are females (about 75 %) because of the frequent release of males (see section 6.1). There is another captive breeding programme of Arabian oryx in Oman that was initiated in early 1980s at the Omani Mammals Breeding Centre in Seeb (Figure 1.1), which currently holds more than 150 oryx (AlRasbi, K., Personal Communication).

In Saudi Arabia, an intensive captive breeding programme for Arabian oryx was established in 1986 in Taif, starting with some oryx from the World Herd held in the USA and from private collections in Saudi Arabia (Abu Zinada et al., 1988; Asmode and Khoja, 1989). In 1990, the first re-introduction of Arabian oryx in Saudi Arabia was achieved, when oryx were released into Mahazat As-Sayd, a fenced protected area of 2,244 km<sup>2</sup> (Greth and Schwede, 1993; Bedin and Ostrowski, 1998; Ostrowski et al., 1998; Strauss, 2002). The population of oryx in Mahazat As-Sayd grew to more than 850 oryx by the end

of 2008. The availability of food, water within the fenced area and absence of poaching activities contributed to an increase of oryx population at Mahazat As-Sayd (Mesochina et al., 2003c; Chassot et al., 2005; Strauss and Anagariyah, 2007; Strauss, 2008). Arabian oryx were re-introduced to the wild in 1995, in Uruq bani Ma'arid, a sandy region close to the Empty Quarter (Ruba AlKhali Desert) (Mesochina et al., 2003a; Strauss, 2003; Chassot et al., 2005). This area was not fenced. The number of oryx in this protected area was estimated as 203 in 2003 (Bedin and Ostrowski, 2003), but has declined because of poaching, environmental stress and conspecific fights (Chassot et al., 2005). By the end of 2008 there were at least 150 Arabian oryx in this area (Strauss and Anagariyah, 2007; Strauss, 2008).

In Jordan, a captive breeding programme of Arabian oryx started in 1978, obtaining some oryx from the World Herd (Abu Jafar and Hays-Shahin, 1988). Later in 1983, most oryx were released into Shaumari Nature Reserve. The area of this reserve is 342 km<sup>2</sup> with a fenced part of 22 km<sup>2</sup> in which the oryx were kept. Because of the Gulf War (1990-1991) and the abundance of livestock of the fleeing Bedouin tribes in the unfenced part of the reserve, oryx could not be released from the fenced area (Harding, 2002; Harding et al., 2007).

The United Arab Emirates (UAE), holds the highest number of Arabian oryx in the world, currently estimated at 3000 individuals or more (AlQuarqaz and Kiwan, 2007). All the Arabian oryx in UAE are captive and held in private collections (AlQuarqaz and Kiwan, 2007). Only recently some Arabian oryx have been released into closely monitored fenced areas in Abu Dhabi (AlQuarqaz and Kiwan, 2007) and Dubai (Alqami, 2006). In Yemen, the Arabian oryx is locally extinct and has never been re-introduced or held in captivity (Al-Safadi, 2000). Seven Arabian oryx from the World Herd were made available to Israel in 1978, and bred in captivity until they were released to the wild in three sites, between 1997 and 2005 (Saltz, 1998; Gilad et al., 2008). The wild population in Israel currently numbers 90-100 individuals (Gilad et al., 2008). There are also some Arabian oryx living in zoos in Europe and USA. In 27 European zoological institutions, a total of 265 individual Arabian oryx were reported to be living according to the European studbook for the Arabian oryx (Ossowski-Mackie and Gilbert, 2008).

Overall, the total number of Arabian oryx in the Middle East is currently estimated at about 8000 oryx but the majority (more than 95 %) of them are held in some form of captivity (Strauss, 2008). They are distributed in different re-introduction programmes and captive breeding programmes (Table 1.1). Szablewski et al. (2006) recorded 2106 individual Arabian oryx from 184 zoological gardens in 30 countries obtained from International Species Information System (International Species Information System, 2002). So, the global total number of Arabian oryx now might approach 10,000 individuals. Despite this, the status of Arabian oryx in the IUCN red list of threatened species, is endangered (IUCN, 2008) according to version 3.1 of IUCN red list categories and criteria (IUCN, 2001).

The challenges that face the conservation of Arabian oryx in the wild include poaching, loss of habitat, natural droughts and the activities of the oil industry (Stanley Price, 1989; Tear et al., 1997; Spalton et al., 1999). Poaching is the primary challenge for re-introduction of Arabian oryx to the wild in Oman (Spalton et al., 1999; Al-Kharousi, 2006). Prolonged drought is a natural threat that challenges the survival of Arabian oryx in the wild (Spalton, 1999). However, Arabian oryx in the wild can walk for long distances in search of food and water in unfenced reserves (Kingdon, 1990).

Country	Programme	Type	Fencing	Year established	Area (sq km)	Number of oryx
Sultanate of Oman	Arabian oryx Sanctuary	Reintroduction	Currently being fenced	1980	2824	10
	Arabian oryx Sanctuary Omani Mammals Breeding Center	Conservation breeding	Fenced	1998	2	250
United Arab Emirates	Um-Azumul-Abu Dhabi Desert Reserve-Private Collections	Reintroduction	Fenced	2005	10000	100+
		Conservation breeding	Fenced	2003	225	?
		Private collections				4000
Kingdom of Saudi Arabia	Mahazat Asayed Uruq-Bani Maarid	Reintroduction	Fenced	1989	2244	850
		Reintroduction	Not fenced	1993	12500	150
Kingdom of Bahrain	Hwar island Shwmari Reserve	Conservation breeding	Not fenced		?	?
Jordan	Wadi Rum Reserve	Reintroduction	Fenced	1978	22	200
	Talila Reserve	Reintroduction	Fenced	2002	760	?
Syria	Adamah Reserve	Reintroduction	Fenced	1991	220	?
	Private Collections	Reintroduction Conservation breeding	Fenced	?	?	?
Qatar	Negev	Reintroduction	Not fenced	1997	?	100

Table 1.1 The countries where Arabian oryx have been re-introduced or there is a captive-breeding programme (Shobrak, 2007; Soorae, 2008).



Figure 1.1 Map to show locations of captive Arabian oryx in Oman. The Omani Mammals Breeding Centre is located in Seeb within the Capital, Muscat. Jaaluni is located in the central region of Oman within the Arabian Oryx Sanctuary in Jiddat Al Harasis area.

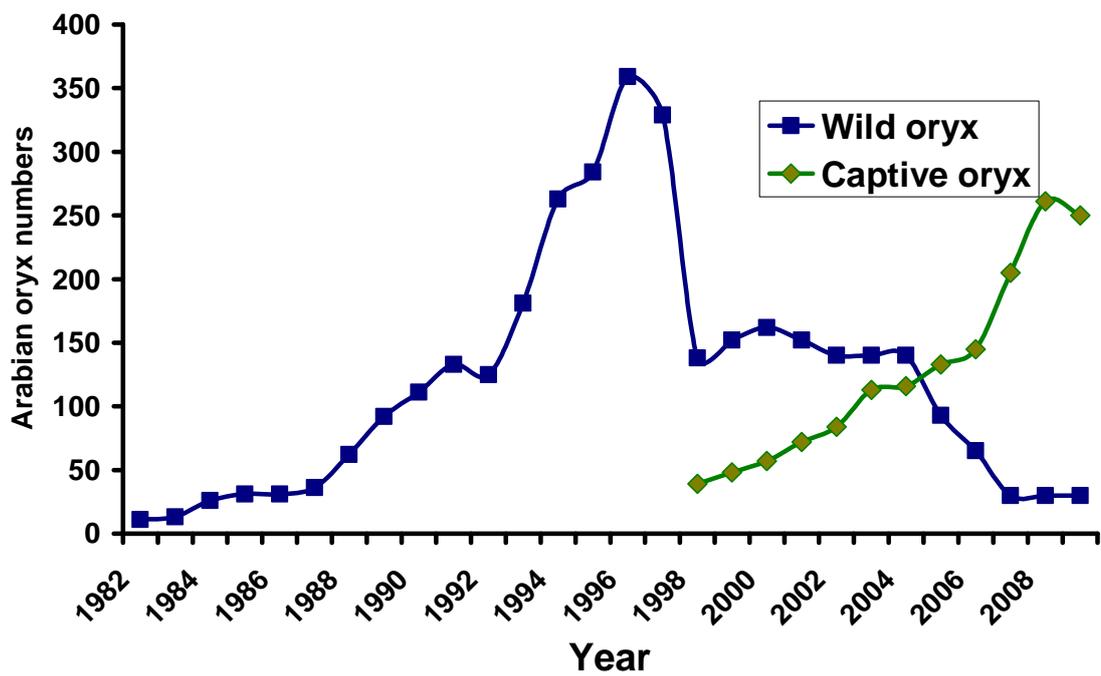


Figure 1.2 The number of Arabian oryx at the Arabian oryx sanctuary in Oman, in the wild (estimated by sight mark re-sight method, Arabian oryx project, unpublished data) and in captivity (counted) between 1982 and 2009.

### **1.1.2 Climate**

Arabian oryx lives in one of the harshest environments in the world in terms of water and food scarcity and at extremely high temperatures which approach 50 °C during summer. The mean monthly temperatures in Muscat and Jaaluni are shown in Figure 1.3 and Figure 1.4, respectively. Also, the data of monthly relative humidity in Muscat and Jaaluni are shown in Figure 1.5 and Figure 1.6, respectively. The Arabian Oryx Sanctuary has very low rainfall which is unpredictable temporally and spatially (Spalton, 1995). For example, Jaaluni received variable amounts of annual rainfall between 1990 and 2008 as shown in Figure 1.7.

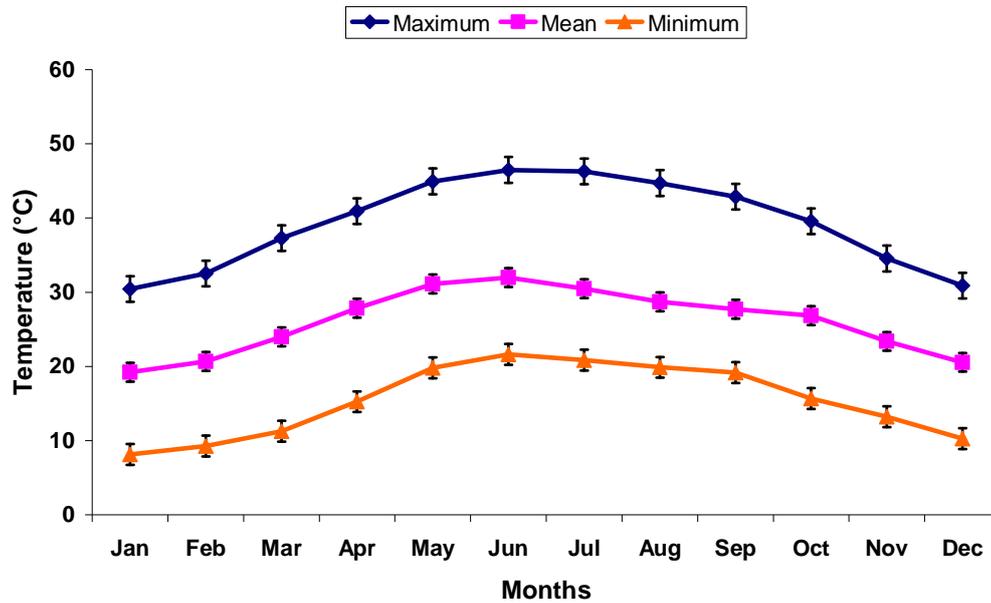


Figure 1.3 The means, minima and maxima of monthly temperatures at Jaaluni from 1980 - 2001. Data are shown as mean  $\pm$  standard error of mean. The data were obtained from Meteorological Department, Directorate General of Civil Aviation and Meteorology, Ministry of Transport and Communications, Oman.

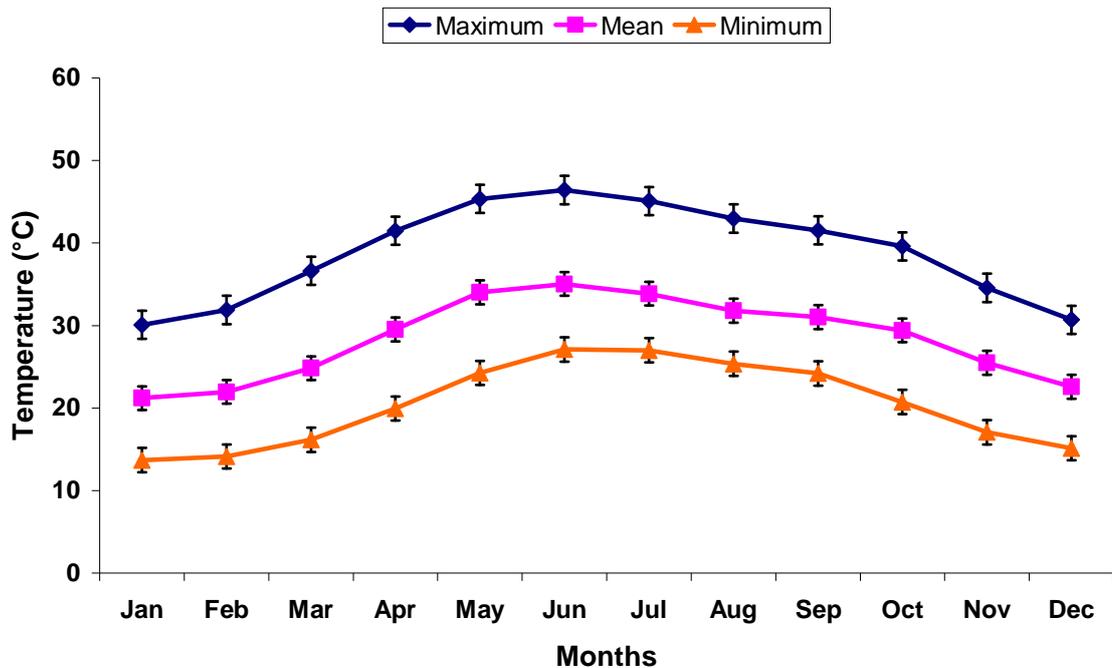


Figure 1.4 The means, minima and maxima of monthly temperatures at Seeb station in Muscat between 1974 and 2003. Data are shown as mean  $\pm$  standard error of mean. The data were obtained from Meteorological Department, Directorate General of Civil Aviation and Meteorology, Ministry of Transport and Communications, Oman.

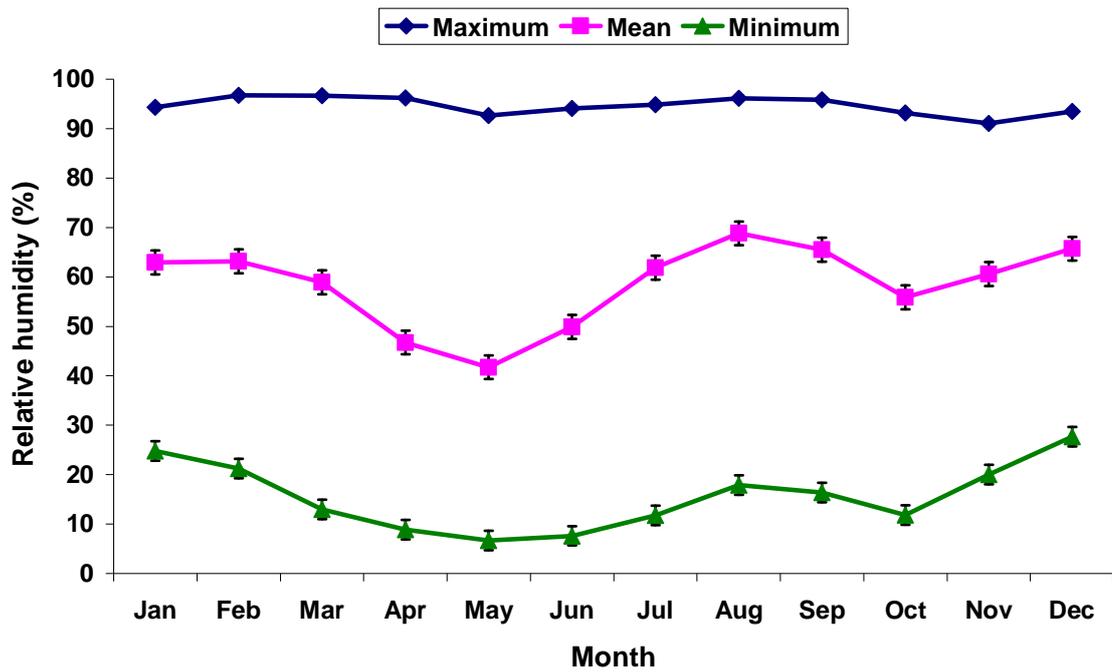


Figure 1.5 The means, minima and maxima of monthly relative humidity at Seeb station in Muscat between 1983 and 2003. Data are shown as mean  $\pm$  standard error of mean. The data were obtained from Meteorological Department, Directorate General of Civil Aviation and Meteorology, Ministry of Transport and Communications, Oman.

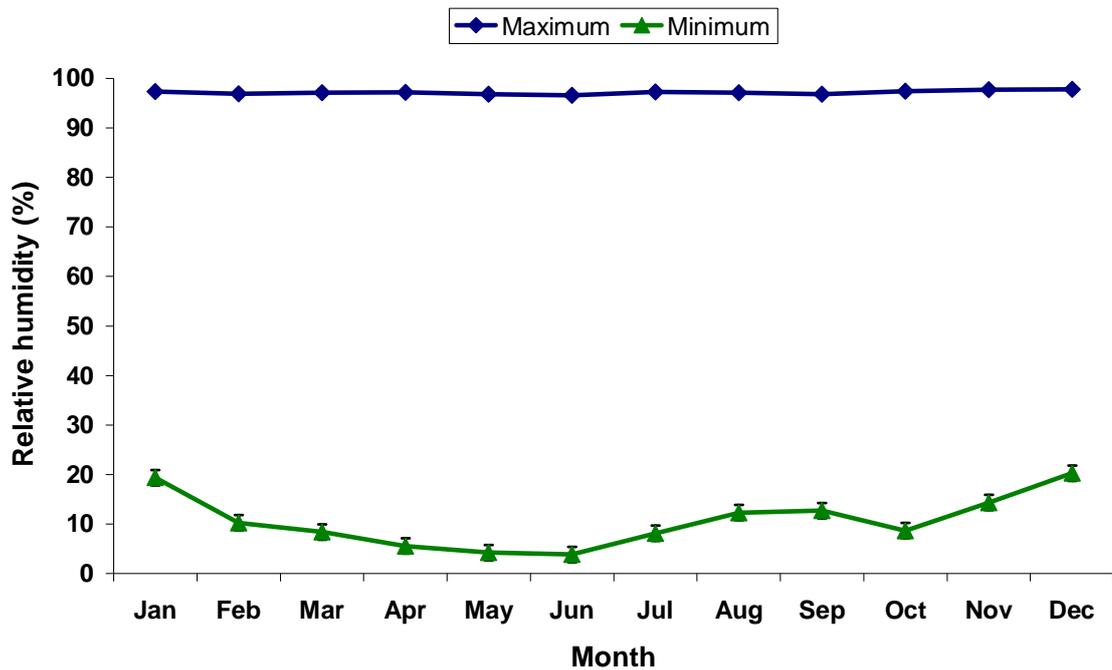


Figure 1.6 The minima and maxima of monthly relative humidity at Jaaluni between 1980 and 2003. Data are shown as mean  $\pm$  standard error of mean. The data were obtained from Meteorological Department, Directorate General of Civil Aviation and Meteorology, Ministry of Transport and Communications, Oman.

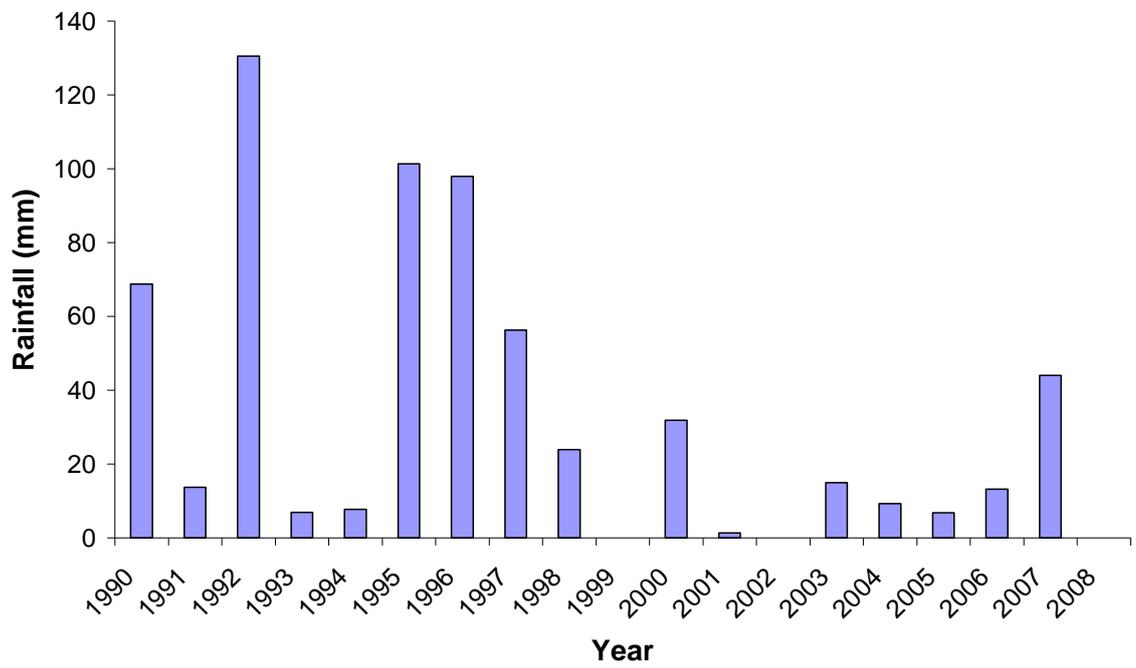


Figure 1.7 Rainfall at Jaaluni between 1990 and 2008, (Al-Kharousi (2003) and AOP, unpublished data).

### **1.1.3 Reproduction**

Natural populations of Arabian oryx are characterised by having a social system with a hierarchy of dominant and subordinate oryx (Ghandour, 1987; Kingdon, 1990; Tear and Ables, 1999). In a wild herd of Arabian oryx, one male is dominant while the rest of the males and females are subordinates. The dominant male usually prevents all subordinates from mating females in the herd, keeping this task exclusively for himself (Thorp, 1964; Kingdon, 1990). The cohesive social structure of the herd in the wild can be fragmented and the herd size will decrease when forage conditions deteriorate a few months after rain (Tear and Ables, 1999).

The Arabian oryx is an aseasonal and opportunistic breeder (Sempere et al., 1996; Ancrenaz et al., 1998). Conception in the wild can occur in any month of the year but peaks following good nutritional conditions after rainfall (Spalton, 1995). The male oryx reaches sexual maturity at around age 7-13 months and will breed in all months (Ancrenaz et al., 1998). In captivity, where food and water are provided, oryx conceive and give birth in all months of the year. Female oryx are polyoestrous, with an average oestrous cycle of 22 days and gestation length of  $260 \pm 5.5$  days (mean  $\pm$  SD) (Sempere et al., 1996; Vie, 1996). On average, wild female oryx calve for the first time at an age of  $28.2 \pm 5.3$  months and in best conditions at 20.7 months old (Spalton, 1995) close to the  $30 \pm 3.6$  months reported for Arabian oryx in captivity (Al-Kharousi, 2003). In captivity, female oryx reach sexual maturity between 13 and 17 months (Blanvillain, 1998) as the presence of males stimulates early maturity (Blanvillain et al., 1997). A non-invasive monitoring of reproduction by measurement of faecal progestins has been developed and found to be reliable in determining the reproductive status of captive Arabian oryx (Ostrowski et al., 2005).

### **1.1.4 Health and welfare of Arabian oryx**

Captive breeding of Arabian oryx proved to be essential for conservation in the period between establishment of the “World Herd” and the re-introduction programmes. The continuation of captive breeding is important to provide a reservoir of oryx for augmenting wild populations and to ensure their viability (Griffith et al., 1989; Tenhumberg et al., 2004).

Luckily Arabian oryx breed easily in captivity unlike some endangered species in Arabia such as the Arabian leopards (*Panthera pardus nimr*), that are very difficult to breed even with using artificial insemination techniques (Edmonds et al., 2006).

To avoid over-crowding in the enclosures and its negative impacts, and to reduce the high cost of feeding the growing numbers of Arabian oryx held in captivity in Oman, a zero-breeding programme was initiated in 2005 by separating males from females and holding them in separate enclosures (Spalton, J.A., Personal Communication).

Despite the importance of captive breeding of wildlife, there are some challenges such as the increasing cost, and potential health problems due to crowding or stress. Maintaining a healthy captive herd requires good feeding, good housing and frequent monitoring of well-being.

A captive herd is vulnerable to the outbreak of infectious diseases because they are restricted to a small area and the likelihood of close contact between individuals and infective agents is greater than for free-ranging animals in the wild. There is evidence that Arabian oryx are susceptible to some agents that affect livestock (Ostrowski et al., 2002a; Frolich et al., 2005). Many captive Arabian oryx collections are kept in close contact with domestic animals, where there is a risk of diseases transmission (Molnar et al., 2005). For example, tuberculosis and brucellosis has been seen to be transferred from domestic animals to captive herds of Arabian oryx in Saudi Arabia (Flamand et al., 1994b; Ostrowski et al., 2002a).

In Saudi Arabia, the first captive breeding program of Arabian oryx that was started in 1986 had a serious outbreak of tuberculosis in the late 1980s (Flamand et al., 1994b; Greth et al., 1994). This led to separation of calves from their mothers and hand rearing of many calves (Flamand et al., 1994a). Later tuberculosis was successfully diagnosed and managed (Greth et al., 1994) and this resulted in eradication of the tuberculosis and re-introduction of a healthy population into Mahazat As-Sayd reserve (Ostrowski et al., 1998).

Many diseases have been reported in Arabian oryx, especially captive ones (Ostrowski and Anagariyah, 2002, 2003). However these surveys of Middle East populations unfortunately

have not continued, even though more health problems might be predicted due to the increasing numbers of captive Arabian oryx.

The most frequently reported non-infectious pathological problem reported are related to digestive disorders, accidental traumas, such as broken legs and injuries because of fighting and jaw abscesses, and breeding problems (Ostrowski and Anagariyah, 2003). The commonly reported infectious diseases in the two surveys were brucellosis, enterotoxaemia caused by *Clostridium* sp., ecto-parasites (ticks, mites causing sarcoptic mange), endoparasites (nematodes, cestodes, *Taenia hydatigena*), and foot and mouth disease. In 2001, an outbreak of foot and mouth disease (FMD) was reported in an Arabian oryx collection in Bahrain (Ostrowski and Anagariyah, 2002). The control of endo-parasites such as nematodes is recommended and infected Arabian oryx in a zoo in Belgium were successfully treated with benzimidazoles (Goossens et al., 2004).

The surveys of diseases of Arabian oryx in the Middle East (Ostrowski and Anagariyah, 2002, 2003) also revealed an absence of some bacterial diseases (tuberculosis, paratuberculosis, botulism, anthrax, caseous lymphadenitis, lyme disease, leptospirosis, chlamydiosis, Q-fever and mycoplasmosis) and some viral diseases (rinderpest, PPR, bluetongue, rift valley fever, lumpy skin, and sheep and goat pox).

Recently some of the diseases that were reported absent has been reported in some countries. Q-fever (caused by *Coxiella burnetii*) has been reported in Arabian oryx and sand and mountain gazelles in Saudi Arabia (Hussein et al., 2010). The World Organisation of Animal Health (OIE) regarded peste des petits ruminants (PPR), sheep and goat pox as present in the UAE (Cardas et al., 2010). Several diseases were diagnosed in th UAE in wild and domestic animals including parasitic (coccidia, ticks and nematodes), viral (FMD, lumpy skin, PPR) and bacterial (clostridia, pasterella, and brucellosis) (Cardas et al., 2010). In 2009, an outbreak of FMD was reported in 2 mixed collections of wild ungulates in the UAE, which included the Arabian oryx that was reported to be affected by the disease (Bailey et al., 2009). One collection was annually vaccinated and the other was unvaccinated against FMD. The vaccinated collection had lower mortality rates and milder signs in comparison to the unvaccinated collection during the FMD outbreak (Bailey et al., 2009). The presence of such fatal infectious diseases in some collections of Arabian oryx

suggests the need for application of strict rules that prevent the spread of infectious diseases to other collections within or outside the country.

At the Omani Mammals Breeding Center, some recent cases of bluetongue in Arabian oryx with unknown origin have been diagnosed (AlRasbi, personal communication, 2010).

Peste des petits ruminants (PPR) disease is distributed across Asia, West, Central and East Africa and has been confirmed as the cause of mortality in free-ranging and captive ibex in Pakistan (Kock et al., 2010). Based on a survey of eight captive and one free-ranging herd of Arabian oryx in Saudi Arabian and UAE, PPR was reported as not present (Frolich et al., 2005). However, it was reported in goat and sheep in the UAE (Cardas et al., 2010), therefore caution should be taken to prevent the transmission to Arabian oryx and other captive-bred wildlife. There is worry that PPR virus threatens various regions across the globe and methods and tools that aid in diagnosis and prevention have recently been reviewed by Banyard et al. (2010).

A survey conducted in Saudi Arabia and UAE of eight captive and one free-ranging herd of Arabian oryx, also showed the exposure of Arabian oryx in some herds to bluetongue virus, epizootic hemorrhagic disease virus, rinderpest virus, bovine respiratory syncytial virus (BRSV), bovine adenovirus 3 (BAV-3), cervid herpesvirus-1, foot-and-mouth disease virus, equine herpesvirus 9, and bovine viral diarrhea virus (Frolich et al., 2005). Based on this survey extra caution should be applied when considering translocation of Arabian oryx and only animals that are health checked and proved to be free of diseases should be moved.

Most Arabian oryx nowadays live in captivity, but the effects of captivity and husbandry practices on the welfare of these animals are largely unknown. The well-being of animals in the wild are unpredictable as it depends on the habitat conditions that vary from time to time and therefore, cannot be controlled by conservation managers. In captivity, their health and welfare is determined in theory by the care they receive.

In captivity, with large numbers of oryx housed together, the normal social hierarchy is less clear. Personal observations suggest that because of the large number in captive herds, dominance status is difficult to maintain as the dominant male gets exhausted from mating

with females and simultaneous fighting with subordinates that attempt to violate the social hierarchy system and this ends up with loss of control of the herd.

Obviously there is a difference in the energy budget of captive and wild male Arabian oryx. The dominant captive male will spend less time feeding and more time mating and fighting with the competing adult males for dominance as the herd in captivity is usually too large to be controlled by a single male. When animals are released to the wild, the large herd will divide into many small herds each dominated by an adult male. This would result in less competition between males which will have more time for foraging.

The increasing number of animals in captivity and crowding may contradict with achieving good welfare status for the captive herd (Broom and Kirkden, 2004). Crowding of animals in captivity leads to aggression between individuals that may cause injuries. Melengestrol acetate, a synthetic progestogen (Petrow, 1970), was found to be effective for controlling aggression in male fringe-eared oryx (*Oryx gazella callotis*) (Patton et al., 2001) and reported to decrease aggression in scimitar-horned oryx (*Oryx dammah*) (Blumer et al., 1992), male muntjacs (*Muntjacus reevesi*) (Stover et al., 1987) and farmed fallow bucks (*Dama dama*) (Wilson et al., 2002). The crowding issue can also be solved by breeding control. If the breeding of animals is not controlled, the numbers will grow more and more as is probably the case for Arabian oryx in many private collections and zoos in the Middle East and elsewhere. Therefore, welfare of the worldwide growing numbers of Arabian oryx in captivity is becoming a concern.

The captive conditions vary among different captive breeding programmes. For example, in private collections, Arabian oryx are often housed with other wildlife such as gazelles and ibex, and have minimal health checkups and little concern for their welfare (Ostrowski and Anagariyah, 2003). In addition, contact of captive Arabian oryx with other species may result in transfer of infectious diseases and is therefore not recommended (Ostrowski and Anagariyah, 2003). Although in the wild they can be expected to have contact with other species or pathogens, lower population densities of animals, means this is much less likely. Governmental breeding programmes typically have better resources and veterinary services than private collections.

The assessment of the welfare of captive animals is very important in order to improve the captive environment and avoid or minimise negative consequences that might result such as impairment of health, poor reproduction, stress, suppression of the immune system and possible mortality (Duncan, 2005; Jordan, 2005; Botreau et al., 2007; Webster et al., 2008). For Arabian oryx in captivity, some management practices might affect the welfare of the animals. For example, their management requires physical practices such as capture, handling and temporary confinement as a preparative step for transportation. Capture of wildlife either physically or using chemical immobilisation, sometimes causes capture myopathy, which is characterised by muscular stiffness, lack of coordination and sometimes death (Vassart et al., 1992; Montan, et al., 2002; Ostrowski and Anagariyah, 2003).

Transportation of Arabian oryx is a common practice used in moving oryx between captive breeding programmes or from captivity to the wild or vice versa. Transport of wildlife is sometimes inevitable but can put the health of animals at risk (Grandin, 1997), causes short term stress (Openshaw, 1993; Fazio and Ferlazzo, 2003), and can jeopardise survival. For example, eight out of forty Arabian oryx died within 2 weeks after transport in Saudi Arabia (Ancrenaz et al., 1995). Measures to mitigate its impacts should be used whenever possible. For example, familiarisation with handling before transport, good transport facilities and the use of long-acting tranquillisers during transportation.

The physical practices used for management of wildlife in captivity such as capture and transport, often require use of chemicals such as immobilisers and tranquillisers. Immobilisation chemicals such as ketamine, xylazine, etorphine and medetomidine are widely used in capture of Arabian oryx (Ostrowski et al., 1993; Ancrenaz, 1994; Ancrenaz et al., 1996; Molnarova et al., 2005), and long-acting tranquillisers such as perphenazine enanthate and haloperidol have been used for calming Arabian oryx during transportation (Ancrenaz et al., 1995; Strauss and Anagariyah, 2007). Chapter 4 examines the physiological effects of perphenazine enanthate, and Chapter 5 examines the physiological effects of xylazine on Arabian oryx.

The confinement of animals before transport or before release to the wild is necessary for familiarisation of animals to handling and closer contact with humans (Ancrenaz et al.,

1995). Exposure of animals to the same stressor e.g. frequent handling by humans, has been shown to reduce the stress response over time, which means that animals become acclimated to the stressor (Bhatnagar and Vining, 2003; Romero, 2004).

The release of animals from captivity to the wild is a difficult transition stage (Letty et al., 2000; Tenhumberg et al., 2004). In captivity, food, water and shade are provided and these might not be adequately available after release to the wild. The period of transition between life in captivity and in the wild and gradual adaptation to the new environment might be a tough and a stressful event (Lyles and May, 1987; Curio, 1996). High mortality rates in rabbits after release to the wild has been attributed to handling trauma and the novelty of the natural environment (Letty et al., 2000). So many issues need to be considered during the planning of the release of captive animals to the wild. However, by using a “soft release” approach for releasing Arabian oryx to the wild of Oman during a time with abundant good vegetation condition, oryx became adapted easily after release (Spalton J.A., Personal Communication). After release these oryx were closely monitored by biologists and rangers and sometimes food and water were provided if needed.

Arabian oryx should always be released when there is adequate vegetation in the release area. The site for release should have trees that offer shade from direct sunlight and shelter for animals to help with adaptive behavioural thermoregulation during the high summer temperatures (Stanley Price, 1986; Seddon and Ismail, 2002; van Heezik et al., 2003).

Social planning is also an important part of re-introduction of a group of animals to the wild. Formation of a socially cohesive herd of Arabian oryx is recommended before the release as one group (Stanley Price, 1989). The re-introduction of Arabian oryx in Oman was the first ever re-introduction of a large mammal to the wild in a location that formally was a part of its historic range (Stanley Price, 1986). The social planning for that re-introduction used information about herd size and structure for fringe-eared oryx (*Oryx beisa callotis*) in Kenya (Stewart, 1962). Another example was the translocation of three socially cohesive troops of 131 olive baboons (*Papio anubis*) in Kenya which resulted in no mortalities (Strum and Southwick, 1986). Before release of any captive animals, a health check-up should be carried out and vaccination against common infectious diseases that

might be acquired from livestock or wild animals in the release area (Woodford and Kock, 1991).

Monitoring and management of animals after release is equally important (Kleiman, 1989; Treydte et al., 2001; Mesochina et al., 2003b). The lack of follow-up in many reintroduction programmes to establish survival and long-term persistence, might decrease the success rate of the programme (Seddon, 1999). Mortalities of Arabian oryx after release to the wild were reported as a result of failure to adapt to the new environment and fighting between males in Saudi Arabia (Mesochina et al., 2003c). Dehydration and malnutrition caused death of some wild Arabian oryx during prolonged drought (Spalton, 1993), even though the Arabian oryx is well known for its ability to survive indefinitely without drinking water relying instead on metabolic water and preformed water in the forage (see section 1.1.5; Williams et al., 2001; Ostrowski et al., 2002b). During prolonged drought, the water content of plants decreases to a minimum and they desiccate or may even die (Spalton, 1995), and this leads to malnutrition, dehydration and can cause deaths of oryx in extreme cases. It was observed that a herd of oryx was capable of changing their habits from drinking daily (in captivity) to total water independence in a single day given sufficiently moist vegetation complex which is hardly available in arid desert (Tear, 1992). As Arabian oryx live in areas with very low unpredictable rainfall, this has a severe impact on reproduction in the wild, reducing conception rate and the chance of survival of the foetus and new born calves (Spalton, 1995).

### **1.1.5 Physiological adaptations of Arabian oryx**

Most Arabian oryx live in the Arabian Peninsula, which is considered a hyper-arid zone, due to extremely high temperatures and low sporadic rainfall (Ghazanfar, 1992). The animals therefore are physiologically adapted to survive these harsh conditions.

#### *1.1.5.1 Water economy and heterothermy*

Arabian oryx held in captivity and provided with water will consume the water. For example, water consumption of captive Arabian oryx held in the Arabian Oryx Sanctuary at Jaaluni varied between 1.3 and 5.01 litres per day in cold and hot months respectively (Stanley Price, 1989). In captivity, Arabian oryx obtain water from drinking water (86.6

%), metabolic water (11.2 %) and preformed water (2.2 %) (Ostrowski et al., 2006). In the wild, however, Arabian oryx can, if necessary, survive indefinitely in hot deserts without drinking (Ghandour, 1987; Williams et al., 2001; Ostrowski et al., 2002b; 2003; 2006) even in Rub' AlKhali (the 'Empty Quarter'), one of the driest regions in the world (Meigs, 1953). In the Mahazat as-Sayd Reserve in Saudi Arabia, other than temporary pools after infrequent rain, the fenced reserve provides no drinking water for the oryx (Ostrowski et al., 2002b). Furthermore, mothers of two calves in Oman were observed lactating for six months before they had a drink of water (Stanley Price, 1986). In an experiment carried out in Mahazat As-Sayd reserve in Saudi Arabia, Arabian oryx were reported to have an extremely low mass-specific water influx rate ( $31.5 \text{ ml kg}^{-0.922} \text{ day}^{-1}$ ), only 32 % that of the camel ( $99.3 \text{ ml kg}^{-0.922} \text{ day}^{-1}$ ) (Maloiy, 1973; Ostrowski et al., 2002b). To conserve water, Arabian oryx employ heterothermy with a maximum daily amplitude of the body temperature reaching  $7.7^\circ\text{C}$  during hot-dry periods (Hetem et al., 2010).

In Saudi Arabia, Arabian oryx in the wild were found to rely on preformed water in the food they consume and the metabolic water that is formed from oxidation of the consumed food (Williams et al., 2001; Ostrowski et al., 2002b; 2003; 2006). In an experiment carried out by Ostrowski et al. (2006) to simulate water scarcity in the desert by reducing water intake of oryx by 70 % over a period of five months, the oryx were found to maintain a constant plasma osmolality. They achieved this by increasing the renal re-absorption of water, increasing urine osmolality and reducing urine volume by 40 %, and excreting faeces with less than 50 % water content.

In the Jiddat AlHarasis, the area where the Arabian Oryx Sanctuary is located, the phenomenon of fog occurs heavily in spring (March/April) and autumn (September/October) and mildly in other months (Stanley Price et al., 1988; Spalton, 1995). The dew that precipitates on leaves of trees and grasses, provides another source of water in that barren landscape (Stanley Price et al., 1988; 1989). There are about 200 plant species on the central desert of Oman and some plants are partially sustained by fog and dew which supplement moisture for growth (Ghazanfar, 2004). Because of the fog, some species of lichens occur and form part of the diet of some mammals in the area like the Arabian gazelle (*Gazella gazella*) (Hawksworth et al., 1984). There are no reports that Arabian oryx

consume these lichens, as oryx mainly graze on the grasses, and also do not browse trees as gazelles do.

#### 1.1.5.2 Nutrition and diet

Gillet et al. (1989) examined the nutritional value of plant species found naturally in Saudi Arabia and favoured by Arabian oryx, and suitable feeding supplement for Arabian oryx in captivity. Alfalfa was found to have a very high total crude protein content (about 20 %) which makes it a very good diet for Arabian oryx in captivity (Gillet et al., 1989). Most plant species in the wild are nutritionally poor (Gillet et al., 1989) and therefore, the Arabian oryx feeds on a wide range of plant species, walking long distances to fulfil its daily requirement (Gillet et al., 1989). As the smallest species of the genus *Oryx*, the Arabian oryx can satisfy itself with less food in the wild than other species of *Oryx* (Gillet et al., 1989). The plant species existing in the Arabian Peninsula vary between countries (Gillet et al., 1989; Ghazanfar, 2004), so the favourite plant species of Arabian oryx foraging in the wild also differs. In Saudi Arabia, in Taif, thirty-two species of grasses, herbs, shrubs and trees were present in an enclosure that had not been grazed for two years following rain. Arabian oryx was observed feeding on seventeen of these species and the most favoured species was *Cynodan dactylon* (Asmodé, 1990). Arabian oryx was also observed digging the plants with its hoofs while feeding in order to get moisture and minerals (mainly sodium) from the roots and tubers (Asmodé, 1990). In Oman, the grass species *Stipagrostis sp.* was the most favoured plant species for Arabian oryx foraging in the wild (Kingdon, 1990; Tear et al., 1997; Spalton, 1999).

At the Arabian Oryx Sanctuary, Oman, the feeding ecology of Arabian oryx was evaluated by analysis of faecal content in comparison to the plant species on site. Arabian oryx were found to feed mainly on 20 of the 41 plant species present. After rainfall, oryx were found to feed mainly on monocotyledons (81 %) whereas in the dominant dry periods they concentrate foraging on dicotyledons (77 %) which have a higher crude protein content (Al-Mahdhoury, 2003).

The adaptation of Arabian oryx for survival with minimal water and food intake makes it a successful large desert mammal capable of living in one of the harshest environments in Arabia. Despite this, with severe prolonged droughts, the nutritional content of plants

deteriorates and water content decreases and can lead to loss of body condition and eventual death of oryx (Spalton, 1995; 1999). The crude protein content of the grass *Stipagrostis* decreases after approximately 200 days following the last rainfall, to less than 6 %, which is the minimum requirement for maintenance of the body condition of Arabian oryx (Spalton, 1995; 1999).

## 1.2 Stress and welfare

As for welfare, there is also no standard definition of stress. Regardless of definitions, one of the potential indicators of good welfare is the absence of distress (Moberg and Mench, 2000). Animals have sophisticated behavioural and physiological mechanisms to deal with stressors. Stress is considered to jeopardise the welfare of animals, only if it results in some significant biological change that places the animal's well-being and/or homeostasis at risk (Moberg and Mench, 2000). A multitude of hormones are involved in initiation of the stress response, such as adrenocorticotrophic hormone (ACTH), glucocorticoids, catecholamines and prolactin (Matteri et al., 2000) and measuring these hormones can be used for assessment of distress and welfare.

The welfare of wildlife in captivity is a concern for the general public as well as scientists, although the definition of welfare is the subject of debate and controversy. According to Hewson (2003), a widely accepted definition of animal welfare is that poor welfare comprises the state of the animal's body and mind and the extent to which its nature (genetic traits manifest in breed and temperament) is satisfied (Duncan and Fraser, 1997). Animal welfare was summarised in a short statement as "fit and feeling good" (Webster et al., 2004). There are many other definitions of welfare but some researchers have said that a precise scientific definition is not possible (Fraser, 1999; Duncan, 2005). While controversy may swirl around as to how to define animal welfare, no one disagrees with the argument that suffering from stress threatens an animal's welfare (Moberg, 2000). Therefore, a potential indicator of good animal welfare is the absence of distress (Möstl and Palme, 2002). The key to determining when stress affects an animal is the biological cost of the stress. The animal experiences distress when the biological cost of coping with the stressor diverts resources away from other biological functions such as maintaining immune competence, reproduction or growth. During distress, the impairment of biological

functions places the animal in a pre-pathological state that renders it vulnerable to a number of pathologies (Moberg, 2000). Glucocorticoids play a crucial role during initial response to stress for an animal's survival, however, prolonged high blood levels of these hormones has severe cost (Wingfield, 2001). Stress and welfare are closely-related terms and sometimes considered as opposites since good welfare cannot be achieved under chronic stress and vice versa (Veissier and Boissy, 2007).

In 1993, the Farm Animal Welfare Council in the UK, developed what is widely known as the Five Freedoms (FAWC, 1993) as a means of assessing welfare. These are:

- 1- Freedom from thirst, hunger and malnutrition – by ready access to fresh water and a diet to maintain full health and vigour.
- 2- Freedom from discomfort – by providing a suitable environment including shelter and a comfortable resting area.
- 3- Freedom from pain, injury and disease – by prevention and rapid diagnosis and treatment.
- 4- Freedom from fear and distress – by ensuring conditions that avoid mental suffering.
- 5- Freedom to express normal behaviour – by providing sufficient space, proper facilities and company of the animal's own kind.

These Five Freedoms provided a concise and comprehensive framework for assessment of welfare in farmed animals (Webster, 2001; Whay et al., 2003; Webster et al., 2004). However, the framework of Five Freedoms has been criticised for its neglect of the process of allostasis (Korte et al., 2007). The concept of allostasis was coined by Sterling (Sterling and Eyer, 1988) to describe the maintaining of stability (homeostasis) through physiological or behavioural changes in response to challenges. In relation to welfare, the concept of homeostasis has been considered too limited because it ignores the hypostimulation that results from an absence of environmental challenges (Korte et al., 2007). Korte et al. (2007) stated that the Five Freedoms are more an ethical viewpoint than a science-based approach and complete 'freedom' is undesirable. Based on the allostasis

concept, the animals have several regulatory mechanisms for thirst, hunger, pain, distress etc, and the complete freedom from these factors could lead to deactivation of regulatory mechanisms leading to a failure to respond adequately to challenges after prolonged deactivation (Korte et al., 2007). Hypostimulation may thus produce negative effects. For instance, neuronal survival depends on whether new neural cells are sufficiently activated by incoming signals (Jacobs et al., 2000) in a process that is referred to as “use it or lose it” (Swaab, 1991). On the other hand, chronic hyperstimulation, because of high allostatic load, leads to development of pathologies due to “wear and tear” effects (McEwen, 2004).

Another example is freedom from distress, which is one of the five freedoms. Distress is usually associated with negative effects and consequences. Glucocorticoids are well known as stress hormones, but they can also be called anti-stress hormones because of their beneficial role during stress (McEwen and Wingfield, 2003). The scientific challenge is to make a cost-benefit analysis by determining under which conditions the costs outweigh the benefits and vice versa (Korte et al., 2007). A third example of the delicate balance is the freedom from hunger compared to *ad libitum* availability of food in farm or zoo animals, which produces problems (Wechsler, 1991). Mammals that are fed on restricted calorie diets live longer and therefore, longevity and hunger are considered part of a healthy life (Korte et al., 2007).

The concept of allostasis explains that high glucocorticoid levels do not always indicate poor welfare as these hormones could increase in response to normal animals’ activities such as mating (Romero, 2002). However, it is widely agreed that a low allostatic load (not very low or zero glucocorticoid levels) is key for good health and good animals welfare (Korte et al., 2007).

### **1.2.1 Assessment of health, stress and welfare**

The assessment of welfare in animals is not straightforward, and with the exception of humans, feelings cannot be measured directly and therefore assessments are indirect for example, by preference and motivational tests (Duncan, 2005). Measurements of impaired biological functioning such as decreased health and increased physiological stress can provide good corroborating evidence that welfare is compromised (Duncan, 2005). Dawkins (2003; 2004) argued that behaviour is a better tool for assessment of welfare than

physical health. Measurement of behaviour is often favoured in assessment of welfare in wild animals as physiological assessments are often more difficult (Jordan, 2005). However, physiological assessment of the welfare of wild animals has been made feasible by recent developments of non-invasive methods of measuring glucocorticoids and their metabolites in urine (Hay and Mormede, 1998; Schoemaker et al., 2004), saliva (Vining et al., 1983; Lebelt et al., 1996) or faecal samples (Möstl and Palme, 2002; Lane, 2006).

Monitoring of overall health is very important for conservation of wildlife and to aid the process, establishment of “normal” or reference values for meaningful parameters in threatened or endangered wild species is critically needed (Deem et al., 2001). Data for reference ranges of relevant parameters in wild animals are very few compared to domestic animals. The data needed includes blood parameters such as complete blood count, serum biochemistry profiles, vitamins and mineral levels (Karesh et al., 1997; Deem et al., 2001). Such data can be used in assessing the viability of a population and its health (Karesh and Cook, 1995). Examples of establishment of reference values for haematological and biochemical parameters are those collated for the Spanish ibex (Perez et al., 2003), donkey (Caldin et al., 2005), working horses (Pritchard et al., 2009) and hairy-nosed wombats (Reiss et al., 2008). Chapter 3 aims to establish reference values for the Arabian oryx for haematological, some ions, biochemical and clinical parameters.

### **1.2.2 Improvement of welfare**

After assessment of welfare and finding that some animals are in poor welfare states, the next important step is to improve the welfare status (Webster et al., 2004; Whay, 2007). Improvement of animal welfare can be done through environmental enrichment (Shepherdson et al., 1998; Young, 2003). For example, the use of an enriched cage system improved the welfare of rabbits as implied from behavioural assessment (Hansen and Berthelsen, 2000). The enrichment of cages by introducing occupational materials to farmed mink (*Mustela vison*) improved their welfare as measured by behavioural and physiological methods. This enrichment resulted in fewer stereotypies and reduced level of faecal glucocorticoid metabolites (Hansen et al., 2007).

### 1.2.3 Catecholamines

Catecholamines such as adrenaline (epinephrine) and noradrenaline (nor-epinephrine) are secreted from the chromaffin cells of the adrenal medulla during stress under stimulation of the sympathetic nervous system. These hormones are secreted during acute stress within seconds and cause cardiovascular responses, such as an increase in heart rate, cardiac output and blood pressure, pupil dilation, and metabolic effects. Metabolic actions increase blood glucose as a result of glycogenolysis and gluconeogenesis in the liver (Norman and Litwack, 1990).

*In vitro* studies in sheep revealed that nor-adrenaline and adrenaline are present not only in plasma (70 %), but also in erythrocytes (30 %) mainly bound to haemoglobin (El-Bahr et al., 2006). Also, about 15 % of the plasma fraction is strongly bound (probably by covalent bonds) to plasma proteins, mainly albumins (70 %) and globulins (30 %) (El-Bahr et al., 2006). Catecholamines have a half-life of about 1 min in the circulation as reported in sheep (Jones and Robinson, 1975).

Intravenous injection of  $^3\text{H}$ -adrenaline and  $^3\text{H}$ -nor-adrenaline, revealed that catecholamines are mainly excreted in the urine (96 %) and little is excreted via faeces. Although metabolites of catecholamines appear to be excreted in the faeces, the quantitative measurement was not found to be possible (El-Bahr et al., 2005). Because of rapid secretion and binding of catecholamines to plasma proteins and erythrocytic haemoglobin, and fast clearance from the circulation, measurement of these hormones in plasma as an indicator of stress might not be reliable (El-Bahr et al., 2005; Palme et al., 2005). However, a greater problem in wild animals is the need for very rapid collection of blood samples, within seconds, which will rarely be possible. Therefore, alternative approaches are needed.

### 1.2.4 Glucocorticoids

Glucocorticoids, also called glucocorticosteroids e.g. cortisol and corticosterone, are secreted during stress from the adrenal cortex within a few seconds to a few minutes after the perception of stress (Sapolsky et al., 2000). Some animals have a mixture of both cortisol and corticosterone and one of them is more dominant than the other in different species (Von Holst, 1998). For example, cortisol is dominant in certain species such as

antelopes, primates, dogs, cats, horses and pigs while corticosterone is dominant in mice, rats, rabbits and birds (Palme et al., 2005). The secretion of glucocorticoids is regulated by the Hypothalamic–Pituitary-Adrenal (HPA) axis. During stress, the hypothalamus releases corticotropin-releasing hormone (CRH), which triggers the anterior lobe of the pituitary (pars distalis) to release adrenocorticotrophic hormone (ACTH). The secreted ACTH stimulates the adrenal cortex to synthesise and secrete cortisol. The elevation in the concentration of plasma cortisol inhibits further release of ACTH by a negative feedback mechanism (Norman and Litwack, 1990; Nussey and Whitehead, 2001; Smith and Dobson, 2002).

#### 1.2.4.1 *Synthesis, circulation and metabolism of glucocorticoids*

Glucocorticoids are synthesised from cholesterol in the adrenal cortex. The majority of cortisol and corticosterone molecules bind in circulation to globulins (90 %) and albumin (6 %), while the remaining 4 % are unbound (Nussey and Whitehead, 2001). The half-life of cortisol in the circulation is about 100 min (Kerrigan et al., 1993). Glucocorticoids are conjugated in the liver and pass through the bile as glucuronates and sulfonates to the gut (Taylor, 1971; Möstl and Palme, 2002). Most of the conjugated metabolites are excreted via urine and some pass via the bile to the gut (Figure 1.8) (Möstl and Palme, 2002). In the gut, they are further metabolised to many different compounds (Macdonald et al., 1983). Some metabolites are partially deconjugated in the gut by bacterial enzymes (Taylor, 1971; Palme et al., 1996) and partially reabsorbed through enterohepatic circulation (Lindner, 1972; Palme et al., 2005). Steroid hormones undergoing enterohepatic circulation are reduced in the liver in the A ring of the steroid (see Figure 1.9)(Groh et al., 1993). Glucocorticoid metabolites are excreted eventually in faeces (Brownie, 1992).

In view of the metabolic processes, the steroid hormones themselves are often absent from the faeces or only present at very low levels. For example, in sheep, after infusion of 1 g of <sup>14</sup>C- cortisol, no cortisol molecules were detected in the faeces (Palme and Möstl, 1997; Möstl et al., 1999; Bahr et al., 2000).

The majority of the immuno-assays used for measurement of cortisol in plasma are specific for cortisol, because monoclonal antibodies raised against cortisol are employed, and these assays cannot be used for measurement of cortisol metabolites in faeces because of poor

cross-reaction with the metabolites (Palme et al., 1999). Therefore, analysis of glucocorticoid metabolites is usually preferred using EIAs that have better cross-reactivity with the faecal metabolites of cortisol/corticosterone (Palme et al., 2005).

#### 1.2.4.2 *Route of excretion of glucocorticoid metabolites*

The primary route for excretion of cortisol in many species is urine (Palme et al., 2005). For example, infusion of  $^{14}\text{C}$ -cortisol showed that 93 %, 59 % and 72 % of radioactivity appeared in the urine of pigs, ponies and sheep, respectively (Palme et al., 1996) and the rest of the radioactivity appeared in the faeces. In dogs, approximately 20 % of cortisol metabolites are excreted in the faeces, while the majority are excreted in the urine (Schatz and Palme, 2001). However, in some species, such as cats, more than 80 % of the cortisol metabolites are excreted in the faeces (Graham and Brown, 1996; Schatz and Palme, 2001).

The small proportion of glucocorticoid metabolites excreted in faeces in some species, does not preclude the measurement of faecal metabolites, as the total faecal content depends on cortisol production rate and the amounts of faeces, and use of a sensitive enzyme immunoassay that enables the measurement of very minute quantities of metabolites (Teskey-Gerstl et al., 2000). The glucocorticoid metabolites accumulate in the faeces over time and result in measurable amounts after efficient extraction using sensitive immuno-assays (Möstl and Palme, 2002).

Glucocorticoids are extensively metabolised (Figure 1.8), and the types of metabolites formed differ considerably between species (Bahr et al., 2000; Schatz and Palme, 2001). In ruminants, 21 metabolites with  $\text{C}_{19}\text{O}_3$  or  $\text{C}_{21}\text{O}_4$  structures were detected in faecal samples using HPLC coupled with mass spectrometry (Möstl et al., 2002; Palme et al., 2005). Similar metabolites are likely to occur in the Arabian oryx, but as yet there have been no investigations of glucocorticoid excretion by the Arabian oryx.



### 1.2.4.3 Circadian and seasonal pattern of secretion of glucocorticoids

The pattern of glucocorticoid secretion has been reported to vary within or about 24 h (ultradian or circadian rhythms, respectively) or on annual basis (seasonal or circannual rhythms) (Ingram et al., 1999). Circadian rhythms of plasma glucocorticoid concentrations are well established in humans (Weitzman et al., 1971), sheep (Fulkerson and Tang, 1979), red deer (*Cervus elaphus*) (Ingram et al., 1999) and have been reported in some studies of cattle (Lefcourt et al., 1993). However, Hudson et al. (1975) did not detect any circadian rhythm of plasma cortisol concentrations in cattle.

In ruminants that have long gut passage times, the faecal glucocorticoid metabolites might not show diurnal differences (Millspaugh and Washburn, 2004). In cattle for example, although some diurnal variations were seen in plasma cortisol, the faecal glucocorticoid metabolites did not show any significant differences (Palme et al., 2003; Touma and Palme, 2005). Despite the absence of diurnal variations in faecal glucocorticoid metabolites in some species, it is recommended to collect faecal samples excreted at the same time of the day if repeated single measurements are made on different days, and when comparing different groups or populations of animals (Touma and Palme, 2005).

Seasonal differences in the basal levels of plasma glucocorticoids have been documented in animals with well-defined breeding seasons as reviewed by Romero (2002). The seasonal changes in the basal levels might be attributed to the availability of food, seasonal weather patterns and/or the presence of mating partners (Lane, 2006). Romero (2002) found that the glucocorticoid concentrations in free-ranging reptiles, birds, amphibians but not mammals, are elevated during their breeding seasons. However, some mammals such as the squirrel monkeys (*Saimiri sciureus*) show higher concentrations of plasma glucocorticoids during mating (Coe and Levine, 1995). Regardless of the existence or absence of circadian or seasonal rhythm, the concentration of cortisol secreted during stress is higher than the basal concentration secreted on a circadian or seasonal basis (Ingram et al., 1999; Landys et al., 2006).

#### 1.2.4.4 The gender differences in glucocorticoid secretion

Pronounced gender differences have been shown in baseline plasma glucocorticoids as well as in the reactivity of the HPA axis to stressors. For example, in ground squirrels, the free plasma concentration of cortisol in adult males was half that of adult females (Boonstra et al., 2001), and in domestic sheep, plasma cortisol was significantly higher in females than males (Turner et al., 2002). Differences in the plasma concentrations of glucocorticoids might be expected to influence the faecal content of glucocorticoid metabolites. This expectation might explain the higher faecal content of glucocorticoid metabolites in the female European hare (*Lepus europaeus*) (Teskey-Gerstl et al., 2000), domestic dog (Schatz and Palme, 2001), African wild dog (*Lycaon pictus*) (Creel, 1997) and cheetah (*Acinonyx jubatus*) (Wielebnowski et al., 2002) or in male laboratory rats (Cavigelli et al., 2005) and Steller sea lion (*Eumetopias jubatus*) (Hunt et al., 2004). Despite that, some studies found no differences in faecal content of glucocorticoids metabolites of males and females as in wolf (*Canis lupus*) (Sands and Creel, 2004), black rhinoceros (*Diceros bicornis*) and white rhinoceroses (*Ceratotherium simum*) (Brown et al., 2001) or red deer (*Cervus elaphus*) (Huber et al., 2003a).

Many factors might be responsible for gender-specific differences in the faecal content of glucocorticoids (Touma and Palme, 2005). The higher capacity of steroid-binding globulins in females and their high affinity to glucocorticoids is thought to be the reason behind the higher levels of faecal glucocorticoid content in females compared to males (Breuner and Orchinik, 2002). The existence of differences between sexes in the faecal content of glucocorticoid metabolites might also result from the reproductive steroid hormones present in males and females, differences in the routes for excretion of glucocorticoid metabolites and differences in the types of metabolites formed by males and females (Touma and Palme, 2005). Sex differences in the amounts of excreted steroids have been reported in cats (Schatz and Palme, 2001), and also for ponies and pigs (Palme et al., 1996). Therefore, possible gender differences should be considered when measuring faecal glucocorticoid metabolites in males and females of a given species.

#### 1.2.4.5 *Lag-time of faecal production*

The rate of faecal production differs between species, for example, small animals like rats and mice usually defecate more frequently than large animals that have relatively long-gut passage time. This applies to hind gut fermenters and most carnivores and reptiles, which defecate rather infrequently (Touma and Palme, 2005). Arabian oryx is a ruminant and a delay in defecation is expected. However, further research is needed to determine the frequency of faecal production especially in relation to the delay of appearance of glucocorticoid metabolites in the faeces. Collection of fresh faecal samples produced at the same time of the day is a challenge. This can be overcome by housing animals in individual pens and collecting the freshest sample in the pen from each animal (Millsbaugh and Washburn, 2003).

Faecal glucocorticoid metabolites reflect the results of events that took place a certain time ago (Palme, 2005). The lag-time between a stressful event and appearance of faecal glucocorticoid metabolites in the faeces ranges from a few hours e.g. about 4 h in chicken (Rettenbacher et al., 2004) to two days e.g. in pigs (Palme et al., 1996), depending on the species and their activity rhythms (Touma et al., 2003; Palme et al., 2005; Touma and Palme, 2005). The lag-time can be accurately determined by radiometabolism studies, by injecting radiolabelled cortisol and monitoring the time of appearance of the peak radioactivity in faecal samples (Palme, 2005). In endangered species, radio-labelled steroids cannot be used for ethical reasons, but the injection of ACTH might give an approximate idea about the lag-time from secretion of glucocorticoids in the circulation and the peak in appearance of glucocorticoid metabolites in faeces (Palme, 2005).

### **1.3 Measurement of glucocorticoids**

The traditional and conventional method of evaluating stress is by measurement of glucocorticoids in plasma or serum (Thompson et al., 1988; Neubert et al., 1996; Saco et al., 2008). However, capture of wild animals for collection of blood samples without disturbance is not easy. The capture itself will stimulate secretion of glucocorticoids and may influence measurements, depending on timing between capture and sampling. In birds and reptiles, collection of blood samples in less than 2 min after physical capture has been

reported to provide measurements that reflect baseline concentrations of corticosterone, and samples collected 2 to 3 min after capture gave near baseline concentrations (Romero and Reed, 2005). The release of glucocorticoids takes 3-5 min in mammals and birds (Romero, 2004). In white-tailed deer (*Odocoileus virginianus*), the serum concentration of cortisol was significantly lower (i.e. baseline) in animals killed instantly by head-shot from a concealed position than in animals immobilised with xylazine and ketamine or those captured by drop-net which was attributed to the time elapsed from perception of capture stress to blood collection (DeNicola and Swihart, 1997).

Collection of blood samples within 2-3 minutes from starting physical capture is a challenge in large mammals such as Arabian oryx unless a team of people that are highly trained for rapid capture are available. Even approaching the animals for the purpose of capture and collection of blood samples might stimulate the production of glucocorticoids several minutes before physical capture succeeds and this reduces the chance of measuring the true baseline levels of glucocorticoids in blood plasma.

About 15 % of the unbound plasma cortisol transfers into saliva (Queyras and Carosi, 2004) and therefore some researchers have used salivary cortisol to assess stress in some species (Vining et al., 1983; Cooper et al., 1989; Lebelt et al., 1996; Berger et al., 1999; Jonsson et al., 2003). There have also been some attempts to measure cortisol metabolites in hair (Koren et al., 2002; Accorsi et al., 2008), but these are not fully developed or widely used.

Measurement of glucocorticoid metabolites in faecal samples is easier because of the feasibility of collecting samples even from wild animals. Another advantage of this approach is that capture of animals is not necessary and therefore the measurements are not affected by the capture, as is the case for plasma or salivary cortisol. Therefore, faecal samples can be used readily for long-term studies of welfare involving repeated measurements (Möstl and Palme, 2002). For example, for more than four months Viljoen et al. (2008) monitored faecal glucocorticoid metabolites before and after transportation of free ranging African elephants (*Loxodonta africana*). The use of faecal glucocorticoid metabolites for monitoring stress has been found to give accurate and reliable information regarding the welfare status of many animal species, without the procedures themselves

causing any kind of distress to the subjects (Möstl and Palme, 2002; Palme et al., 2005; Lane, 2006).

Ideally, faecal samples collected for measurement of glucocorticoid metabolites should be collected at known times after defecation and from known individuals (Millspaugh and Washburn, 2004). For best results, faecal samples should be collected shortly after defecation and stored immediately in a freezer at – 20 °C or below (Lynch et al., 2003; Millspaugh and Washburn, 2004; Palme, 2005). However, in field conditions, fresh samples and immediate storage in a freezer is not always possible. In this case, a storage experiment that mimics the delay between collection and storage using fresh faecal samples should be performed (Palme, 2005). The collected faecal samples should be homogenised before taking a portion for extracting faecal glucocorticoid metabolites (Palme et al., 1996), because within-sample variations in faecal glucocorticoid metabolites might exist as it has been reported, for example in white-tailed deer (*Odocoileus virginianus*) (Millspaugh and Washburn, 2003).

The exposure of faecal samples to rain can significantly increase the concentration of glucocorticoid metabolites (Washburn and Millspaugh, 2002). In addition, ambient temperature may influence results. An increase in faecal glucocorticoid metabolites (11,17 dioxoandrostanes) in faecal samples incubated at room temperature occurred, due to the presence of bacterial enzymes e.g. desmolase, which removes the side chain from C-21 steroids converting them to C-19 steroids (Figure 1.9) (D'Arcy et al., 1971; Möstl et al., 1999). In contrast, a decrease in the concentration of faecal glucocorticoid metabolites was found in samples stored in solar or conventional ovens for seven days (Terio et al., 2002). Therefore, faecal samples that have been exposed to rain or high ambient temperatures and humidity should be avoided. Storage of faecal samples in a freezer soon after collection is always recommended (Khan et al., 2002; Möstl and Palme, 2002; Millspaugh and Washburn, 2004; Palme, 2005; Palme et al., 2005; Touma and Palme, 2005). If immediate freezing is not possible, samples can be kept cold in a field pack and transported back to the field camp and then stored in a freezer (Ziegler and Wittwer, 2005). Heating faecal samples at 95 °C for 20 min in a water bath was found to deactivate bacterial enzymes and the measurement of faecal glucocorticoid metabolites gave stable constant readings when heated faecal samples were stored at ambient temperatures afterwards (Möstl et al., 1999;

2005). So, heating faecal samples that are collected in the wild, at 95°C for 20 min can be an alternative approach if samples cannot be frozen or kept in field packs.

The majority of faecal glucocorticoid metabolites are not extractable in diethylether, indicating the predominance of conjugated or polar unconjugated metabolites which was confirmed by separation by reverse-phase High Performance Liquid Chromatography (HPLC) (Teskey-Gerstl et al., 2000). Because of the presence of some polar unconjugated metabolites besides the majority of non-polar metabolites, 80 % methanol has been found to yield the highest levels of recovery of radioactivity in extracting faecal samples, for example after infusion of sheep with <sup>14</sup>C-cortisol (Palme and Möstl, 1997; Möstl et al., 1999; Palme, 2005).

There are many enzyme immuno-assays (EIAs) and radioimmuno-assays (RIAs) that have been used for measurement of faecal glucocorticoid metabolites. The methods for production of antibodies for measurement of steroids using EIAs or RIAs have been described in detail (Abraham, 1974; Kellie et al., 1975; Niswender et al., 1975; Cook and Beastall, 1987; Möstl et al., 2005). Briefly, the steroid of interest is linked to a protein such as bovine serum albumin (BSA), through a bridge such as hemisuccinate or carboxymethyloxime, to form an immunogen that is injected into an animal for example a rabbit, for production of steroid-specific antibodies (Möstl et al., 2005).

Immuno-assays which are specific for cortisol, without any cross-reactivities, cannot be used for measurement of faecal cortisol metabolites. Most immuno-assays with monoclonal antibodies probably have a degree of cross-reaction with other steroid metabolites. For example, some studies used cortisol specific immuno-assays in chimpanzees (Whitten et al., 1998), cheetah (Jurke et al., 1997), baboons (Weingrill et al., 2004) and carnivores (Young et al., 2004) and measured some faecal glucocorticoid metabolites. The detected measurements probably reflected the cross-reaction of the antibody with cortisol metabolites, because of the reported absence of cortisol itself in faeces (Palme et al., 2005).

Because of the low faecal content or absence of cortisol, and higher levels of metabolites, some group specific enzyme immuno-assays for glucocorticoids metabolites have been established. These group specific EIAs cross-react with a group of metabolites sharing functional groups (Möstl and Palme, 2002). For example, Palme and Möstl (1997)

established an enzyme immuno-assay that cross-reacts with 11,17 dioxoandrostanes. Möstl et al. (2002) also established an enzyme immuno-assay that measures cortisol metabolites with 3 $\alpha$ , 11-oxo or 3 $\alpha$ ,11 $\beta$  dihydroxy functional groups. These two EIAs were deliberately established to be group specific. However, a commercially available radioimmuno-assay kit for corticosterone (07-120102; ICN Biomedicals Inc) has also been found to cross-react with cortisol metabolites in many mammalian species (African elephant, black rhinoceros, Roosevelt elk, gerenuk, scimitar-horned oryx, Alaskan sea otter, Malayan sun bear, cheetah, clouded leopard and longtailed macaque) (Wasser et al., 2000), although which metabolites are detected, has not been fully evaluated. Therefore, this corticosterone radioimmuno-assay has the properties of the group specific immuno-assays.

### **1.3.1 Selection of assays for faecal glucocorticoids metabolites**

Some researchers compared different immuno-assays for the measurement of faecal glucocorticoid metabolites in several species and they found that immuno-assays that worked well in one species, sometimes do not work well for others (Millsbaugh and Washburn, 2004). For example, Wasser et al. (2000) compared four different immuno-assays for the measurement of faecal glucocorticoid metabolites and found that only one (ICN corticosterone RIA) gave good results in a wide range of mammalian and avian species, including proboscideans, three classes of carnivores (mustelid, bear, and felid), two classes of ruminants (cervid and bovid), a perissodactyl (rhinoceros), some Old World primates, and owls. Goymann et al. (1999) also compared four different EIAs in spotted hyenas (*Crocuta crocuta*) and again found that only the ICN corticosterone RIA gave satisfactory cross-reactivity with glucocorticoid metabolites. Morrow et al. (2002) also compared two EIAs in dairy cattle for the measurement of faecal glucocorticoid metabolites and found that both assays detected stimulation of adrenal activity after an ACTH stimulation test (see Chapter 2, section 2.2.1). Four different assays were also tried for evaluation of adrenal activity in male elephants and one worked well in the measurement of faecal glucocorticoid metabolites while other immuno-assays did not (Ganswindt et al., 2003). For this reason, the immuno-assays should be evaluated for suitability for any species that has not studied for faecal glucocorticoid metabolites such as the Arabian oryx.

The ICN corticosterone RIA was first used in measuring faecal glucocorticoid metabolites in Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) by Miller et al. (1991) and detected an increase in faecal glucocorticoids after ACTH administration, within 24 h after injection. Although the antibody was raised specifically against corticosterone, it presumably cross-reacted with other glucocorticoid metabolites in faeces (Graham and Brown, 1996; Millspaugh and Washburn, 2004). This RIA has been widely used for measurement of faecal glucocorticoid metabolites in many species for example, African elephants (*Loxodonta africana*) (Foley et al., 2001), oldfield mice (*Peromyscus polionotus*) (Good et al., 2003), spotted hyenas (*Crocuta crocuta*) (Goymann et al., 1999), African wild dogs (*Lycaon pictus*) (Creel et al., 1997; Monfort et al., 1998), wild baboons (*Papio cynocephalus*) (Lynch et al., 2003), gorillas (*Gorilla gorilla gorilla*) (Peel et al., 2005), chinchilla (*Chinchilla lanigera*) (Ponzio et al., 2004), cheetah (*Acinonyx jubatus*) (2002; Terio et al., 2004), blue fox (*Alopex lagopus*) (Sanson et al., 2005), white-tailed deer (*Odocoileus virginianus*) (Washburn and Millspaugh, 2002), Steller sea lions (*Eumetopias jubatus*) (Hunt et al., 2004; Mashburn and Atkinson, 2004), dairy cattle (Morrow et al., 2002), several avian and mammalian species (Wasser et al., 2000) and several species of felids (Graham and Brown, 1996, 1997), primates (Heistermann et al., 2006) and carnivores (Young et al., 2004). This assay has also been used to measure FGM of the scimitar-horned oryx, as mentioned earlier, but only 2 oryx were used in an ACTH challenge test and results were only presented for one oryx (Wasser et al., 2000). Therefore, the results should be further validated for this species and may not be applicable in similar species such as the Arabian oryx.

Two enzyme immuno-assays are validated for measurement of specific groups of faecal steroid metabolites and use antibodies that cross-react with specific groups of metabolites of glucocorticoids originating in the faeces. These assays measure 11,17-dioxoandrostanes (first description Palme and Möstl, 1997) and 3 $\alpha$ ,11 oxo or 3 $\alpha$ , 11 $\beta$  dihydroxy cortisol metabolites (first description, Möstl et al., 2002). The assays were developed at the Institute of Biochemistry, University of Veterinary Medicine, Austria, Vienna. The antibodies used in the assays were raised against the same standard (11-oxoeticholanolone) as an immunogen. The EIA described by Palme and Möstl (1997) used an antibody against 11-oxoethicholanole coupled at position C-3 (therefore measuring 11, 17-dioxoandrostanes),

while, the EIA first described by Möstl et al.(2002) raised an antibody against 11-oxoethicholnolone coupled at C-17 (hence measuring 3 $\alpha$ , 11-oxo and 3 $\alpha$ , 11 $\beta$ -dihydroxy cortisol metabolites). The institute has since pioneered the use of faecal steroids for assessment of reproductive status and adrenocortical activity and established many enzyme immuno-assays for these purposes that suit a variety of species of birds and mammals (Baltic et al., 2005; Möstl et al., 2005; Palme et al., 2005; Touma and Palme, 2005).

The enzyme immuno-assay measuring 11,17-dioxoandrostanes developed by Palme and Möstl (1997), has been widely applied for the monitoring the welfare of many species, for example, cats and dogs (Schatz and Palme, 2001), roe deer (*Capreolus capreolus*) (Dehnhard et al., 2001), red deer (*Cervus elaphus*) (Sauerwein et al., 2004), African elephants (*Loxodonta africana*) (Stead et al., 2000; Ganswindt et al., 2003), horses (Merl et al., 2000), ponies and pigs (Möstl et al., 1999), okapi (*Okapia johnstoni*) (Schwarzenberger et al., 1998), hares (*Lepus europaeus*) (Teskey-Gerstl et al., 2000), Barbary macaques (*Macaca sylvanus*) (Wallner et al., 1999), cattle (Palme et al., 2000; Morrow et al., 2002), ruminants (Palme et al., 1999) and sheep (Palme and Möstl, 1997).

The alternative enzyme immuno-assay that measures 3 $\alpha$ ,11-oxo-cortisol metabolites (Möstl et al., 2002) has also been widely used for measurement of faecal glucocorticoid metabolites in ruminants (Möstl et al., 2002), red deer (*Cervus elaphus*) (Huber et al., 2003a; 2003b), African elephant (*Loxodonta africana*) (Ganswindt et al., 2003; 2004, 2005), guinea pig (*Cavia aperea f. porcellus*) (Bauer et al., 2008) and several species of primates (Heistermann et al., 2006). In the present studies, the two enzyme immuno-assays developed by Palme and Möstl (1997) and Möstl et al. (2002) were chosen for analysis of faecal glucocorticoid metabolites of Arabian oryx because these two assays are group-specific i.e. deliberately established to cross-react with a group of metabolites. In contrast, the ICN corticosterone RIA was developed specifically to cross-react with corticosterone with no guarantee of cross-reaction of cortisol metabolites of the Arabian oryx. However, in future studies, this ICN RIA could also be assessed for its suitability for measurement of faecal glucocorticoid metabolites of Arabian oryx.

### **1.3.2 Analytical validation**

The analytical validation of an immuno-assay includes the determination of accuracy, specificity (cross-reactions), sensitivity, precision and parallelism of the dose-dependent relationship of the standard and the unknown (Möstl et al., 2005). This validation is done during the first establishment of any assay. The two enzyme immuno-assays established by Palme and Möstl (1997) and Möstl et al. (2002) have been analytically validated.

### **1.3.3 Biological and physiological validation**

An immuno-assay that can detect faecal glucocorticoid metabolites in one species cannot be assumed to be valid for measurements in another species and therefore, each immuno-assay has to be biologically and physiologically validated during the first application in a certain species (Möstl et al., 2005; Touma and Palme, 2005). Physiological validation is defined as the pharmacological induction of physiological changes in circulating glucocorticoid levels e.g. by administration of ACTH or dexamethasone, that stimulates or potentially suppresses the HPA-axis, respectively (Touma and Palme, 2005). Biological validation is achieved by ascribing changes in the circulating levels of glucocorticoids to particular events such as capture, immobilisation and transportation (Touma and Palme, 2005).

## **1.4 Aims of the study**

As Arabian oryx face events that potentially affect their welfare in the wild, in captivity or while being translocated there is a need for development of methods that monitor and improve their welfare. While recognising that welfare is a multidisciplinary field and that the thorough assessment of welfare involves evaluation of physiological, behavioural, clinical and genetic aspects; the present studies focuses primarily on the physiological aspects of welfare. The studies described in this thesis aim to develop and apply physiological tools for monitoring the welfare of Arabian oryx. The longer-term aim is to improve their welfare and ensure their viability for conservation. For this, the studies aimed to develop for the first time, a non-invasive method for assessment of stress using faecal glucocorticoid metabolites. In Chapter 2, two enzyme immuno-assays were validated for measuring faecal glucocorticoid metabolites in Arabian oryx by investigation of the effects

of an ACTH challenge test and by a dexamethasone suppression test. The measurement of faecal glucocorticoid metabolites was employed in assessment of stress during road transport and after release of Arabian oryx to the wild by road transport, as described in Chapter 6. The studies also aimed to establish for the first time reference ranges for ions, haematological, biochemical and clinical parameters from oryx that have not been injected with restraint chemicals, to help in the diagnosis of disease and evaluation of the overall health of Arabian oryx (Chapter 3). Physiological evaluation of the effects of management tools such as tranquillisation with a long acting tranquilliser, perphenazine enanthate (Chapter 4) and immobilisation with xylazine (Chapter 5) on physiological parameters of the derived reference ranges in Arabian oryx, was a further objective of the studies. Chapter 7 draws the results together in relation to future studies of welfare and conservation of this species in a broader context.

## Chapter 2

### **Measuring faecal glucocorticoid metabolites as a non-invasive method for assessment of welfare in Arabian oryx**

#### **2.1 Introduction**

Cortisol is the key hormone that is widely used for physiological assessment of welfare in animals (Broom and Johnson, 1993b; Moberg and Mench, 2000; Möstl and Palme, 2002). During stress, cortisol is secreted by the adrenal cortex in large quantities (Wingfield and Ramenofsky, 1999). Traditionally, for monitoring stress, cortisol is measured in blood samples. Collection of blood samples is invasive, and capture of animals itself causes cortisol secretion. In addition, capture for collection of blood samples is not always feasible in wild animals or those kept housed in large enclosures. Therefore, an alternative to measurement of cortisol in blood samples is preferred.

One of the best alternatives to blood cortisol is measurement of faecal glucocorticoid metabolites (Möstl and Palme, 2002). The cortisol is metabolised in the liver, then in the gut and then excreted through the faeces and/or urine (Möstl and Palme, 2002). For the measurement of cortisol metabolites in faeces, many different enzyme immuno-assays have been used (see Chapter 1, section 1.3.1). In the present study, two EIAs were used for measurement of faecal glucocorticoid metabolites of Arabian oryx. The first EIA was first described by Palme and Möstl (1997) and is referred to in this thesis as EIA I. The second EIA was first described by Möstl et al.(2002), and is referred to in this thesis as EIA II. Both assays have been proven to give good results in many species as described in detail in Chapter 1 (section 1.3). EIA I was successfully applied, for example, in cattle and sheep (Palme et al., 1999; Palme et al., 2000), roe deer (Dehnhard et al., 2001), hares (Teskey-Gerstl et al., 2000), elephants (Stead et al., 2000), primates (Wallner et al., 1999; Bahr et al., 2000) and horses (Möstl et al., 1999; Merl et al., 2000). EIA II was successfully applied in cattle (Möstl et al., 2002), red deer (Huber et al., 2003a; Huber et al., 2003b) and elephants (Ganswindt et al., 2003). Both EIA I and EIA II have been analytically validated by (Palme and Möstl, 1997) and (Möstl et al., 2002), respectively.

The antibodies of the two assays were raised against the same standard (5 $\beta$ -androstan-3 $\alpha$ -ol-11,17-dione) (Figure 2.1) but the standard was bound to bovine serum albumin at different positions in the steroid molecule, either at carbon-3 for EIA I (Figure 2.2), as described by Palme and Möstl (1997) or at carbon-17 for EIA II (Figure 2.3), as described by Möstl et al. (2002). Therefore, the antibody for EIA I cross-reacts with metabolites that contain 11 and 17-oxo groups, while, the antibody of EIA II cross-reacts with metabolites that have 3 $\alpha$  - hydroxy and 11-oxo or, 11 $\beta$ - hydroxy groups.

Even though EIA I and EIA II have been successfully used for measurement of faecal glucocorticoid metabolites for monitoring of stress in many species, sometimes these EIAs do not work for some species or measure more metabolites in one sex but not the other. For example, EIA I was tried for measurement of faecal cortisol metabolites in spotted hyenas (*Crocuta crucota*) but did not work (Goymann et al., 1999). In elephants, EIA II gave good results but EIA I did not (Ganswindt et al., 2003). In new applications in species that have not been examined so far, it is important to assess both assays. EIA I detected much higher faecal glucocorticoid metabolites in cats than dogs since cortisol metabolites in cats were excreted mainly via faeces while in dogs these metabolites were mainly excreted via urine (Schatz and Palme, 2001). The route of excretion (urine or faeces) varies from one species to another (Palme et al., 2005). Furthermore, in male dogs, much higher faecal content of glucocorticoid metabolites were measured by EIA I than in females, which could be attributed to male androgen metabolites cross-reacting with the antibody of EIA I (Schatz and Palme, 2001). Therefore, there is a need to validate the EIAs before application in new species. As neither EIA I nor EIA II have previously been applied in Arabian oryx (*Oryx leucoryx*), they must be physiologically validated.

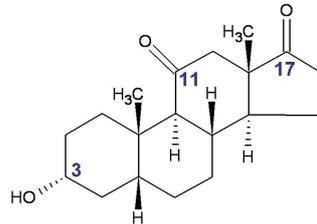


Figure 2.1 The standard (5β-Androstane-3α-ol-11,17-dione) used for raising antibodies in both EIA I and EIA II.

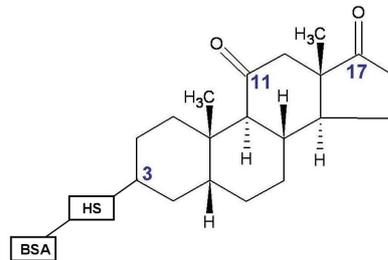


Figure 2.2 The antigen injected into rabbits for raising the antibodies used in EIA I. 5β-Androstane-11,17-dione-3-hemisuccinate (HS): bovine serum albumin (BSA).

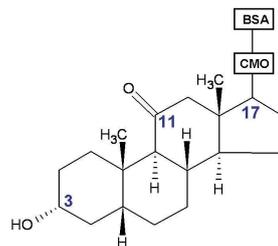


Figure 2.3 The antigen injected into rabbits for raising the antibodies used in EIA II. 5β-Androstane-3α-ol-11-one-17-carboxymethyloxime (CMO): bovine serum albumin (BSA).

ACTH challenge and dexamethasone suppression tests have been widely used for validation of enzyme immuno-assays in many species (reviewed by Touma and Palme, 2005). ACTH stimulates the adrenal cortex to secrete glucocorticoids, while dexamethasone (a synthetic steroid) may suppress the hypothalamic-pituitary-adrenal axis by a negative feedback mechanism, hence reducing the production of glucocorticoids. The ability of an EIA to detect changes in the activity of the adrenal cortex after ACTH or dexamethasone administration and the resultant increase or decrease of secreted glucocorticoids, respectively, can provide evidence that the measured metabolites originate from the adrenal cortex of the injected animal. Therefore, these approaches can physiologically validate the application of the EIA in that particular species.

Another aspect to be considered is the stability of faecal glucocorticoid metabolites after collection of fresh faecal samples and the time of storage in a freezer. It is always recommended that faecal samples should be collected shortly after defecation and stored at -20 °C or below as soon as possible (Khan et al., 2002; Palme, 2005). Stored in the freezer, the faecal glucocorticoid metabolites are reported to remain stable (Millspaugh and Washburn, 2004; Palme et al., 2005; Touma and Palme, 2005). Faecal glucocorticoid metabolites can be altered or degraded by bacterial enzymes after defecation depending on the environmental conditions if faecal samples are not stored in the freezer (Möstl and Palme, 2002; Möstl et al., 2005; Palme, 2005). Sometimes the immediate storage of faecal samples in a freezer is not possible. For example, for monitoring faecal glucocorticoid metabolites of animals in the wild, the storage of collected faecal samples in a freezer is inevitably delayed for many hours or longer. For this reason, an experiment to evaluate the stability of faecal glucocorticoid metabolites in Arabian oryx over a couple of days at ambient temperature was conducted.

This chapter aims at physiologically validating two enzyme immuno-assays (EIA I and EIA II) for potentially measuring faecal glucocorticoid metabolites in Arabian oryx through the activation of the HPA axis. It also examines the stability of the faecal glucocorticoid metabolites of Arabian oryx measured by EIA I and II during incubation of faecal samples at 30 °C for up to three days.

## **2.2 Materials and methods**

### **2.2.1 ACTH challenge test**

Eight Arabian oryx (4 males and 4 females) housed individually in holding pens of 2.6 x 6.2 m (Figure 2.4) at the Omani Mammals Breeding Centre, were cornered at the back of the holding pen and injected intramuscularly with 0.5 mg (50 IU or 17  $\mu$ mol) of synthetic ACTH (Synacthen at 0.25 mg/ml, Novartis Pharma GmbH, Vienna, Austria) as described by Palme et al. (1999). Synacthen is a synthetic corticotrophic preparation that displays all of the pharmacological properties of endogenous mammalian ACTH (Watson et al., 1998). It is a long-chain polypeptide composed of the first 24 of the 39 amino acids contained in naturally occurring ACTH. It contains tetracosactrin (as hexa-acetate salt) with an amino acid sequence Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-Gly-Lys-Lys-Arg-Arg-Pro-Val-Lys-Val-Tyr-Pro.

This experiment started at night time (11:00 pm) in order to collect samples until the next evening. Fresh faecal samples were collected at the time of ACTH injection and about every 2 h for 20 h. Later, a single fresh faecal sample from each animal was collected about 33 h post-injection of ACTH.

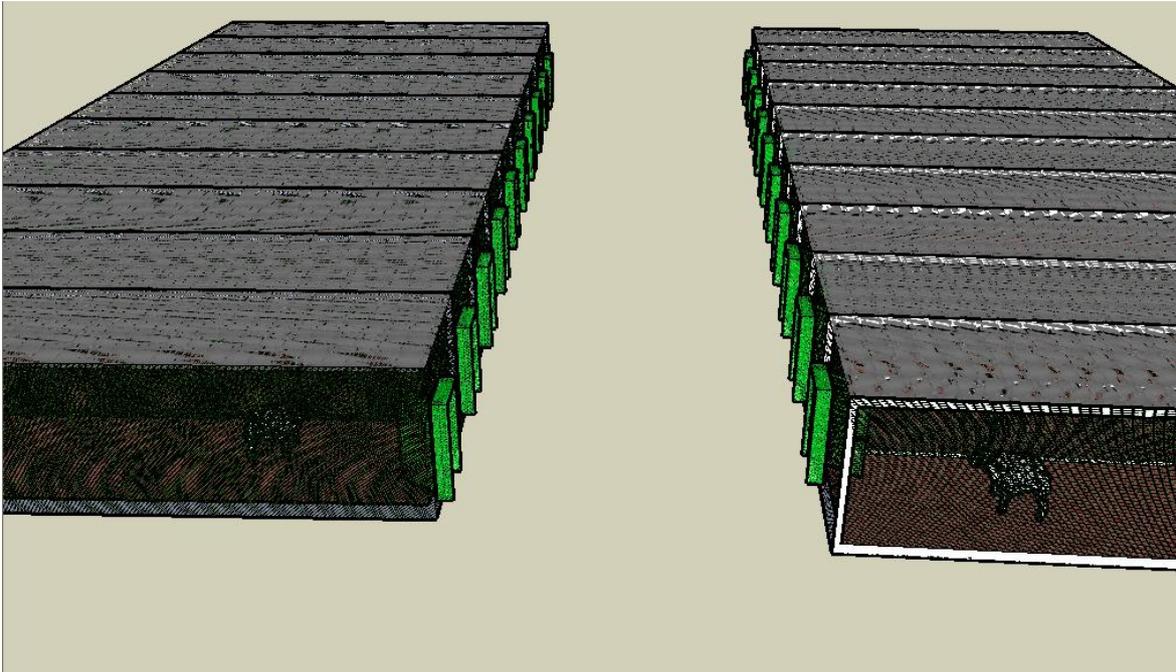


Figure 2.4 3D-Illustration of the 20 holding pens at the Omani Mammals Breeding Centre, Seeb, Oman.

### **2.2.2 Dexamethasone suppression test**

Sixteen Arabian oryx (8 females and 8 males) were physically captured (i.e. without using immobilising chemicals) and injected intramuscularly with 4 ml (204  $\mu$ mol) of dexamethasone (Colvasone at 2 mg/ml, Norbrook, Carlisle, UK) similar to previous studies in pigs (5 mg) (Möstl et al., 1999). Faecal samples were collected at the time of injection and 1, 3 and 7 days after injection.

### **2.2.3 Stability of faecal glucocorticoid metabolites**

For evaluation of the stability of faecal glucocorticoid metabolites, 8 fresh faecal samples were collected from 4 male and 4 female Arabian oryx. They were placed in plastic bags, labelled and placed immediately on a cool box containing ice. Each sample was homogenised using a blender (Panasonic, MX-J210GN), subdivided into 5 sub-samples within about 1 h post collection and one sub-sample was stored in a freezer at  $-80^{\circ}\text{C}$

immediately after subdivision. The other four sub-samples from each homogenised sample of faeces from 8 oryx were transferred to an incubator with the temperature at 30 °C and held at this temperature for 4, 12 or 24 h and 3 days after collection. At each time point, each sub-sample was stored in a - 80 °C freezer until extraction.

#### **2.2.4 Collection of faecal samples**

Faecal samples were always collected from the floor of the holding pen, in plastic sampling bags. Disposable hand gloves were used during collection. Bags were labelled with the time of collection, date and treatment. The samples were placed immediately on a cool-box containing ice and were stored in a - 20 °C freezer within less than 1 h at the Omani Mammals Breeding Centre. The samples were then transferred in a cool-box containing ice, within less than 1 h, to a - 80 °C freezer at Sultan Qaboos University, Muscat, Oman.

#### **2.2.5 Extraction of faecal glucocorticoid metabolites**

The glass tubes (10 ml, Pyrex) and polypropylene microcentrifuge tubes (1.5 ml) used for extraction of faecal samples were acid-washed. For acid washing, the tubes were rinsed once in distilled water and placed in an acid wash bath (10 % HCl) for 3 days. Tubes were then rinsed three times with tap water and then three times with distilled water and then dried and stored in sealed containers until use.

Faecal samples were homogenised using a blender (Panasonic, MX-J210GN) and 0.5 g (weighed to 0.1 mg, Mettler AE 160, Leicester, UK) of the homogenised faecal sample, was transferred into an acid-washed glass tube (10 ml, Pyrex). Five ml of 80 % methanol (Sigma Aldrich, HPLC grade diluted with double distilled water) were added to each tube. The tubes were vigorously mixed on a hand-held vortex for 1 min. Tubes were then centrifuged (2500 g, 15 min, at 4 °C, Eppendorf Centrifuge 5804R, Germany). A volume of 0.5 ml of the supernatant was removed from each tube, transferred into an acid-washed polypropylene microcentrifuge tube and dried under a stream of nitrogen gas. For drying, tubes were placed in a built-in rack with tubing connected to a pure nitrogen gas generator (N<sub>2</sub> gas quality suitable for liquid chromatography mass spectrometry (LCMS), Peak Scientific Instruments, UK). The faecal extracts were dried within 12 h and the tubes were closed tightly and stored in a freezer at -80 °C until analysis.

The faecal glucocorticoid metabolites were measured with EIA I and EIA II at the laboratory of Professor Rupert Palme and Professor Erich Möstl, University of Veterinary Medicine in Vienna, Austria.

### **2.2.6 Buffers and solutions for enzyme immuno-assay**

Buffers and solutions for enzyme immuno-assays were prepared at the Institute of Biochemistry, University of Veterinary medicine, Vienna, Austria, by specialised technicians as follows.

#### *2.2.6.1 Assay buffer*

The assay buffer was prepared by dissolving 2.42 g tris-hydroxyaminomethane (Merck), 17.9 g NaCl (Merck), 1 g bovine serum albumin (Sigma Aldrich) and 1 ml tween 80 (Merck) in 1 litre of double-distilled water and the pH was adjusted to 7.5 with HCl, and then filtered through a cartridge column (SEP-PAK C18, Millipore).

#### *2.2.6.2 First coating buffer*

Coating buffer was prepared by dissolving 1.59 g Na<sub>2</sub>CO<sub>3</sub> (Merck) and 2.93 g NaHCO<sub>3</sub> (Merck) in 1 litre of double-distilled water, and pH adjusted to 9.6 with HCl.

#### *2.2.6.3 Second coating buffer*

The second coating buffer was a mixture of 3.146 g tris-hydroxyaminomethane (Merck), 23.3 g NaCl (Merck), 13 g bovine serum albumin (Sigma Aldrich) and 1.3 g sodium azide (Merck) dissolved in 1.3 litre of double-distilled. The pH was adjusted to 7.5 with HCl and the solution was passed through a cartridge column (SEP-PAK C18, Millipore).

#### *2.2.6.4 Washing solution*

The washing solution was prepared with 0.5 ml tween 20 (Merck) in 2.5 l double-distilled water.

#### 2.2.6.5 *Substrate buffer for peroxidase*

The substrate buffer for peroxidase was prepared by dissolving 1.36 g of sodium acetate (Merck) in 1 litre of double-distilled water. The pH was adjusted to 5.0 with 5 % citric acid (Merck).

#### 2.2.6.6 *Enzyme solution for streptavidin-reaction*

The enzyme solution was prepared in 30 ml assay buffer (section 2.2.6.1) by adding 1  $\mu$ l of streptavidin-peroxidase-conjugate (Boehringer) and then mixing on a magnetic stirrer a few minutes before use.

#### 2.2.6.7 *Substrate solution for peroxidase*

The substrate solution for peroxidase was prepared in 30 ml of substrate buffer (section 2.2.6.5) by adding 0.5 ml (0.4 %) of 3,3', 5,5'-tetramethylbenzidine (Fluka), 0.1 ml of 0.6 % H<sub>2</sub>O<sub>2</sub> (Merck) and 5 ml double-distilled water. The solution was then mixed on a magnetic stirrer a few minutes before use.

### **2.2.7 Enzyme immuno-assays reagents**

#### 2.2.7.1 *Preparation of samples*

The dried extracts were reconstituted by adding 0.4 ml of methanol (Sigma Aldrich), shaken for 10 seconds on a vortex mixer and then 0.1 ml of distilled water was added about 30 minutes later and the tubes were shaken by hand for 10 seconds. The extracts were diluted by taking 50  $\mu$ l aliquots and adding 450  $\mu$ l of assay buffer (section 2.2.6.1).

#### 2.2.7.2 *Preparation of the standard*

To prepare the stock solution, 1 mg of the standard (5 $\beta$ -Androstane-3 $\alpha$ -ol-11, 17-dione) was dissolved in 1 ml of methanol, and mixed using a vortex mixer. An aliquot of 10  $\mu$ l of this stock solution was diluted with 20 ml of assay buffer (section 2.2.6.1) and mixed for 2 min in an ultrasonic bath. Then aliquots of 50  $\mu$ l each containing 25,000 pg of the standard were transferred into new vials.

The vials of 50 µl of diluted standard were further diluted by adding 150 µl of assay buffer and shaken using a vortex mixer. This was serially-diluted with assay buffer with a ratio of 1:2.5 for seven times. The concentration range was from 500 pg to 2 pg per 10 µl. These standards were used for both EIA I and EIA II.

#### 2.2.7.3 *Coating antibody*

The coating antibody was anti-rabbit IgG (Möstl et al., 2002).

#### 2.2.7.4 *Specific steroid antibodies*

The antibodies that were used in this study were raised in rabbits against 5β-androstane-11, 17-dione-3-HS coupled with BSA for EIA I (Palme and Möstl, 1997) and against 5β-androstane-3α-ol-11-one-17-CMO coupled with BSA for EIA II (Möstl et al., 2002). The stock solutions of these two antibodies were prepared in the same way as described for the standard (section 2.2.7.2).

#### 2.2.7.5 *Biotin-labelled steroids*

The biotin-labelled steroids used were 5β-androstane-11, 17-dione-3-glucoronide coupled with N-biotinyl-1, 8-diamino-3,6-dioxaoctane (DADOO-biotin)) for EIA I (Palme and Möstl, 1997) and (5β-androstane-3α-ol-11-one-17-CMO coupled with biotinyl-3,6,9-trioxaundecanediamine (LC-biotin)) for EIA II (Möstl et al., 2002). The stock solutions of these two biotin-labelled steroids were prepared as described for the standard (section 2.2.7.2).

### 2.2.8 **Protocol of enzyme immuno-assay**

First, the micro-titer plates (F96, Maxisorp, Nunc, Denmark) were coated with a coating solution containing 25 µg of coating antibody (section 2.2.7.3) added to 25 ml of the first coating buffer (section 2.2.6.2). Then, in each well of the plates, 250 µl of the coating solution was dispensed. These plates were incubated at room temperature overnight. The following day the solution was discarded and each well was refilled with 300 µl of the second coating buffer (section 2.2.6.3). The plates were washed three times with washing solution (section 2.2.6.4) and then the solution was removed. In each well, 50 µl per well of

the diluted extracts of samples (section 2.2.7.1) were placed in duplicate. Similarly, 50 µl of the serially diluted standards (section 2.2.7.2) were placed in duplicate into separate wells. Then 100 µl of the biotin-labelled steroids (differing according to the type of EIA, section 2.2.7.5) were dispensed to all wells. Following this, 100 µl of specific steroid antibodies (differing according to the type of EIA, section 2.2.7.4) were added to all wells. The plates were covered with parafilm and shaken mildly overnight at 4 °C. The plates were then washed with cold (4 °C) washing solution (section 2.2.6.4). Then, 250 µl of the enzyme substrate solution containing streptavidin (section 2.2.6.6) was dispensed in each well, and plates incubated for 45 min at 4 °C on a micro-titer plate shaker. The plates were then washed again with cold (4 °C) washing solution (section 2.2.6.4). Then 250 µl of substrate solution for peroxidase (section 2.2.6.7) were dispensed in each well, covered and incubated for 45 min at 4 °C. The reaction was stopped by 10 % H<sub>2</sub>SO<sub>4</sub> (Merck). The absorbance of colour produced was measured using an automated micro-titre plate reader (Digiscan, ASYS, Vienna, Austria) using a reference filter of wavelength 620 nm and a measuring filter of 450 nm. Results were calculated by a software program (Mikrowin) connected to the plate reader.

## **2.2.9 Procedural validations**

### *2.2.9.1 Blanks*

During the extraction of faecal samples, 12 procedural blanks were prepared. Blank tubes containing no sample were treated exactly as the tubes in which faecal samples were extracted. The readings obtained using either EIA I or EIA II were negligible (~1 pg/well compared to 4.7 to 310.3 pg/well for faecal samples).

### *2.2.9.2 Standard curves*

Standard curves for EIA I and EIA II are shown in Figure 2.5 and Figure 2.6, respectively.

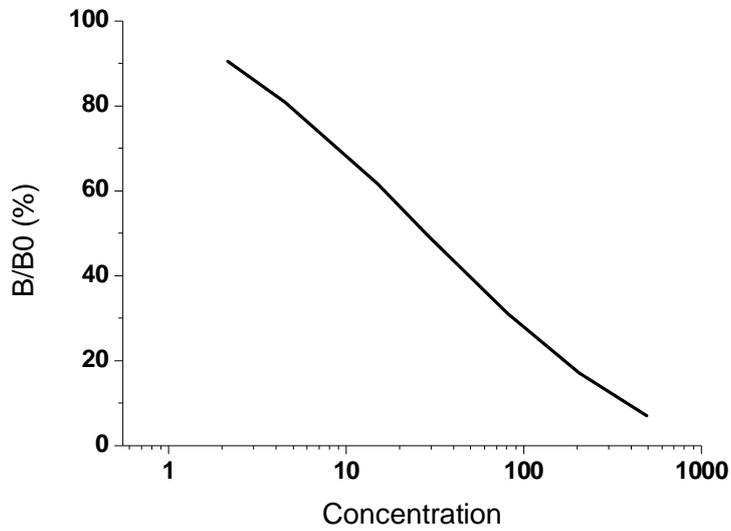


Figure 2.5 Standard curve of EIA I.

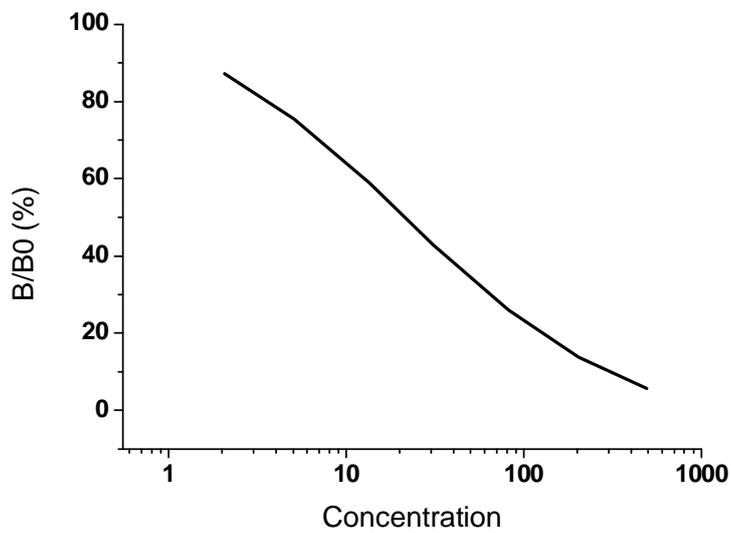


Figure 2.6 Standard curve of EIA II.

### 2.2.10 Statistical analysis

Statistical analyses were carried out using (SigmaStat, version 3.5). Faecal samples after the ACTH challenge experiment were collected at different times after injection as they became available. Therefore, the time points were categorized in 2 hour intervals (0-2, 2-4 etc up to 32-34 h). Trials were designed to test the data for EIA I with two way repeated measures ANOVA in order to compare data between sexes and at different time points simultaneously. However, the data for EIA I were not normally distributed, and normality was not achieved by transformations (square, ln, log<sub>10</sub>, reciprocal, exponential, square root, arcsin square root, rank). Therefore data for EIA I were tested with a one way repeated measures ANOVA. The data for faecal glucocorticoid metabolites after the injection with ACTH at different time points were compared to the control (baseline data at 0-2 h). Comparison of data in males and females for EIA I at each time point was achieved by t-tests or Mann-Whitney tests for parametric and non-parametric data, respectively.

For ACTH experiment, EIA II data were tested with two way repeated measures ANOVA followed by multiple comparisons versus control (baseline data at 0-2 h) using Holm-Sidak method taking sex as a factor.

The data for faecal glucocorticoid metabolites after dexamethasone injection were not normally distributed but were transformed to normality by natural logarithm (ln) for EIA I and reciprocal transformation for EIA II. The transformed data were then analysed by two way repeated measures ANOVAs, followed by multiple comparisons of data after injection with the control basal data at time point 0-2 h and for gender differences.

The data for the experiment of evaluating the stability of faecal glucocorticoid metabolites were not normally distributed, but were transformed to normality by natural logarithm (ln) for EIA I and by logarithm (log<sub>10</sub>) for EIA II. The data were then analysed by two way repeated measures ANOVAs followed by multiple comparisons (Holm-Sidak method).

Values in text and graphs are always shown as mean  $\pm$  standard error unless indicated otherwise.

## 2.3 Results

### 2.3.1 ACTH challenge test

#### 2.3.1.1 EIA I

There were no differences between males and females at sampling time points post ACTH injection, except at 6–8 h (t-test,  $P = 0.009$ ) and at 16–18 h (Mann Whitney test,  $P = 0.029$ ), hence, data for both males and females were combined. There were significant increases of immuno-reactive faecal glucocorticoid metabolites between 8 and 20 h after injection of Arabian oryx with ACTH, as measured by EIA I (Figure 2.7). The mean basal value for faecal glucocorticoid metabolites measured in samples collected at the time of ACTH injection was  $273 \pm 44$  ng/g faeces, using data from both sexes. The mean peak value for faecal glucocorticoid metabolites after ACTH injection was  $2515 \pm 303$  ng/g faeces ( $n = 8$  oryx) and occurred in a mean time of  $14 \pm 1$  h after ACTH injection. The peak to basal ratio was  $11 \pm 3$  in individual animals, based on data for both sexes. Generally, both males (Figure 2.8) and females (Figure 2.9) showed a clear increase in the faecal glucocorticoid metabolites after ACTH injection. One male (M11) (Figure 2.8), however, had an exceptionally low response to ACTH injection.

#### 2.3.1.2 EIA II

There were no significant differences between males and females at any sampling time points and therefore data for both sexes were combined. The immuno-reactive faecal glucocorticoid metabolites measured by EIA II, increased significantly between 8 and 20 h post ACTH injection (Figure 2.10). The male (M11) had much lower response and late peak (Figure 2.11), which resembled its pattern in EIA I (Figure 2.8). The mean basal value of faecal glucocorticoid metabolites measured at the time of injecting ACTH for both sexes was  $383 \pm 49$  ng/g faeces and the mean peak value post ACTH injection was  $3386 \pm 612$  ng/g faeces, reached in a mean time of  $14 \pm 1.5$  h. The peak to basal ratio of individual animals was  $9 \pm 1$  ( $n = 8$  oryx).

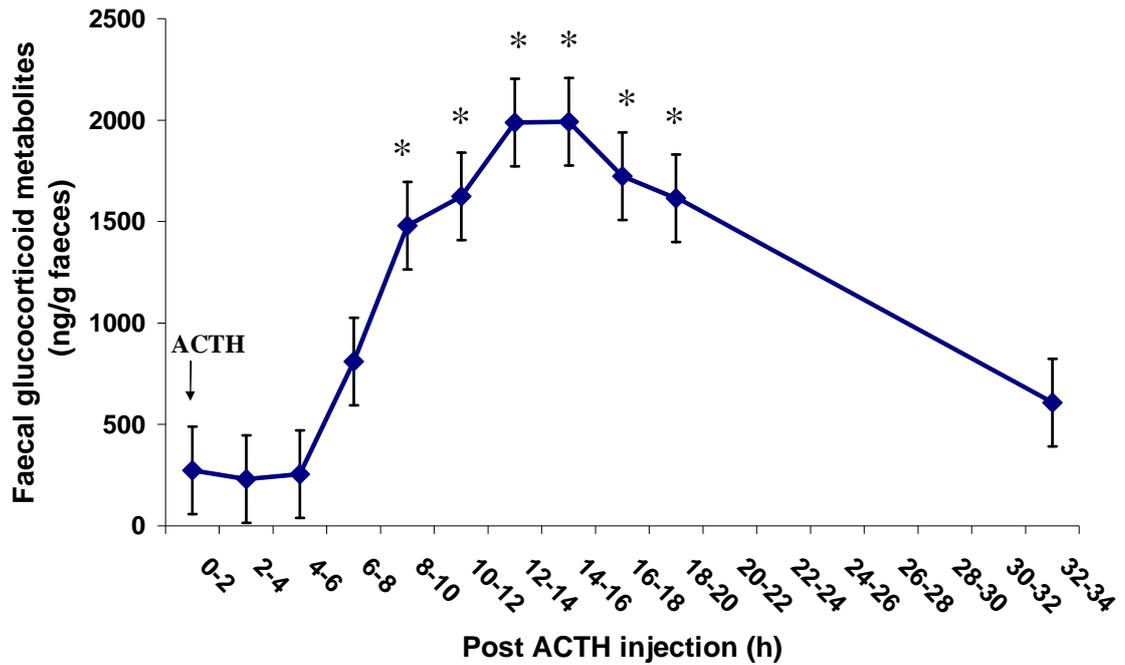


Figure 2.7 Immuno-reactive faecal glucocorticoid metabolites measured by EIA I in 8 Arabian oryx (4 females and 4 males) after injection with ACTH at 0 h. Data are shown as mean  $\pm$  standard error. Asterisks (\*) show time points where immuno-reactive faecal glucocorticoid metabolites were significantly different from basal (0-2 h) values. Data were analysed by a one way repeated measures ANOVA,  $P < 0.001$ .

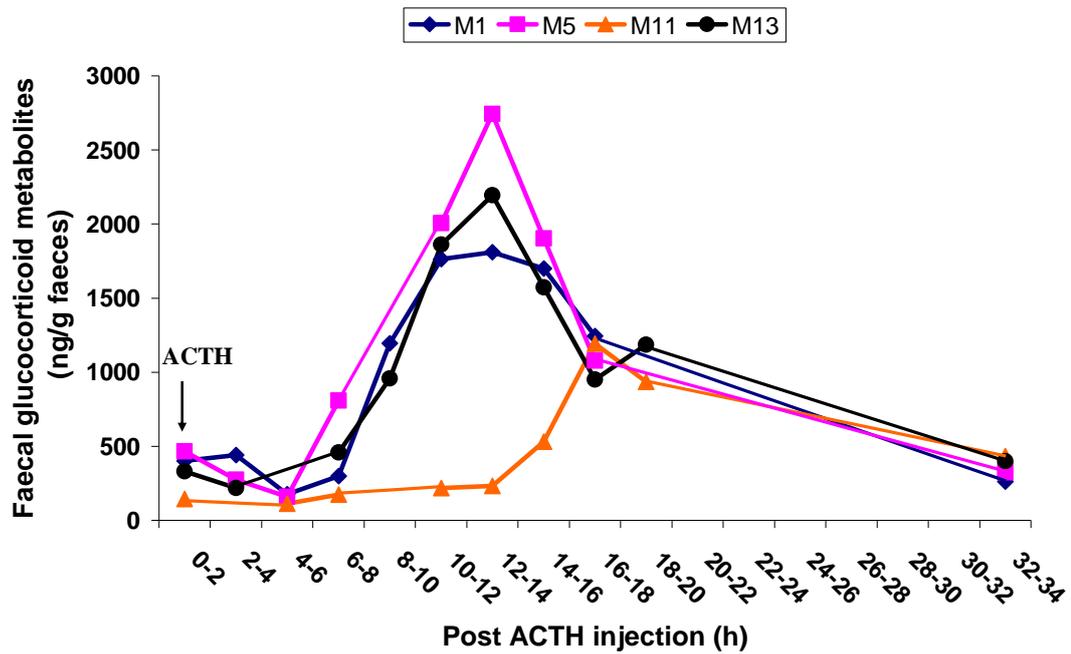


Figure 2.8 Immuno-reactive faecal glucocorticoid metabolites measured by EIA I in 4 male Arabian oryx after injection with ACTH at 0 h.

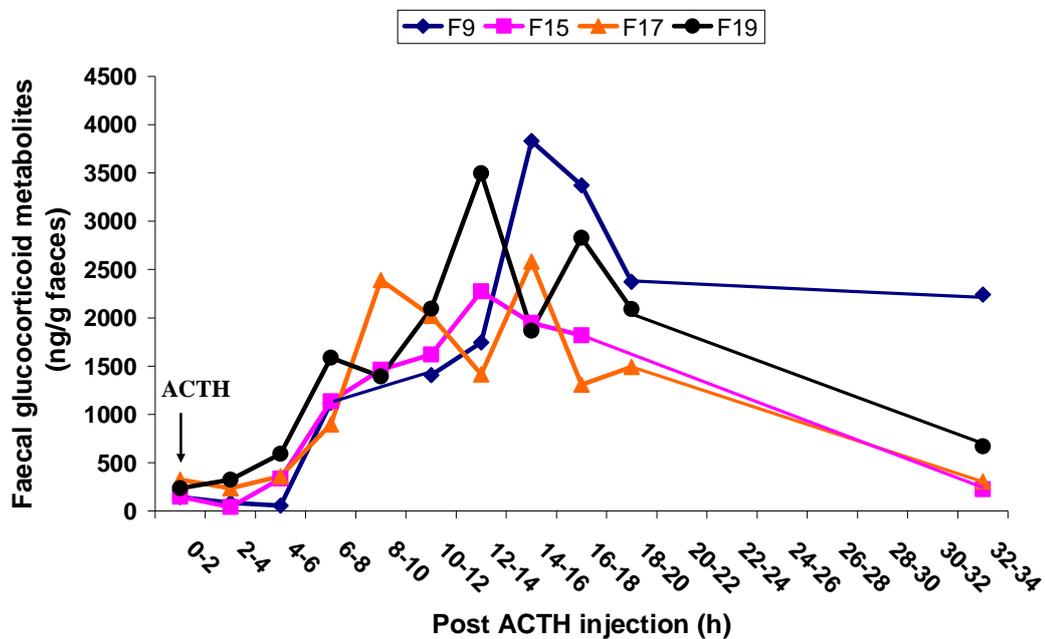


Figure 2.9 Immuno-reactive faecal glucocorticoid metabolites measured by EIA I in 4 female Arabian oryx after injection with ACTH at 0 h.

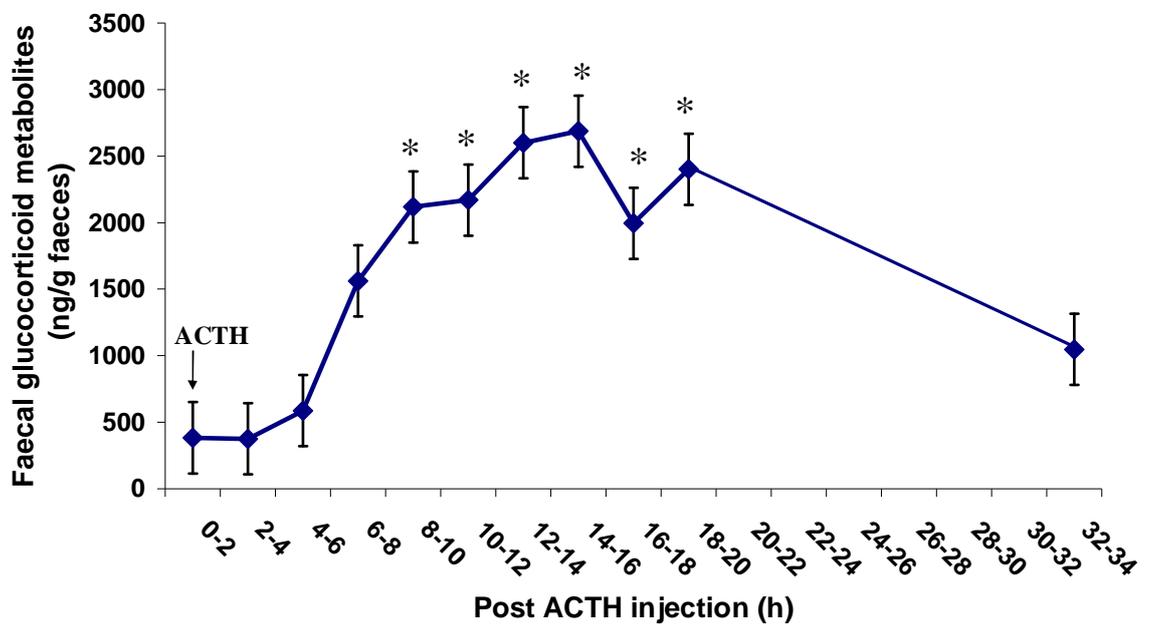


Figure 2.10 Immuno-reactive faecal glucocorticoid metabolites of 8 Arabian oryx (4 males and 4 females) after injection of ACTH (arrow), measured by EIA II. Data are shown as mean  $\pm$  standard error. Asterisk (\*) shows significantly different from basal values (0-2 h), Two way repeated measures ANOVA, followed by multiple comparisons versus control using Holm-Sidak method, \*P <0.001.

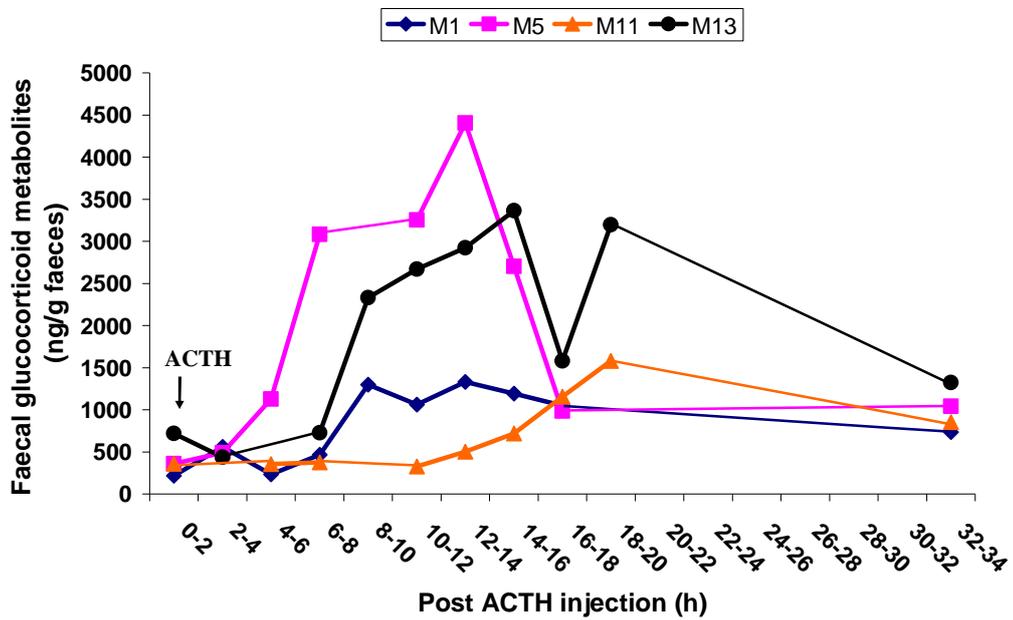


Figure 2.11 Immuno-reactive faecal glucocorticoid metabolites measured by EIA II in 4 male Arabian oryx after injection with ACTH at 0 h.

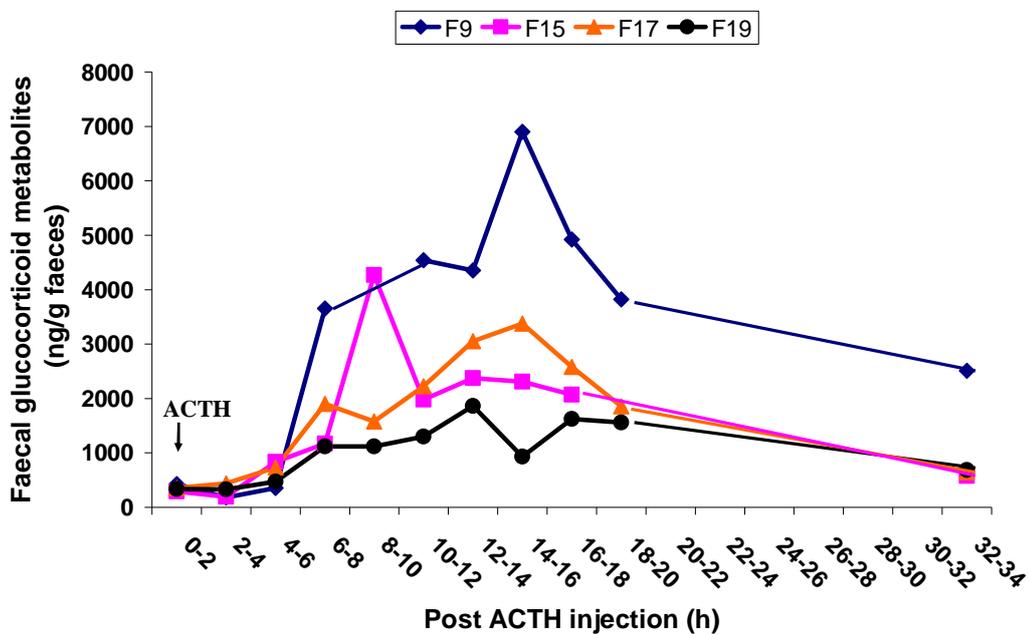


Figure 2.12 Immuno-reactive faecal glucocorticoid metabolites measured by EIA II in 4 female Arabian oryx after injection with ACTH at 0 h.

### 2.3.2 Dexamethasone suppression test

There was no significant difference between male and female oryx ( $P = 0.593$ ), therefore data for both sexes were combined. In both EIA I and EIA II, the immuno-reactive faecal glucocorticoid metabolites decreased significantly one day after the injection of dexamethasone (Figure 2.13 and Figure 2.14 respectively).

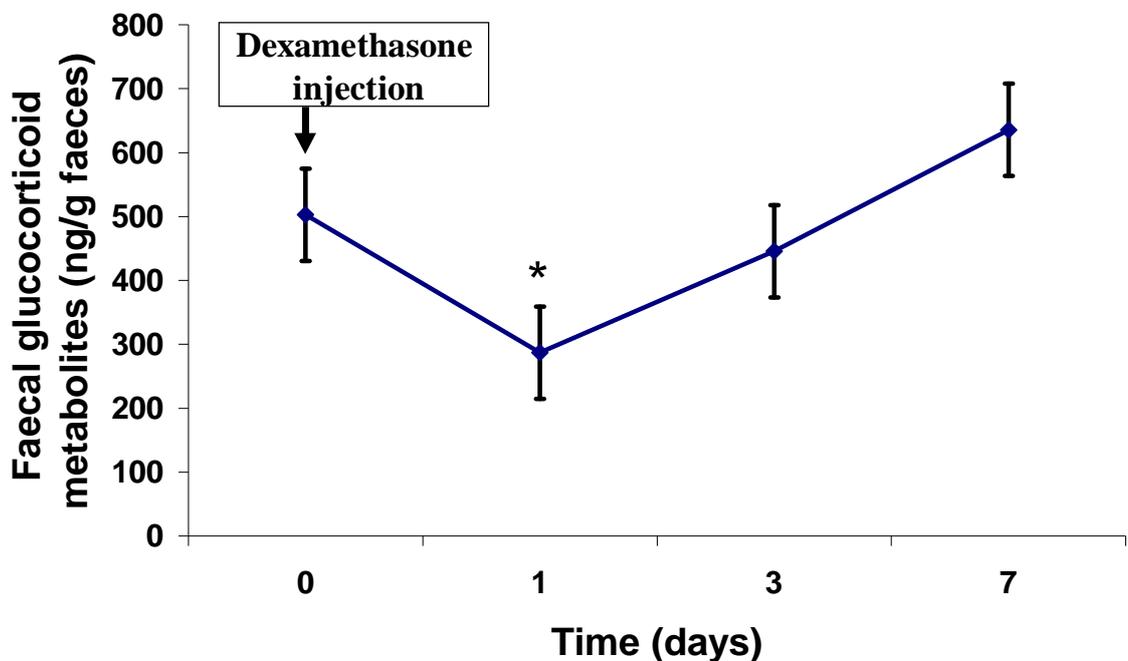


Figure 2.13 Immuno-reactive faecal glucocorticoid metabolites measured by EIA I in 8 female and 8 male Arabian oryx after injection with dexamethasone at 0 h. Data are shown as mean  $\pm$  standard error. Asterisk shows significant decrease compared to the data at 0 h, two way repeated measures ANOVA, followed by multiple comparisons versus control using Holm-Sidak method, \*  $P < 0.001$ .

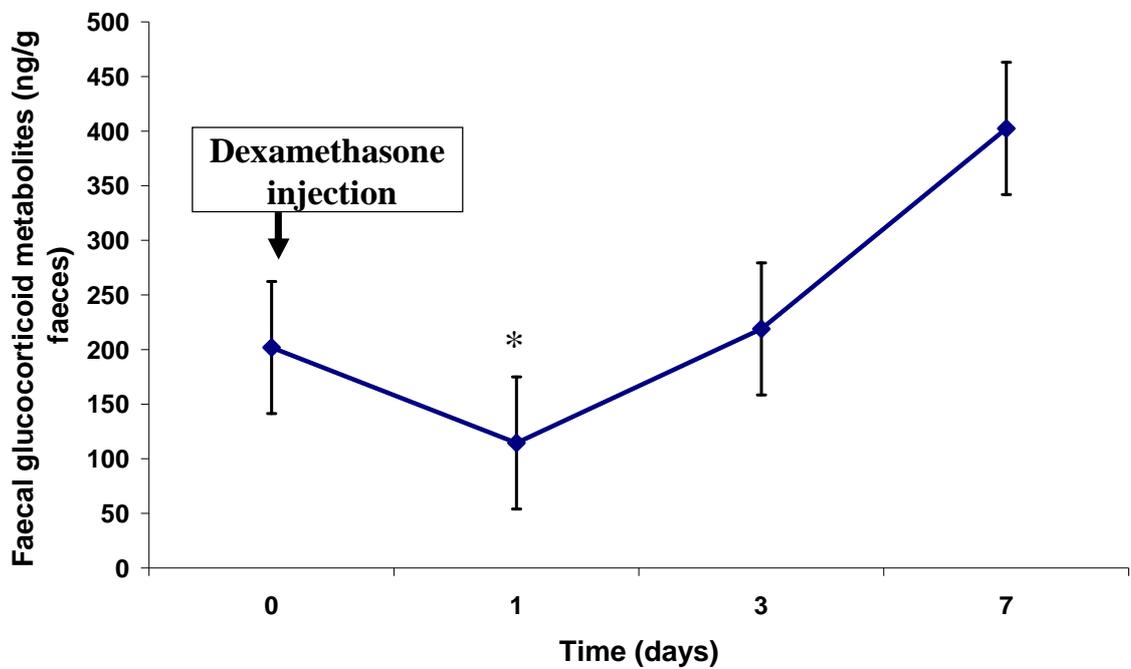


Figure 2.14 Immuno-reactive faecal glucocorticoid metabolites measured by EIA II in 8 female and 8 male Arabian oryx after dexamethasone injection at 0 h. Data are shown as mean  $\pm$  standard error. Asterisk shows significant decrease compared to the value at injection 0 h, two way repeated measures ANOVA, followed by multiple comparisons versus control using Holm-Sidak method, \*  $P = 0.006$ .

### 2.3.3 Stability of faecal glucocorticoid metabolites

There was no significant differences in measurements of faecal glucocorticoid metabolites from male and female Arabian oryx at each time point for either EIA I or EIA II ( $P = 0.721$ ) and therefore the data were combined. The faecal glucocorticoid metabolites measured by EIA I were stable for at least 24 h after collection and incubation at 30 °C and they decreased significantly after 3 days of incubation (Figure 2.15). On the other hand, the faecal glucocorticoid metabolites measured by EIA II were stable for at least 4 h after collection and incubation at 30 °C and they decreased progressively reaching significantly lower levels at 12 h, with further decreases after 24 h and 3 days after collection (Figure 2.16).

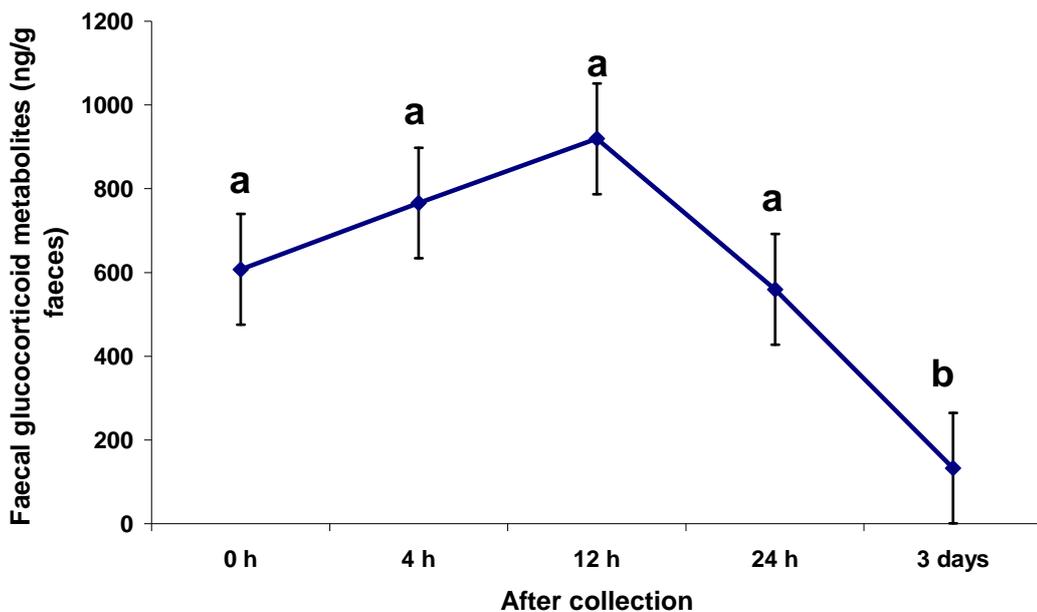


Figure 2.15 Immuno-reactive faecal glucocorticoid metabolites measured by EIA I in 4 male and 4 females Arabian oryx. Eight faecal samples were divided into 5 sub-samples incubated at 30 °C for 4, 12, 24 h and 3 days. Data are shown as mean  $\pm$  standard error. Data points with different letter above error bars are significantly different from each other, two way repeated measures ANOVA, followed by multiple comparisons using Holm-Sidak method,  $P < 0.001$ .

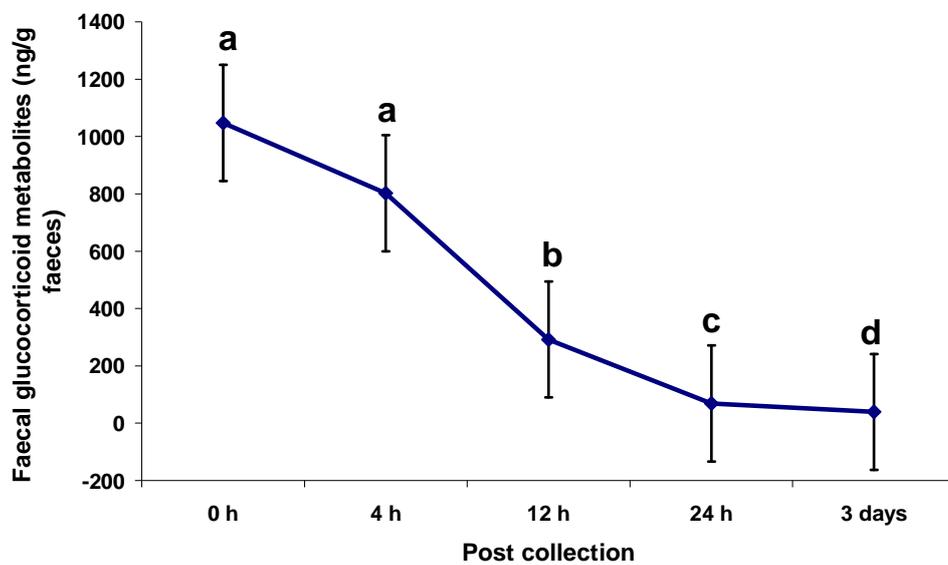


Figure 2.16 Immuno-reactive faecal glucocorticoid metabolites measured by EIA II in 4 male and 4 females Arabian oryx. Eight faecal samples were divided into 5 sub-samples incubated at 30 °C for 4, 12, 24 h and 3 days. Data are shown as mean  $\pm$  standard error. Data points with different letter above error bars are significantly different from each other, two way repeated measures ANOVA, followed by multiple comparisons using Holm-Sidak method,  $P < 0.001$ , except for 12 h versus 24 h and 4 h versus 12 h,  $P < 0.01$ .

## 2.4 Discussion

Most published studies of ACTH challenge tests aiming to stimulate adrenocortical activity and increase faecal glucocorticoid metabolites use very few animals, one or two in many cases (reviewed by Touma and Palme, (2005) as typical examples. However, with small sample sizes, detection of statistical differences that are truly present may be compromised and using of a large sample size strengthens the likelihood of detecting true statistical differences. The relatively small number of oryx used for the ACTH challenge test and storage experiments ( $n = 8$ ) was in line with welfare recommendations of the famous three R's coined by Russell and Burch (1959) which include reduction of the number of animals used to obtain information. In addition, continuous collection of faecal samples for 22 h after injection, from a large mammal (oryx) housed in individual housing pens made it difficult to use more than 8 oryx.

Although the number of oryx used was small, it was sufficient to demonstrate a subsequent increase in faecal glucocorticoid metabolites after ACTH administration. Both EIA I and EIA II detected an ACTH-induced increase and dexamethasone-induced decrease in faecal glucocorticoid metabolites that followed from the changes in circulating glucocorticoids. Therefore, it can be concluded that the antibodies used in both EIAs successfully cross-reacted with faecal corticosteroid metabolites of Arabian oryx and the data reflected the expected changes in the adrenocortical activity. This physiologically validates both EIAs and suggests that either assay could be successfully used for the measurement of faecal glucocorticoid metabolites in Arabian oryx.

The changes in the day-to-day diet of animals are unlikely to affect levels of glucocorticoids, but drastic changes in nutritional status leading to starvation have a significant effect on the levels of glucocorticoids (Lane, 2006). This is because starvation is perceived as stressor and also during starvation glucocorticoids are released to breakdown of muscle protein once body fat and carbohydrate have been depleted. Overall, the effect of diet on the concentration of faecal glucocorticoid metabolites is not well known and further research is needed to evaluate this (Millspaugh and Washburn, 2004; Palme, 2005; Palme et al., 2005). The oryx in the present study were provided with similar food in excess at

feeding time, on a regular basis, as a cautious measure to avoid any effect of dietary regime on the excreted glucocorticoid metabolites, but as a result there was frequent excretion of faeces. To avoid difficulties in identification of which oryx produced particular faecal pellets, animals were housed in individual holding pens. The freshest faecal sample was collected from each animal at the same time of the day (early morning) and the older samples were avoided. Then the samples were homogenised before extraction to ensure balanced distribution of metabolites within the faecal sample.

Generally, there was no difference between males and females in responding to the ACTH challenge test, dexamethasone suppression test and in the stability of faecal glucocorticoid metabolites in both EIA I and EIA II. In the ACTH experiment, males and females differed only at time points 6-8 h and 16-18 h after ACTH injection as measured by EIA I. The difference between males and females at 6-8 h after ACTH injection might be due to differences in the initial response of the adrenal cortex to ACTH, which was followed by a clearly significant increase in the faecal glucocorticoid metabolites with a delay in appearance of 6-8 h. The time point 16-18 h (Figure 2.7) represents the end of the adrenal cortex response to ACTH and this probably reflects variation among individuals at this time point, particularly given the small number of animals employed, rather than real differences between male and female response patterns.

One male (M11) showed exceptionally low values of faecal glucocorticoid metabolites at most sampling time points in both values generated using EIA I (Figure 2.8) and EIA II (Figure 2.11). It was unclear why this male had low values but it is possible that it did not receive an identical injection of ACTH as the experiment was started at night time using torch light. However, both EIAs detected a similar pattern of very low levels of faecal glucocorticoid metabolites in this male. Therefore, this gives extra validation that both EIAs have the ability to detect similar changes in the adrenocortical activity of Arabian oryx.

After injection of ACTH, cortisol secretion is stimulated. Then the cortisol is metabolised in the liver and the gut and its metabolites are excreted later in the faeces (Möstl and Palme, 2002). There is a delay from ACTH injection until the appearance of faecal metabolites which is called lag time (Palme et al., 2005). The determination of lag time from secretion

of cortisol in the blood until its excretion in faeces is important for determination of the duration between a stress event and the appearance of changes in faecal glucocorticoid metabolites. This helps in experimental design for monitoring of stress using faecal glucocorticoid metabolites. From this study, the delay from injection of ACTH until the first significant increase was 8-10 h while the peak effect was at about 14 h after ACTH injection in both EIA I and EIA II. The continuous elevation of faecal glucocorticoid metabolites for about 4-5 h after the first response may be due to minor disturbance during the frequent collection of faecal samples. Overall, the lag time for Arabian oryx in the present studies of captive oryx ranged from 8 to 15 h after ACTH injection for both EIA I and II. This range of lag time is quite wide. It is probable that in wild free-ranging Arabian oryx, the lag time will be greater since defecation will occur less frequently (Lane, 2006). But in any case, exact determination of the lag time between secretion of glucocorticoids and the appearance of metabolites in the faecal samples, would require radiometabolism studies. This would involve injection of radiolabelled  $^{14}\text{C}$ -cortisol and measurement of the peak radioactivity in faecal samples. Such studies are ethically difficult because Arabian oryx is an endangered species and measurements involving radioisotopes are very unlikely to be permitted.

From the experiment evaluating the stability of faecal glucocorticoid metabolites, it can be concluded that the metabolites measured by EIA I differ from those measured by EIA II. The metabolites measured by EIA I more stable than those measured by EIA II. The data analysis suggested that FGM measured by EIA were stable for at least 24 h (Figure 2.15) while those measured by EIA II were stable only for 4 h (Figure 2.16). However, the absolute timings need to be considered with caution given the small number of samples studied. In particular, the trend in FGM measurements in EIA II between 0 h and 4 h may indicate declining levels soon after collection and future studies using a larger number of samples are needed to gain more accurate information.

The antibody used in EIA I cross-reacts with metabolites that contain 11 and 17-oxo groups (Figure 2.2), while, the antibody used in EIA II cross-reacts with metabolites that have 3-hydroxy and 11-oxo groups (Figure 2.3). Stability of faecal glucocorticoid metabolites might be therefore attributed to the double bond of oxo-groups between oxygen and carbon

at positions 11 and 17 of the steroid structure and present in the metabolites measured by EIA I (11,17-dioxoandrostanes).

Despite differences in the stabilities of faecal glucocorticoid metabolites, both EIA I and EIA II worked successfully in Arabian oryx. However, EIA I has a major advantage over EIA II, as the metabolites measured by EIA I are stable for a longer period of time at ambient temperature. Hence, EIA I can be reliably applied for monitoring stress of Arabian oryx in the wild when storage of faecal samples in a freezer is likely to be delayed for several hours. Even though EIA I gave stable readings for at least 24 h, storing faecal samples after collection as soon as possible in a -20 °C freezer or below is still recommended as a sensible precautionary measure.

It has been reported that heating of faecal samples in a water bath at 95 °C for 20 min, inactivates bacterial enzymes and prolongs the stability of faecal glucocorticoid metabolites at ambient temperature (Möstl et al., 1999). This method of storage should be investigated for Arabian oryx and if found to prolong the stability of faecal glucocorticoid metabolites, it will be an extra advantage for using EIA I that measures quite stable metabolites for at least 24 h or even for EIA II that measures metabolites with at least 4 h of stability.

## **2.5 Summary and conclusions**

In conclusion, EIA I and EIA II proved to work successfully in Arabian oryx for non-invasive measurement of faecal glucocorticoid metabolites. These assays can help conservation biologists to measure stress of this endangered species, both in the wild and captivity. These assays can improve understanding of the welfare status of captive Arabian oryx in relation to management procedures and captivity conditions. These assays have been employed in this study for evaluation of the effects of tranquillisation of Arabian oryx with perphenazine enanthate (Chapter 4) and transport and release of oryx to the wild (Chapter 6).

## **Chapter 3**

### **Reference values for haematological, biochemical and physiological parameters in Arabian oryx**

#### **3.1 Introduction**

Maintaining good health and freedom from disease are essential elements of good animal welfare (Botreau et al., 2007). One of the ‘Five freedoms’ for animal welfare is Freedom from pain, injury and disease by prevention and rapid diagnosis and treatment (FAWC, 1993). The role of veterinary medicine is important for conservation of wildlife through assessment and monitoring of health in the wild or under captive conditions (Kirkwood, 1993; Karesh and Cook, 1995). Maintaining good health of wildlife particularly under captive conditions is of high importance. Examples of health problems are injuries, endo and ecto-parasites, lameness, infectious diseases, diarrhoea and chronic stress.

For assessment and monitoring of health of animals, one of the important things is to establish reference or expected range of values for various parameters which include data for blood parameters such as complete blood count, serum biochemistry profile, ions, hormones and clinical parameters e.g. heart rate, respiratory rate and body temperature (Karesh et al., 1997; Deem et al., 2001). These reference values aid in the diagnosis of certain pathologies (Kaneko et al., 1997; Deem et al., 2001). For example, haematological data such as haemoglobin and neutrophil count are used for the diagnosis of pathologies such as anaemia and bacterial infections, respectively (Hawkey, 1991; Junquera and Carnerio, 2005).

The values used for diagnosis or screening of diseases are often called “normal values” (Walton, 2001). Because these values might vary according to the sex, age or in captive or wild individuals of the same species, the term “reference values” should be used to avoid the confusion of using the term “normal values” (Walton, 2001; Solberg, 2006). Reference values are often given as the range between minimum and maximum values, which might include extreme outliers. Therefore, the International Federation of Clinical Chemistry

(IFCC) recommended the use of the central 95 % reference ranges defined as the 2.5 and 97.5 percentiles (Harris and Boyd, 1995; Lumsden, 1998; Walton, 2001; Horn and Pesce, 2003; Solberg, 2006; Dimauro et al., 2008). Haematological and biochemical reference values have been established for a wide range of animals. For example, reference values for haematological and/or biochemical parameters were established for chital deer (*Axis axis*) (Chapple et al., 1991), southern chamois (*Rupicapra pyrenaica*) (Lopez-Olvera et al., 2006a), Tamar wallaby (*Macropus eugenii*) (McKenzie et al., 2002), Parma wallabies (*Macropus parma*) (Clark et al., 2003), Ragusana donkeys (Caldin et al., 2005), hairy-nosed wombats (*Lasiorninus krefftii*) (Reiss et al., 2008), Spanish ibex (*Capra pyrenaica*) (Perez et al., 2003), black-faced impala (*Aepyceros melampus petersi*) (Karesh et al., 1997), primiparous cows (Abeni et al., 2005), working horses (Pritchard et al., 2009) and some species of wild ruminants in captivity (Peinado et al., 1999).

Most Arabian oryx around the world (more than 95 %) live in some form of captivity nowadays. The total number of Arabian oryx in Arabia is currently estimated at about 8000 oryx (Strauss, 2008). Holding of Arabian oryx in captivity requires close monitoring for health and well-being. The availability of accepted reference values for haematology, biochemistry, ions and hormones and clinical parameters will help in the diagnosis of diseases within the population.

The reference values of some haematological, biochemical, ions and other physiological parameters such as heart and respiratory rates vary according to the sex and age of the same species (Bush et al., 1983; Jain, 1986; Lopez-Olvera et al., 2006a). The variation might also depend on the method of capture and whether immobilising or tranquilising chemicals have been used or whether animals have been captured with or without using chemicals, as reported in red deer (*Cervus elaphus*) (Marco and Lavin, 1999) and koalas (*Phascolarctos cinereus*) (Hajduk et al., 1992). Bush et al. (1983) evaluated the differences of haematological and some serum chemistry values between neonate (less than a month old), juvenile (less than a year old) and adult (more than a year old) scimitar-horned oryx. Fifteen of 29 measured parameters were found to differ significantly between neonates and adults.

Some haematological and biochemical parameters, and data for body temperature, heart and respiratory rates were reported for neonates of Arabian oryx (Bounous-Dalton and Hood, 1980; Ferrell et al., 2001). There are clear differences in some haematological parameters between some of the seven age-groups reported for scimitar-horned oryx (*Oryx dammah*) (Hawkey and Hart, 1984). Ferrell et al. (2001) demonstrated important differences in biomedical parameters between neonates of closely related species of hippotragini: Arabian oryx (*Oryx leucoryx*), Addax (*Addax nasomaculatus*), scimitar-horned oryx (*Oryx dammah*) and sable antelope (*Hippotragus niger*). Therefore, the reference values established for neonates of one species could not be applied in neonates of other species. Reference values of adult animals are also not recommended as a reference for neonates of the same species or other closely related species (Ferrell et al., 2001).

Available information for haematological and biochemical parameters, serum ions and osmolality, hormones and clinical parameters (e.g. body temperature, heart rate and respiratory rate) in adult Arabian oryx have all been obtained either from immobilised animals (with data combined for both sexes) (Vassart and Greth, 1991; Greth et al., 1993; Ancrenaz et al., 1996) or from a single female that experienced capture myopathy (Vassart et al., 1992). In 1986, when there was an outbreak of tuberculosis in a recently reintroduced herd of Arabian oryx in Saudi Arabia (Vassart and Greth, 1991) the first haematological and serum biochemical values for Arabian oryx were established during a campaign for eradication of the tuberculosis. The oryx that were used for establishment of the reference values were captured by immobilisation and tranquillisation. The idea of establishing reference ranges was excellent; however, as samples were collected from animals that might have tuberculosis and that were captured after chemical injection, the values reported need to be considered with care. Other available values for Arabian oryx of more than 50 parameters measured by 17 different institutions have been collected in a database (International Species Information System, 2002), however, the data combine data for both sexes and all age groups. In this database, the number of samples per parameter varied from a single sample for some parameters to 265 samples for others with variable numbers of datapoints per animal. Because of mixing data from all age groups and sexes and using variable numbers of samples per animal, these values should also be considered with care.

One study that was looking particularly at the blood-gas and acid-base parameters also investigated a limited number of haematological parameters and serum ions from non-immobilised and non-tranquillised Arabian oryx (Kilgallon et al., 2008). However, they combined the data for 14 males and 5 females. Ostrowski et al. (2006) also reported some biochemical parameters for Arabian oryx, but did not specify the method of capture and whether it was with or without immobilisation.

So all available information for haematological, biochemical or other physiological data for adult Arabian oryx were obtained from either immobilised oryx in a mixed sex group, or after unspecified methods of capture or analytical methods. The use of chemical restraint e.g. immobilisation and tranquillisation, has been reported to depress some blood parameters in white-tailed deer (*Odocoileus virginianus*) compared to restraint without using chemicals (Seal et al., 1972; Presidente et al., 1973; Wesson III et al., 1979; Kocan et al., 1981). The effect of tranquillisation on some haematological, biochemical and clinical parameters, ions and some hormones in Arabian oryx are discussed in chapter 4 of this thesis. The effects of immobilisation on these parameters in Arabian oryx are discussed in chapter 5.

In this chapter, I present data for some haematological and biochemical parameters, serum ions and hormones, respiratory and heart rates and body temperature for male and female adult Arabian oryx that were captured physically without chemical immobilisation or tranquillisation. The differences between sexes were examined and the effects of the speed of capture on these parameters were highlighted. The blood cell-types in Arabian oryx were examined for the first time by transmission electron and light microscopes as measure to validate the haematological analytical method (See Appendix II).

During the course of these studies, 36 different adult Arabian oryx (24 males and 12 females) were investigated. The total number of oryx at the Omani Mammals Breeding Centre was 146 by the end of 2008, which means that about 25 % of the oryx at the centre were included in the studies. The sampled oryx represent about 8 % of Oman's total population of captive and wild oryx (430 individuals, at the end of 2008).

## **3.2 Materials and methods**

Thirty six adult Arabian oryx (24 males and 12 females) were used for the establishment of reference values. Twenty oryx (16 males and 4 females, numbered 1-20 in Table 3.1) were sampled between September 2006 and April 2007, while sixteen oryx (8 males and 8 females, numbered 21-36 in Table 3.1) were sampled in January and February 2008. All reference values presented in this chapter were obtained from oryx that were captured physically without using chemical immobilisation or tranquillisation.

During the studies reported in this thesis, several blood samples were collected from each oryx but only one sample per animal was used in the establishment of reference values. For calculating mean reference values in this chapter, a value for each parameter was obtained for each oryx from the final occasion when they were blood sampled without chemical pre-treatment (such as an immobilising agent: see Chapter 5 or tranquilliser: see Chapter 4). There were several reasons for selecting data from the last time of handling and blood sampling. During the early captures of each oryx, the animals were nervous. The animals became more familiar to handling after several captures and were calmer and therefore likely to be less stressed by the capture. The second reason was that with time, the team of people helping in capture became more experienced and handling time was reduced. Therefore, the speed of capture of Arabian oryx handled in 2006 and 2007 (Animals 1-20 in Table 3.1) was slower than those handled in 2008 (animals 21-36 in Table 3.1). Also, rapid collection of blood samples may be advantageous for parameters that might change if sampling is delayed, for example, serum glucocorticoids (Romero and Reed, 2005).

### **3.2.1 Capture and confinement**

Arabian oryx at the Omani Mammals Breeding Centre are held in two enclosures of areas 37,331 m<sup>2</sup> and 12,674 m<sup>2</sup>. Adult Arabian oryx were randomly selected based on inclusion and exclusion criteria from the paddocks and captured by immobilisation or sometimes physically, without using chemicals, and transferred to small holding pens (Figure 2.4). The inclusion criteria included adults of age between 1 and 5 years, apparently healthy and from both sexes and the exclusion criteria included calves, old oryx, dominant males, pregnant and lactating females, and apparently sick animals. The immobilisation involved darting

oryx using a blowpipe with a combination of 1.5 ml ketamine (Vetalar, 100 mg/ ml, Pharmacia) and 1.5 ml medetomidine (Domitor, 1 mg/ ml, Novartis) and reversal by 3 ml atipamezole (Antisedan, 5 mg / ml, Novartis). Food and water were provided for the oryx in the morning and evening. They are fed with hay, fresh alfalfa (lucerne), concentrate animal feed pellets (Barakat, Oman Flour Mills, Muscat, Oman), and sometimes with some cabbage and lettuce.

### **3.2.2 Capture for collection of blood samples**

All samples were collected in the morning between 7 and 10 am. To capture an oryx in the holding pen at the Omani Mammals Breeding Centre, a team of 6-10 people was required. Oryx have long sharp horns that are dangerous to humans. Therefore, the animal was cornered at the back of the pen by a plywood board as a shield to reduce the area of movement and facilitate capture. The oryx were blind-folded immediately after capture and blood samples were collected (section 3.2.4) by a qualified veterinarian. This was followed by measurement of heart and respiratory rates and body temperature (section 3.2.3).

### **3.2.3 Measurement of heart rate, respiratory rate and body temperature**

After the collection of blood samples and while the animal was being held firmly with legs stretched horizontally to the ground, body temperature, and respiration and heart rates were measured. Rectal body temperature was measured by using a digital thermometer (Thermoval, Hartmann, Heidenheim, Germany). Heart and respiratory rates were measured using a stethoscope and a stopwatch.

No	Animal ID	Sex	Date of blood sampling
1	998	female	30/08/2006
2	13A	male	18/09/2006
3	995	female	25/12/2006
4	996	female	25/12/2006
5	997	female	25/12/2006
6	10A	male	25/12/2006
7	1A	male	25/12/2006
8	5A	male	25/12/2006
9	10B	male	20/02/2007
10	16B	male	20/02/2007
11	17B	male	20/02/2007
12	19B	male	20/02/2007
13	1B	male	20/02/2007
14	20B	male	20/02/2007
15	10C	male	16/04/2007
16	16C	male	16/04/2007
17	17C	male	16/04/2007
18	19C	male	16/04/2007
19	1C	male	16/04/2007
20	20C	male	16/04/2007
21	F15	female	20/01/2008
22	M1	male	03/02/2008
23	M3	male	03/02/2008
24	M5	male	03/02/2008
25	M9	male	03/02/2008
26	M10	male	04/02/2008
27	M14	male	04/02/2008
28	M12	male	04/02/2008
29	M10B	male	04/02/2008
30	F12	female	05/02/2008
31	F14	female	05/02/2008
32	F16	female	05/02/2008
33	F18	female	05/02/2008
34	F17	female	06/02/2008
35	F19	female	06/02/2008
36	F20	female	06/02/2008

Table 3.1 List of individual Arabian oryx from which the reference values were obtained. The list is ordered by the date of blood sampling.

### **3.2.4 Collection of blood samples**

Blood samples were collected from the jugular vein using a disposable 50 ml syringe with a 18 G x 1.5” needle (Becton Dickinson) and then transferred into vacutainer (Becton Dickinson, 7 ml) tubes containing anticoagulant (EDTA) for haematological analysis, anticoagulant (fluoride oxalate) for analysis of plasma lactate and tubes without anticoagulant for analysis of serum ions, osmolality, hormones and biochemistry, by the passive force of the vacuum. This method was preferable to direct collection into vacutainers which in early trials was found to be unreliable for collection of required amount of blood in the various types of vacutainer tubes as well as taking longer overall. The inner diameter (ID) of the needle employed in the present studies 18 G (ID: 0.838 mm) was wider than the 21G (ID: 0.514 mm) commonly used for blood sampling (Lopez-Olvera et al., 2006b), which minimised cell damage and erythrocyte haemolysis.

The collected samples were labelled and placed in a cool-box containing ice, until processing within 3-6 h after collection.

The mean time from capture to completion of blood sampling for oryx numbered 21-36 (Table 3.1) studied in 2008 was  $122 \pm 8$  seconds (2.03 min). This time interval was not systematically recorded for oryx numbered 1-20 (Table 3.1) that were sampled earlier in 2006 and 2007, but the sampling took relatively longer (about 5-10 min per animal).

### **3.2.5 Haematology**

Haematological analysis was done within about 3 h after blood collection, using an automated blood analyser (Cell-DYN 4000, Abbott Diagnostics Santa Clara, CA, USA) at the Sultan Qaboos University Hospital, Muscat, Sultanate of Oman.

According to the manual for the auto-analyser Cell-DYN 4000 system, the identification of white blood cell types (neutrophils, basophils, eosinophils, monocytes and lymphocytes) is based on the size, cytoplasmic granularity and nuclear lobularity. The measurements of size and the number of erythrocytes and platelets are based on the degree of electrical impedance they produce.

The inter- and intra-variations of data for haematological parameters were measured for 8 males using duplicate samples (Table 3.2) as recommended by (Murray et al., 1993).

<b>Parameters</b>	<b>Intra-variation</b>	<b>Inter-variation</b>
WBC	1.77	19.48
Neutrophils	1.08	4.92
Lymphocytes	3.36	12.10
Monocytes	126.20	247.09
Eosinophils	20.61	28.45
Basophils	21.83	44.90
RBC	1.34	4.69
Haemoglobin	0.80	6.82
Haematocrit	1.29	7.58
MCV	0.23	6.66
MCH	1.58	6.38
MCHC	1.64	1.40
Platelets	36.31	74.24

Table 3.2 Inter- and intra-variations ( %) of haematological parameters calculated for 8 male Arabian oryx after measurement by Cell-Dyn 4000.

The instrument used for haematological analysis was calibrated for measurement of human blood, and could not be calibrated specifically for Arabian oryx. As a check of the validity of haematological data, the morphology and ultrastructure of Arabian oryx blood cells were examined by light microscopy and electron microscopy, and compared with those of humans (Junquera and Carnerio, 2005; Gartner and Hiatt, 2007) as outlined in Appendix 1.

### **3.2.6 Processing of blood samples**

Blood samples for the analysis of hormones, ions, osmolality and biochemical parameters were centrifuged at 2500 g for 15 min. Serum and plasma were transferred into polypropylene micro-centrifuge tubes, which were stored at - 80 °C until analysis.

### **3.2.7 Analysis of ions, hormones and biochemical parameters**

At the Department of Biochemistry, Sultan Qaboos University Hospital, Serum ions (calcium, phosphorus, magnesium) and a range of biochemical parameters (serum glucose, urea, albumin, total protein and plasma lactate) were analysed with an auto-analyser (Cobas

Integra 800, Roche, Switzerland) using absorbance photometry (enzymes and substrates). This instrument was also used in the potentiometric mode (measuring electrical potential) to determine serum concentrations of sodium, potassium and chloride.

Serum hormones were analysed by immuno-assays (Access® immuno-assay system, Beckman Coulter Inc.). The studied parameters were cortisol (kit number 33600, Beckman Coulter), free thyroxine (T<sub>4</sub>) (kit number 33880, Beckman Coulter), free triiodothyronine (T<sub>3</sub>) (kit number A13422, Beckman Coulter) and insulin (kit number 33410, Beckman Coulter).

Serum samples were thawed to room temperature before analysis of all ions, biochemical and hormonal parameters. The plasma for the measurement of lactate was kept on ice until analysis.

The instruments that measured blood ions, biochemistry and hormones (Cobas Integra 800 and Access, respectively) were calibrated as a routine practice within the Department of Clinical Biochemistry at Sultan Qaboos University Hospital. As a further check on the validity of measurements for Arabian oryx blood samples, a blood sample was subjected to dilution with normal saline (sodium chloride (0.9 %, w/v)) by preparation of different ratios of serum : saline: 0 % serum (0.0 ml : 1 ml), 25 % serum (0.25 ml : 0.75 ml), 50 % serum (0.50 ml : 0.50 ml), 75 % serum (0.75 ml : 0.25 ml) and 100 % serum (1.0 ml : 0.0 ml). The dilution of serum with saline resulted in the expected decline in the concentration of selected representative parameters (Table 3.3). Interassay and intraassay variability in the concentrations of ions, biochemical parameters and hormones calculated from 4 duplicated samples are shown in (Table 3.4).

### **3.2.8 Osmolality**

Serum osmolality was determined by freezing point depression using 20 µl of serum in a micro-osmometer (Advanced Micro-osmometer 3300, Advanced Instruments, Inc., Norwood, MA, USA).

### **3.2.9 Statistics**

As most samples (34 out of 36, see Table 3.1) were collected between the months (December and April, see Table 3.1) which is a cooler duration than other months in Muscat (see Figure 1.3), no comparison could be made for potential seasonal effects on the measured parameters.

The statistical differences between sexes (male versus female) and the speed of capture (slow versus fast) were compared by two way ANOVAs with gender and speed as factors, followed by multiple comparison tests (Holm-Sidak method). Where data could not be tested by two way ANOVAs because of missing values or failure to achieve normality, data were analysed by t-tests or Mann-Whitney tests, as appropriate. Data are presented as means, standard deviation of mean (SD), standard error of mean (SEM) and 2.5 and 97.5 percentiles. Within the discussion section of this chapter, the data from the present studies are presented as mean and standard error of mean, unless stated otherwise.

<b>Dilution factor</b>	<b>unit</b>	<b>100%</b>	<b>75%</b>	<b>50%</b>	<b>25%</b>	<b>0%</b>
Potassium	mmol/L	5.70	4.30	2.60	1.30	0.20
Calcium	mmol/L	2.34	1.77	1.02	0.49	0.00
Phosphorus	mmol/L	2.10	1.62	0.96	0.49	0.00
Urea	mmol/L	5.00	3.80	2.30	1.30	0.10
Glucose	mmol/L	4.87	3.70	2.26	1.14	0.01
Total protein	g/L	69	54	32	16	0
Albumin	g/L	47	36	21	10	0
Cortisol	nmol/L	87.00	71.00	44.00	23.00	2.00
Free triiodothyronine	pmol/L	3.19	3.03	2.50	1.77	0.00
Insulin	mIU/L	0.50	0.40	0.30	0.10	0.00

Table 3.3 Dilutions of a sample with normal saline (NaCl) and the resultant correspondent decrease in the concentration of some blood ions, biochemical and hormonal parameters. All parameters declined as a response to the dilution.

<b>Parameters</b>	<b>Intra-variation</b>	<b>Inter-variation</b>
Sodium	13.80	15.73
Potassium	12.92	11.56
Chloride	13.39	13.80
Urea	12.32	26.32
Calcium	14.38	6.32
Total protein	13.37	15.28
Glucose	13.45	16.54
Albumin	14.48	8.91
Phosphorus	14.15	11.70
Cortisol	7.66	22.70
Free T <sub>4</sub>	7.73	9.02
Free T <sub>3</sub>	21.79	12.86
Insulin	30.30	56.84

Table 3.4 Inter- and intra-variations ( %) of serum ions, biochemistry and hormones calculated from 4 duplicated samples.

### 3.3 Results

#### 3.3.1 Haematology

The haematological data for male and female Arabian oryx are presented in (Table 3.5). Statistical differences between male and female oryx and between oryx captured slowly or fast, in the haematological parameters are shown in (Table 3.6). The white blood cell count ( $P = 0.018$ ) and the percentage of neutrophils ( $P < 0.001$ ) were significantly higher in males than females while the percentages of lymphocytes ( $P < 0.001$ ) and basophils ( $P = 0.047$ ) were significantly lower (Table 3.5 and Table 3.6). All reported haematological parameters except white blood cells and the percentages of neutrophils, lymphocytes and basophils, did not differ between males and females (Table 3.5 and Table 3.6).

The speed of capture, whether relatively slow (animals 1-20 in Table 3.1) or relatively fast (animals 21-36 in Table 3.1) was statistically tested to investigate effects on haematological parameters (Table 3.6). Haematological parameters did not differ in oryx captured slowly from those captured more rapidly, except for a significant difference in the total count of leukocytes ( $P = 0.008$ ) (Table 3.6).

Data for parameters that did not differ between sexes and/or in relation to the speed of capture were combined and are presented in Table 3.7. However, the values that differed significantly between males and females (white blood cell count and the percentages of neutrophils, lymphocytes and basophils) are also included in Table 3.7 for the sake of completion of percentage data. The reference values of haematological parameters that take into consideration the sex differences and the speed of capture are presented (Table 3.7).

Parameter	Sex	Unit	n	Mean	SD	SEM	2.5 %	97.5 %
WBC	Males	$\times 10^9/L$	24	8.03	1.60	0.33	5.11	11.20
	Females	$\times 10^9/L$	12	6.93	1.48	0.43	4.26	9.64
Neutrophils	Males	%	24	69.66	5.90	1.21	57.10	78.40
	Females	%	12	58.52	6.60	1.91	45.60	67.20
Lymphocytes	Males	%	24	25.50	5.13	1.05	17.10	36.10
	Females	%	12	34.20	7.50	2.16	24.60	49.10
Monocytes	Males	%	24	0.69	1.27	0.26	0.00	4.05
	Females	%	12	0.98	1.04	0.30	0.00	3.02
Eosinophils	Males	%	24	2.15	1.30	0.27	0.41	5.48
	Females	%	12	3.07	1.64	0.47	0.40	6.02
Basophils	Males	%	24	2.01	1.35	0.28	0.00	4.21
	Females	%	12	3.23	0.90	0.26	1.80	4.99
RBC	Males	$\times 10^{12}/L$	24	12.22	0.75	0.15	10.70	13.70
	Females	$\times 10^{12}/L$	12	12.17	0.59	0.17	11.20	13.10
Haemoglobin	Males	g/dL	24	18.29	1.26	0.26	15.50	20.60
	Females	g/dL	12	18.05	0.98	0.28	15.90	19.30
Haematocrit	Males	%	24	54.80	4.97	1.01	47.10	70.30
	Females	%	12	52.60	2.88	0.83	47.50	56.60
MCV	Males	fL	24	44.80	2.25	0.46	41.60	51.20
	Females	fL	12	43.23	1.71	0.49	40.50	45.90
MCH	Males	pg/cell	24	14.98	0.74	0.15	13.10	16.70
	Females	pg/cell	12	14.86	0.70	0.20	13.60	15.90
MCHC	Males	g/dL	24	33.47	1.59	0.32	29.00	35.30
	Females	g/dL	12	34.33	0.70	0.20	33.60	36.00
Platelets	Males	$\times 10^9/L$	24	215.64	109.51	22.35	44.90	429.00
	Females	$\times 10^9/L$	12	157.97	177.78	51.32	38.30	663.00

Table 3.5 Reference values of haematological parameters for male and female oryx. n, number of animals; SD, standard deviation of mean; SEM, standard error of mean; 2.5 and 97.5 percentiles; WBC, white blood cells (leukocytes); RBC, red blood cells (erythrocytes) MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; and MCHC, mean corpuscular haemoglobin concentration.

Parameter	Males versus females		Fast versus slow capture		Transformation
WBC	Significant	P = 0.018	Significant	P = 0.008	normal
Neutrophils	Significant	P < 0.001	NS	P = 0.363	normal
Lymphocytes	Significant	P < 0.001	NS	P = 0.721	normal
Monocytes	NS	P = 0.680	NS	P = 0.508	Arcsin Square Root
Eosinophils	NS	P = 0.616	NS	P = 0.127	normal
Basophils	Significant	P = 0.047	NS	P = 0.062	normal
RBC	NS	P = 0.817	NS	P = 0.782	normal
Haemoglobin	NS	P = 0.879	NS	P = 0.629	normal
Haematocrit	NS	P = 0.806	NS	P = 0.219	normal
MCV	NS	P = 0.564	NS	P = 0.079	normal
MCH	NS	P = 0.581	NS	P = 0.808	normal
MCHC	NS	P = 0.925	NS	P = 0.235	rank
Platelets	NS	P = 0.391	NS	P = 0.400	normal

Table 3.6 Statistical comparison for differences between sexes and the speed of capture on haematological parameters using two way ANOVA. Differences are considered significant when  $P < 0.05$ . NS, non-significant; WBC, white blood cells (leukocytes); RBC, red blood cells (erythrocytes) MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; and MCHC, mean corpuscular haemoglobin concentration. When data were not normally distributed, they were transformed using the given type of transformation to achieve normality.

Parameter	Sex	Unit	n	Mean	SD	SEM	2.5%	97.5%
WBC	Both	x10 <sup>9</sup> /L	36	7.67	1.63	0.27	4.45	11.11
	<b>Males</b>	<b>x10<sup>9</sup>/L</b>	<b>24</b>	<b>8.03</b>	<b>1.60</b>	<b>0.33</b>	<b>5.11</b>	<b>11.20</b>
	<b>Females</b>	<b>x10<sup>9</sup>/L</b>	<b>12</b>	<b>6.93</b>	<b>1.48</b>	<b>0.43</b>	<b>4.26</b>	<b>9.64</b>
Neutrophils	Both	%	36	65.95	8.06	1.34	46.19	78.20
	<b>Males</b>	<b>%</b>	<b>24</b>	<b>69.66</b>	<b>5.90</b>	<b>1.21</b>	<b>57.10</b>	<b>78.40</b>
	<b>Females</b>	<b>%</b>	<b>12</b>	<b>58.52</b>	<b>6.60</b>	<b>1.91</b>	<b>45.60</b>	<b>67.20</b>
Lymphocytes	Both	%	36	28.40	7.23	1.21	17.25	47.91
	<b>Males</b>	<b>%</b>	<b>24</b>	<b>25.50</b>	<b>5.13</b>	<b>1.05</b>	<b>17.10</b>	<b>36.10</b>
	<b>Females</b>	<b>%</b>	<b>12</b>	<b>34.20</b>	<b>7.50</b>	<b>2.16</b>	<b>24.60</b>	<b>49.10</b>
Monocytes	Both	%	36	0.78	1.19	0.20	0.00	4.02
Eosinophils	Both	%	36	2.46	1.47	0.24	0.40	5.90
	Both	%	36	2.42	1.34	0.22	0.00	4.82
Basophils	<b>Males</b>	<b>%</b>	<b>24</b>	<b>2.01</b>	<b>1.35</b>	<b>0.28</b>	<b>0.00</b>	<b>4.21</b>
	<b>Females</b>	<b>%</b>	<b>12</b>	<b>3.23</b>	<b>0.90</b>	<b>0.26</b>	<b>1.80</b>	<b>4.99</b>
RBC	Both	x10 <sup>12</sup> /L	36	12.20	0.70	0.12	10.74	13.57
Haemoglobin	Both	g/dL	36	18.21	1.17	0.19	15.59	20.56
Haematocrit	Both	%	36	54.10	4.46	0.74	47.20	68.20
MCV	Both	FL	36	44.28	2.19	0.37	40.61	50.56
MCH	Both	pg/cell	36	14.94	0.72	0.12	13.21	16.59
MCHC	Both	g/dL	36	33.76	1.41	0.24	29.13	35.87
Platelets	Both	x10 <sup>9</sup> /L	36	196.42	136.29	22.72	38.32	611.52

Table 3.7 Reference values for haematological data of 24 male and 12 female Arabian oryx. The parameters that differ significantly between males and females are shown in **bold**. n, number of animals ; SD, standard deviation of mean; SEM, standard error of mean; 2.5 and 97.5 percentiles; WBC, white blood cells; RBC, red blood cells (erythrocytes) MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; and MCHC, mean corpuscular haemoglobin concentration.

### 3.3.2 Biochemistry, ions, osmolality, hormones and clinical parameters

The values for biochemical and clinical parameters, ions, osmolality and hormones for male and female oryx are shown separately in Table 3.8. The statistical difference between data for male and female oryx and between groups captured relatively quickly (within 2 min) compared to oryx captured more slowly (5 to 10 min) were tested by two way ANOVAs (results shown in Table 3.9). Where data could not be analysed by two way ANOVAs (not normally distributed) comparisons were made by t-tests or Mann-Whitney (Table 3.10).

Most parameters in Table 3.8 did not differ significantly between males and females. This applied for serum potassium, calcium, total protein, albumin, insulin (Table 3.9), chloride, magnesium, lactate, free thyroxine, body temperature, heart rate and respiratory rate (Table 3.10). The only parameters that differed significantly between males and females (see Table 3.8) were sodium ( $P = 0.027$ ), urea ( $P < 0.001$ ), glucose ( $P < 0.001$ ), free triiodothyronine ( $P = 0.016$ ) and cortisol ( $P < 0.001$ ) (Table 3.9), phosphorus ( $P = 0.044$ ) and osmolality ( $P = 0.049$ ) (Table 3.10). Males have significantly higher values of sodium, phosphorus, osmolality, urea, glucose and free triiodothyronine and significantly lower cortisol than females (Table 3.8, Table 3.9 and Table 3.10). The values that did not differ significantly between sexes were combined and are presented in Table 3.11. The combined means of parameters that differed significantly between males and females are also included in this table with different font style (Table 3.11) to use in the discussion section of this chapter in comparing data with published data.

Some parameters differed only between sexes (phosphorus, osmolality, urea, glucose and free triiodothyronine ( $T_3$ ) (Table 3.12 A), or only in relation to the speed of capture (free thyroxine ( $T_4$ ), total protein and albumin) (Table 3.12 B) or differed in relation to both speed of capture and sexes (cortisol and sodium) (Table 3.12 C).

Many parameters did not differ between slowly and fast captured oryx (potassium, calcium, urea, glucose, free triiodothyronine, insulin (Table 3.9), chloride and phosphorus (Table 3.10). However, magnesium, osmolality, lactate, body temperature, heart rate and respiratory rate (Table 3.10) have missing data for slowly captured oryx and therefore comparison with fast captured ones was not possible. The concentrations of total protein

and albumin were lower in fast captured (animals 21-36 in Table 3.1) while free thyroxine ( $T_4$ ) was higher, than in slowly captured oryx (animals 1 -20 in Table 3.1) (see Table 3.12 B). Among males, the concentration of sodium was significantly higher after fast capture than after slow capture ( $P = 0.003$ , two way ANOVA) but was not significantly different within females ( $P = 0.602$ , two way ANOVA). The concentration of cortisol was significantly lower in fast captured oryx than slowly captured oryx within males ( $P = 0.022$ , two way ANOVA) and within females ( $P = 0.014$ , two way ANOVA).

	Parameter	Sex	Unit	n	Mean	SD	SEM	2.5%	97.5%
Ions and osmolality	Sodium	Males	mmol/L	24	151.00	13.17	2.69	137.00	188.00
		Females	mmol/L	12	140.83	5.36	1.55	132.00	148.00
	Potassium	Males	mmol/L	24	6.28	1.38	0.28	4.20	10.10
		Females	mmol/L	12	6.03	0.93	0.27	4.50	7.50
	Chloride	Males	mmol/L	24	107.00	9.83	2.01	97.00	135.00
		Females	mmol/L	12	101.25	4.09	1.18	95.00	107.00
	Calcium	Males	mmol/L	24	2.30	0.25	0.05	1.95	2.93
		Females	mmol/L	12	2.26	0.14	0.04	2.05	2.48
	Magnesium	Males	mmol/L	16	1.20	0.14	0.04	0.98	1.39
		Females	mmol/L	4	1.18	0.08	0.04	1.09	1.27
Phosphorus	Males	mmol/L	24	2.71	0.52	0.11	1.94	4.26	
	Females	mmol/L	12	2.38	0.52	0.15	1.82	3.58	
Osmolality	Males	mosmo/L	16	309.53	10.53	2.63	286.50	325.00	
	Females	mosmo/L	4	298.13	1.97	0.99	296.50	301.00	
Biochemistry	Urea	Males	mmol/L	24	6.47	1.80	0.37	3.80	10.70
		Females	mmol/L	12	5.02	1.84	0.53	3.50	8.90
	Glucose	Males	mmol/L	24	6.08	1.94	0.40	3.85	10.76
		Females	mmol/L	12	5.14	2.21	0.64	3.46	11.48
	Lactate	Males	mmol/L	8	9.91	3.89	1.38	5.03	18.07
		Females	mmol/L	8	12.00	4.52	1.60	7.13	21.80
	Total protein	Males	g/L	24	71.67	8.99	1.84	56.00	98.00
		Females	g/L	12	63.42	3.83	1.10	57.00	68.00
Albumin	Males	g/L	24	52.29	8.11	1.65	41.00	77.00	
	Females	g/L	12	46.00	4.55	1.31	40.00	54.00	
Hormones	Cortisol	Males	nmol/L	21	102.71	54.33	11.86	4.00	213.00
		Females	nmol/L	12	123.17	42.81	12.36	69.00	208.00
	Free T <sub>4</sub>	Males	pmol/L	17	9.26	1.73	0.42	7.00	12.70
		Females	pmol/L	12	10.07	1.96	0.57	6.00	12.80
	Free T <sub>3</sub>	Males	pmol/L	16	4.41	1.49	0.37	2.41	8.69
		Females	pmol/L	12	4.04	1.31	0.38	0.73	5.77
Insulin	Males	mIU/L	17	0.61	0.27	0.07	0.40	1.50	
	Females	mIU/L	12	0.73	0.31	0.09	0.20	1.30	
Clinical	Body temperature	Males	°C	8	38.29	0.62	0.22	37.50	39.30
		Females	°C	8	38.49	0.56	0.20	37.30	39.20
	Heart rate	Males	bpm	8	101.00	23.62	8.35	80.00	150.00
		Females	bpm	8	113.00	21.19	7.49	90.00	142.00
	Respiratory rate	Males	bpm	8	34.00	9.91	3.51	20.00	48.00
		Females	bpm	8	43.50	9.67	3.42	30.00	54.00

Table 3.8 Blood ions, osmolality, biochemistry, hormonal and clinical parameters for male and female Arabian oryx. n, number of animals ; SD, standard deviation of mean; SEM, standard error of mean; 2.5 and 97.5 percentiles; T<sub>3</sub>, triiodothyronine, T<sub>4</sub>, thyroxine; bpm, breaths per min (for respiratory rate) or beats per min (for heart rate).

Parameter	Transformation	Males versus females		Fast versus slow capture	
Sodium	Rank	Significant	P = 0.027	Significant	P < 0.001
Potassium	Normal	NS	P = 0.102	NS	P = 0.279
<b>Chloride</b>	<b>NP</b>				
Calcium	Normal	NS	P = 0.061	NS	P = 0.215
<b>Magnesium</b>	<b>Missing values</b>				
<b>Phosphorus</b>	<b>NP</b>				
<b>Osmolality</b>	<b>Missing values</b>				
Urea	Normal	Significant	P < 0.001	NS	P = 0.297
Glucose	Reciprocal	Significant	P < 0.001	NS	P = 0.317
<b>Lactate</b>	<b>Missing values</b>				
Total protein	Reciprocal	NS	P = 0.898	Significant	P = 0.006
Albumin	In	NS	P = 0.229	Significant	P = 0.038
Cortisol	Normal	Significant	P < 0.001	Significant	P = 0.030
<b>Free T<sub>4</sub></b>	<b>NP</b>				
Free T <sub>3</sub>	Normal	Significant	P = 0.016	NS	P = 0.749
Insulin	Rank	NS	P = 0.371	NS	P = 0.093
<b>Body temperature</b>	<b>Missing values</b>				
<b>Heart rate</b>	<b>Missing values</b>				
<b>Respiratory rate</b>	<b>Missing values</b>				

Table 3.9 Statistical comparison for differences between sexes and the speed of capture on ions, osmolality, biochemistry, hormones and clinical parameters using two way ANOVA. The parameters in **bold font** were not tested because they were either not normally distributed or they have missing values. Therefore, they were tested with t-test or Mann-Whitney U test (Table 3.10). Differences were considered significant when  $P < 0.05$ ., NS, non-significant, NP, non-parametric; T<sub>3</sub>, triiodothyronine and T<sub>4</sub>, thyroxine.

Parameter	Males versus females			Slow versus fast capture		
Chloride	Mann Whitney	P = 0.052	NS	Mann Whitney	P = 0.554	NS
Magnesium	Mann Whitney	P = 0.601	NS	Missing values		
Phosphorus	Mann Whitney	P = 0.044	significant	t-test	P = 0.588	NS
Osmolality	t-test	P = 0.049	significant	Missing values		
Lactate	t-test	P = 0.337	NS	Missing values		
Free T <sub>4</sub>	t-test	P = 0.250	NS	t-test	P = 0.044	significant
Body temperature	t-test	P = 0.510	NS	Missing values		
Heart rate	t-test	P = 0.303	NS	Missing values		
Respiratory rate	t-test	P = 0.073	NS	Missing values		

Table 3.10 Statistical analysis using t-test or Mann Whitney test, for differences between sex and speed of capture, on the parameters that could not be tested by two way ANOVA. NS, non-significant and T<sub>4</sub>, thyroxine.

<b>Parameter</b>	<b>Unit</b>	<b>n</b>	<b>Mean</b>	<b>SD</b>	<b>SEM</b>	<b>Median</b>	<b>2.5%</b>	<b>97.5%</b>
<b>Sodium<sup>x</sup></b>	<b>mmol/L</b>	<b>36</b>	<b>147.61</b>	<b>12.11</b>	<b>2.02</b>	<b>146.00</b>	<b>132.22</b>	<b>187.78</b>
Potassium	mmol/L	36	6.19	1.24	0.21	6.15	4.20	9.77
Chloride	mmol/L	36	105.08	8.74	1.46	104.00	95.00	133.46
Calcium	mmol/L	36	2.29	0.22	0.04	2.21	1.97	2.92
Magnesium	mmol/L	20 <sup>a</sup>	1.19	0.13	0.03	1.20	0.98	1.39
<b>Phosphorus<sup>y</sup></b>	<b>mmol/L</b>	<b>36</b>	<b>2.60</b>	<b>0.54</b>	<b>0.09</b>	<b>2.54</b>	<b>1.85</b>	<b>4.11</b>
<b>Osmolality<sup>y</sup></b>	<b>mosmo/L</b>	<b>20<sup>a</sup></b>	<b>307.25</b>	<b>10.49</b>	<b>2.35</b>	<b>307.75</b>	<b>286.50</b>	<b>325.00</b>
<b>Urea<sup>y</sup></b>	<b>mmol/L</b>	<b>36</b>	<b>5.98</b>	<b>1.91</b>	<b>0.32</b>	<b>5.85</b>	<b>3.52</b>	<b>10.44</b>
<b>Glucose<sup>y</sup></b>	<b>mmol/L</b>	<b>36</b>	<b>5.76</b>	<b>2.05</b>	<b>0.34</b>	<b>5.11</b>	<b>3.53</b>	<b>11.32</b>
Lactate	mmol/L	16 <sup>b</sup>	10.95	4.22	1.05	9.89	5.03	21.80
<b>Total protein<sup>z</sup></b>	<b>g/L</b>	<b>36</b>	<b>68.92</b>	<b>8.56</b>	<b>1.43</b>	<b>67.50</b>	<b>56.22</b>	<b>96.46</b>
<b>Albumin<sup>z</sup></b>	<b>g/L</b>	<b>36</b>	<b>50.19</b>	<b>7.66</b>	<b>1.28</b>	<b>49.00</b>	<b>40.22</b>	<b>75.90</b>
<b>Cortisol<sup>x</sup></b>	<b>nmol/L</b>	<b>33<sup>c</sup></b>	<b>110.15</b>	<b>50.74</b>	<b>8.83</b>	<b>109.00</b>	<b>6.24</b>	<b>212.20</b>
<b>Free T<sub>4</sub><sup>z</sup></b>	<b>pmol/L</b>	<b>29<sup>d</sup></b>	<b>9.59</b>	<b>1.84</b>	<b>0.34</b>	<b>9.30</b>	<b>6.06</b>	<b>12.79</b>
<b>Free T<sub>3</sub><sup>y</sup></b>	<b>pmol/L</b>	<b>28<sup>e</sup></b>	<b>4.25</b>	<b>1.40</b>	<b>0.27</b>	<b>4.14</b>	<b>0.83</b>	<b>8.57</b>
Insulin	mIU/L	29 <sup>d</sup>	0.66	0.29	0.05	0.60	0.22	1.48
Body temperature	°C	16 <sup>b</sup>	38.39	0.58	0.15	38.50	37.30	39.30
Heart rate	bpm	16 <sup>b</sup>	107.00	22.55	5.64	98.00	80.00	150.00
Respiratory rate	bpm	16 <sup>b</sup>	38.75	10.66	2.66	38.00	20.00	54.00

Table 3.11 Combined data of male and female oryx for ions, osmolality, biochemistry, hormones and clinical parameters. Note: the parameters in **bold and italics font** differed significantly within sexes (<sup>y</sup>), or in relation to speed of capture (<sup>z</sup>) or within both categories (<sup>x</sup>). Data missing for oryx <sup>a</sup> (21-36), <sup>b</sup> (1-20), <sup>c</sup> (10,12,15), <sup>d</sup> (7,10, 12, 14, 15, 17 and 19), <sup>e</sup> (7,10, 12, 14, 15, 17, 18 and 19) as numbered in Table 3.1. n, number of animals; SD, standard deviation of mean; SEM, standard error of mean; 2.5 and 97.5 percentiles; T<sub>3</sub>, triiodothyronine, T<sub>4</sub>, thyroxine; bpm, breaths per min (for respiratory rate) or beats per min (for heart rate).

(A)

Parameter	Males	Females
Phosphorus (mmol/L)	2.71 ± 0.11 (n = 24)	2.38 ± 0.15 (n = 12)
Osmolality (mosmo/L)	309.53 ± 2.63 (n = 16)	298.13 ± 0.99 (n = 12)
Urea (mmol/L)	6.47 ± 0.37 (n = 24)	5.02 ± 0.53 (n = 12)
Glucose (mmol/L)	6.08 ± 0.40 (n = 24)	5.14 ± 0.64 (n = 12)
Free T <sub>3</sub> (pmol/L)	4.41 ± 0.37 (n = 24)	4.04 ± 0.38 (n = 12)

(B)

Parameter	Fast capture	Slow capture
Total protein (g/L)	68.19 ± 2.89 (n = 16)	69.50 ± 1.20 (n = 20)
Albumin (g/L)	48.81 ± 2.70 (n = 16)	51.30 ± 0.82 (n = 20)
Free T <sub>4</sub> (pmol/L)	10.21 ± 0.33 (n = 16)	8.84 ± 0.59 (n = 13)

(C)

Parameter	Sex	Fast capture	Slow capture
Sodium (mmol/L)	male	162.50 ± 6.22 (n = 8)	145.25 ± 0.99 (n = 16)
	female	140.88 ± 2.20 (n = 8)	140.75 ± 1.93 (n = 4)
Cortisol (nmol/L)	male	73.25 ± 15.41 (n = 8)	120.85 ± 12.09 (n = 13)
	female	100.00 ± 15.41 (n = 8)	169.50 ± 21.79 (n = 4)

Table 3.12 The parameters that significantly differed in relation to speed of capture (A), sexes (B) or both categories (C). Data are presented as mean ± standard error of mean. n, number of animals, T<sub>4</sub>, thyroxine and T<sub>3</sub>, triiodothyronine.

### **3.4 Discussion**

The main aim of this chapter was to establish reference values for haematological, biochemical hormonal and clinical parameters. These parameters are the main parameters assessed in analytical laboratories and made available to veterinarians to assess health and abnormalities in the animals that they treat.

In the present study, sex hormones (estradiol, progesterone and testosterone) and luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were also measured, but the results were erratic and are therefore not presented. The selected animals were non-pregnant and non-lactating females, and non-dominant males. Therefore reproductive hormones were not considered important.

Creatine kinase was not measured in the present study but would be a useful parameter for inclusion in future studies as an indicator of muscle damage and capture myopathy (Vassart et al., 1992). Additional parameters that might be useful in future studies are aspartate aminotransferase (AST) and alanine aminotransferase (ALT) for monitoring liver function (Kramer and Hoffmann, 1997).

In the present study, all animal used in assessing reference ranges were from the Omani Mammals Breeding Center within easy reach of the analytical laboratories at Sultan Qaboos University. No samples from the other captive population in Oman, at Jaaluni (the Arabian Oryx Sanctuary) were included because of the complexities of dealing with samples collected 630 km from the laboratory. Capturing and collecting blood samples from a few individuals per day and travelling the very long distance for analysis of unfrozen samples was not feasible for this study. However, ideally to obtain reference values for the population of Arabian oryx in the Oman, values for samples obtained from the Jaaluni population and free-ranging animals should be incorporated in later studies.

The International Federation of Clinical Chemistry (IFCC) recommends a sample size of at least 120 to establish reference values (International Federation of Clinical Chemistry, 1987; Linnet, 2000; Solberg, 2006). However, sample size must take account of the total number of animals in a population, particularly for endangered species. The total number of

Arabian oryx in Oman now (2010) is less than 500 individuals so IFCC recommendations would have required sampling of more than 24 % of the then current Omani population of oryx. As an endangered species, Arabian oryx must be handled with maximum care in a way that keeps them in good welfare and reduces stress such as capture stress. For example, capture myopathy that has been experienced by some captured Arabian oryx (Greth and Vassart, 1989; Vassart et al., 1992). Although the sample size in the present study (36) was smaller than the IFCC recommend, it is in line with the 'three R's' that encourage reduction of the number of animals used to obtain information (Russell and Burch, 1959).

The samples in the present study were exclusively from Arabian oryx housed at the Omani Mammals Breeding Center, which is a Royal Property of H M Sultan Qaboos bin Said. Thirty-six individuals were made available for the present study out of about 150 individuals present at the center at the time of the study, which represents a relatively high proportion, about a quarter (24 %) of the herd at the center. Application of the exclusion criteria (calves, old, lactating and pregnant females and sick animals) would in any case not have provided the 120 individuals that the IFCC recommend. In future studies, the inclusion of animals from Jaaluni would provide data for a total number of oryx that is closer to the recommended 120 for establishing reference values as well as incorporating a different sub-population of Omani Arabian oryx. Applying exclusion and inclusion criteria focused the study on apparently healthy adult male and female Arabian oryx. This helps in the establishment of reference values for adult Arabian oryx of specified age group that are not affected by social status, like dominant males, or by the reproductive status, for example of pregnant and lactating females.

One of the challenges of comparing the data from the present study with previous studies that report haematological, biochemical or other physiological parameters for Arabian oryx or other closely related species, is that most if not all these studies used chemical restraint (e.g. immobilisation, sedation, and tranquillisation). There is a high possibility that reported values were affected by restraint chemicals (Cross et al., 1988; Dehghani et al., 1991; Marco and Lavin, 1999) and therefore, the values reported by those studies might differ from the reference values reported in present studies. Immobilisation has great effects on many haematological and biochemical parameters as found in Arabian oryx using xylazine (Chapter 5). Chemical capture by immobilisation caused significant changes in

haematological and biochemical parameters compared to three physical capture methods in bighorn sheep (*Ovis canadensis*) (Kock et al., 1987). Therefore, the literature that reported haematological and biochemical parameters from immobilised oryx are not discussed in this chapter. Future researchers who measure blood parameters of Arabian oryx should consider the method of capture (i.e. with or without immobilisation) and the time from capture to collection of the blood sample, before comparing their results with the reference values obtained in the present studies. Further research is needed to look more closely at the effect of time of blood sample collection after the start of handling to elucidate any differences. Chapters 4 and 5, respectively, consider the effects of tranquillisation with perphenazine enanthate and immobilisation with xylazine on haematological, biochemical and other physiological parameters. These chemicals were chosen as case studies for the effect of tranquilisation and immobilisation on the mentioned parameters.

In this chapter, the reference values obtained are compared with those reported in the few studies that did not use restraint chemicals. It might be argued that if Arabian oryx and the closely related species are mostly captured by chemical means, why not obtain reference values from those animals?. The difficulty is that many types of restraint chemicals are used and each has a different mechanism of action and therefore effects on the haematological, biochemical or other physiological parameters vary according to the type of chemical. For this reason, obtaining reference values from animals captured without chemical means provides the most representative reference values that are closest to “normal”.

### **3.4.1 Haematology**

Before discussing the haematological parameters that have been established in these studies, the analytical method of measuring haematological parameters, the Cell-Dyn 4000, should be considered. This instrument has been evaluated and proved reliable for counting blood cells (Grimaldi and Scopacasa, 2000) and has been widely used for measurement of haematological parameters in animals such as in rhesus macaques (*Macaca mulatta*) (Winterborn et al., 2008) and rats (de la Mano et al., 2004), but has not been previously used for Arabian oryx blood samples.

The instrument at Sultan Qaboos University Hospital was calibrated for human subjects, hence it was important to assess its suitability for counting blood cells of Arabian oryx. There was no instrument that could be calibrated for dedicated use in analysis of Arabian oryx haematological parameters. The lack of information about the sizes of blood cells led to an examination of the size and morphology of Arabian oryx blood cells in comparison to human blood cells since the instrument identifies blood cell types based on the size, cytoplasmic granularity and nuclear lobularity. There are no previous studies that have looked at the morphology of Arabian oryx blood cell types. Thus, the morphology and ultrastructure of Arabian oryx erythrocytes and differential leukocytes and thrombocytes were examined by light and transmission electron microscopy in the present study (See Appendix II). Comparative haematology studies showed that most mammalian species shared similar features of leukocytes and erythrocytes, with a few exceptions like Camelidae that has oval flat erythrocytes and deer and some carnivores with sickled erythrocytes (Hawkey, 1991). The present study reveals that the morphology of Arabian oryx blood cell types is very similar to those of humans (See Appendix II), although oryx erythrocytes were smaller than that of humans, and in future studies of Arabian oryx blood samples, calibration of the analytical instrument would be desirable.

Bush et al. (1983) evaluated haematological and some serum chemistry values of neonate (less than a month old), juvenile (less than a year old) and adult (more than a year old) scimitar-horned oryx, and found significant differences between neonates and adults in 15 out of 29 parameters. In the present study, only adult Arabian oryx were investigated. Haematological values of neonate Arabian oryx have been measured (Bounous-Dalton and Hood, 1980; Ferrell et al., 2001). Comparing haematological values for adult and neonatal Arabian oryx, values of red blood cell counts, haematocrit and haemoglobin concentrations were clearly lower in neonates than in the adults included in the present study (Table 3.13 and Table 3.14).

The mean leukocyte counts in the present study are comparable to those of neonates reported by Bounous-Dalton and Hood (1980) (Table 3.13). However, Ferrell et al. (2001) reported a lower median WBC count than that obtained in the present study (Table 3.14). The values of MCV, MCH and MCHC are comparable between neonates and adult Arabian oryx (Bounous-Dalton and Hood, 1980) (Table 3.13). However, there were substantial

differences in red blood cell count, haemoglobin and haematocrit of neonates (Bounous-Dalton and Hood, 1980) and the data in the present study of adult oryx. These might represent difference between adults (the present study) and neonates (Bounous-Dalton and Hood study). This idea is supported by the evidence of a lower haematocrit (27 %) in neonates than adults (Ferrell et al., 2001) with a similar hematocrit in neonate oryx to those found by Bounous-Dalton and Hood (1980). However, Ferrell et al. (2001) did not measure red blood cell count and haemoglobin of neonates to make further comparison.

The study of Kilgallon et al. (2008) is the only study that investigated haematological parameters of adult Arabian oryx without using any immobilising or tranquilising chemicals. Comparison of the values reported by Kilgallon et al. (2008) and the present study is presented in Table 3.15. This comparison indicates that the white blood cell data are very close to each other, but other parameters vary between the two studies. The mean percentages of neutrophils and monocytes are higher and the mean haematocrit, percentages of lymphocytes, eosinophils and basophils are lower in Kilgallon et al. (2008)'s study than in the present study. The neutrophilia and lymphopenia reported by Kilgallon et al. (2008) might be due to more stress during capture in that study compared to the present study. Kilgallon et al. (2008) moved oryx from paddocks through corridors and into a handling chute, which involved more manipulation. Stress of handling results in excessive secretion of glucocorticoids, that cause neutrophilia and lymphopenia (Iseki et al., 1991; Burton et al., 1995; Sapolsky et al., 2000). The main difference between the present study and that of Kilgallon et al., (2008) is that the oryx that were used in the present studies were housed for a prolonged period in holding pens and exposed to a series of handling events, while those used by Kilgallon et al. (2008) had not been exposed to previous handling. Familiarisation of animals by frequent exposure to handling is likely to play a role in reducing the physiological response to handling over time and allow derivation of more valid reference data (Broom and Johnson, 1993a). Cortisol and catecholamine-mediated distortions of haematology and blood biochemistry have been reported in captured impala and red deer (Marco and Lavin, 1999).

The mean haematocrit reported by Kilgallon et al. (2008) (41.79 %, Table 3.15) and ISIS (2002) (44.00 %, Table 3.16) are lower than those measured in the present study (54.10 %). The intra-variation between samples for haematocrit in the present study within duplicate

samples was 1.29 % and inter-variation between animals was 7.58 % (Table 3.2) which are below the satisfactory accepted percentage of variation (10 %) suggested by Murray et al. (1993). Arabian oryx in the holding pens are provided with water twice a day (morning and evening) in a water container, but they might be dehydrated by the time of handling, as water was provided after handling. The higher haematocrit in the present study might indicate dehydration of the oryx. Serum osmolality is another indicator of hydration status but urine osmolality is found to be superior indicator of hydration status than serum osmolality or haematocrit (Shirreffs, 2003). In the present study, the urine osmolality was not examined and therefore, further investigations are recommended to look at the correlation between hydration status, haematocrit, serum osmolality and urine osmolality. Stress is known to increase haematocrit by causing splenic contraction and therefore increasing the number of red blood cells in circulation (Stewart and McKenzie, 2002), and consequently causing increase in haematocrit. Probably the stress of capture caused an increase in haematocrit in Arabian oryx in the present study. The previous studies such as those of Kilgallon et al. (2008) probably used more stressful approaches of capture than the present study but they reported much lower haematocrit. Further investigations are needed to explain this apparent discrepancy.

Parameter	Unit	Bounous-Dalton and Hood, 1980				The present study			
		n	Means	SD	SEM	n	Mean	SD	SEM
WBC	$\times 10^9/L$	17	7.3	1.87	0.45	36	7.67	1.63	0.27
Neutrophils	%	17	61.4	9.61	2.33	36	65.95	8.06	1.34
Lymphocytes	%	17	37.2	9.15	2.22	36	28.40	7.23	1.21
Monocytes	%	17	0.74	0.64	0.16	36	0.78	1.19	0.20
Eosinophils	%	17	0.57	0.82	0.20	36	2.46	1.47	0.24
Basophils	%	17	0.0		0.00	36	2.42	1.34	0.22
RBC	$\times 10^{12}/L$	19	6.6	0.8	0.18	36	12.20	0.70	0.12
Haemoglobin	g/dL	17	11.0	1.37	0.33	36	18.21	1.17	0.19
Haematocrit	%	17	33.9	4.24	1.03	36	54.10	4.46	0.74
MCV	fL	19	50.1	4.56	1.05	36	44.28	2.19	0.37
MCH	pg/cell	19	16.5	3.72	0.85	36	14.94	0.72	0.12
MCHC	g/dL	19	32.7	2.64	0.61	36	33.76	1.41	0.24

Table 3.13 Comparison of haematological values for neonates as reported by Bounous-Dalton and Hood (1980) and for adults as reported in the present study. n, number of animals; SD, standard deviation of mean; SEM, standard error of mean; WBC, white blood cells; RBC, red blood cells (erythrocytes) MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; and MCHC, mean corpuscular haemoglobin concentration.

Parameter	Ferrell et al., (2001)				The present study			
	Median	Min	Max	n	Median	Min	Max	n
WBC ( $\times 10^9/L$ )	5.73	4.18	9.25	10	7.45	4.26	11.20	36.00
Neutrophils (%)	65.97	43.80	111.34	9	66.60	45.60	78.40	36.00
Lymphocytes (%)	23.56	20.24	49.39	9	27.10	17.10	49.10	36.00
Monocytes (%)	2.48	0.73	2.74	9	0.10	0.00	4.05	36.00
Eosinophils (%)	0.00	0.00	1.08	9	2.08	0.40	6.02	36.00
Basophils (%)	0.00	0.00	0.00	9	2.69	0.00	4.99	36.00
Haematocrit (%)	38.5	30.0	42.0	10	53.10	47.10	70.30	36.00

Table 3.14 Comparison of haematological values for neonates as reported by Ferrell et al. (2001) and for adults as reported in the present study. WBC, white blood cells; min, minimum; max, maximum and n, number of animals.

Acute stress has been found to cause large, rapid and reversible changes in the distribution of leukocytes in peripheral blood, such as increase in neutrophils and decrease of lymphocytes as found in rats (Dhabhar et al., 1996). The studies reported in this chapter showed significant differences between male and female Arabian oryx in the white blood cell count, percentages of neutrophils, lymphocytes and basophils. Therefore, these parameters should be reported and presented for each sex separately. No previous studies have reported haematological parameters for male and female Arabian oryx separately. Therefore, the present study is the first to look into the differences between sexes and the first to present separate reference values for haematological parameters that differ significantly between sexes. Some sex differences in haematological parameters were found between males and females of southern chamois (*Rupicapra pyrenaica*) such as a higher neutrophil count in males (Lopez-Olvera et al., 2006a), as seen in oryx in the present study. However, in some species there are no sex differences in haematological parameters exposed to capture. For example, Rispat et al. (1993) found no significant difference between 36 males and 35 females of Yucatan micropigs in all haematological parameters except in platelets count. Thus, sex differences are species dependant and it seems that each species has to be studied to elucidate these differences.

For the oryx captured slowly (animals 1-20 in Table 3.1) or more rapidly (animals 21-36 in Table 3.1), the only haematological parameter that differed between these two groups was the white blood cell count. The leukocyte count was significantly higher ( $P = 0.008$ , two way ANOVA, Table 3.6) in oryx captured and blood sampled within 2 min ( $8.07 \pm 0.37 \times 10^9/L$ ,  $n = 16$ , 8 males and 8 females) in comparison with oryx captured and blood sampled more slowly (5-10 min) ( $6.09 \pm 0.41 \times 10^9/L$ ,  $n = 20$ , 16 males and 4 females). This difference in leukocytes counts was not reflected in the differential leukocytes counts.

Parameter	Kilgallon et al., (2008)				The present study			
	n	Mean	SD	SEM	n	Mean	SD	SEM
WBC ( $\times 10^9/L$ )	19	7.31	1.99	0.46	36	7.67	1.63	0.27
Neutrophils (%)	19	76.29	9.02	2.07	36	65.95	8.06	1.34
Lymphocytes (%)	19	19.73	7.85	1.80	36	28.40	7.23	1.21
Monocytes (%)	19	3.08	1.28	0.29	36	0.78	1.19	0.20
Eosinophils (%)	19	0.33	0.27	0.06	36	2.46	1.47	0.24
Basophils (%)	19	0.57		0.00	36	2.42	1.34	0.22
Haematocrit (%)	19	41.79	1.84	0.42	36	54.10	4.46	0.74

Table 3.15 Comparison between some haematological parameters reported by Kilgallon et al., (2008) and the present study. SD, standard deviation of mean, SEM, standard error of mean, n, number of animals and WBC, white blood cells.

Parameter	Unit	ISIS, 2002			The present study			
		Mean	SEM	Samples	Animals	Mean	SEM	N
WBC	$\times 10^9/L$	6.87	0.15	250	137	7.67	0.27	36
Neutrophils	%	70.69	0.14	214	112	65.95	1.34	36
Lymphocytes	%	23.24	0.05	214	112	28.40	1.21	36
Monocytes	%	2.58	0.01	166	97	0.78	0.20	36
Eosinophils	%	2.74	0.02	152	89	2.46	0.24	36
Basophils	%	1.12	0.01	43	34	2.42	0.22	36
RBC	$\times 10^{12}/L$	10.14	0.19	159	95	12.20	0.12	36
Haemoglobin	g/dL	15.70	0.22	158	98	18.21	0.19	36
Haematocrit	%	44.00	0.01	265	145	54.10	0.74	36
MCV	fL	45.20	0.59	152	93	44.28	0.37	36
MCH	pg/cell	15.50	0.19	135	79	14.94	0.12	36
MCHC	g/dL	34.40	0.28	152	96	33.76	0.24	36
Platelets	$\times 10^9/L$	330.00	30.00	35	26	196.42	22.72	36

Table 3.16 Comparison between some haematological parameters reported by the database of ISIS (2002) and the present study. SEM, standard error of mean, n, number of animals, WBC, white blood cells; RBC, red blood cells (erythrocytes) MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; and MCHC, mean corpuscular haemoglobin concentration.

### **3.4.2 Biochemistry, ions, osmolality, hormones and clinical parameters**

The reference values for potassium, chloride, calcium, magnesium, lactate, insulin, body temperature, heart rate and respiratory rate showed no significant differences in the sexes or in relation to capture speed. Therefore, the combined values for these parameters can be used as reference values for both sexes.

Kilgallon et al. (2008) reported the concentrations of potassium and calcium in serum for a mixed group male and female Arabian oryx captured by non-chemical means as  $4.75 \pm 0.19$  mmol/L and  $1.16 \pm 0.01$  mmol/L, respectively (n = 19, 14 males and 5 females). These values are slightly lower but comparable to those obtained in the present study for mixed groups of both sexes ( $6.19 \pm 1.24$  mmol/L and  $2.29 \pm 0.04$  mmol/L respectively) and values did not differ significantly in males and females (Table 3.11).

The concentrations of chloride, magnesium, lactate, insulin, body temperature, heart rate and respiratory rate have not previously reported for oryx captured without chemical restraint. However, the values for chloride, magnesium and body temperature in the ISIS database (International Species Information System, 2002), after unknown methods of capture, and for unknown sex and age groups are comparable to those in the present study (Table 3.17).

Ideally, to get more accurate measurements for the baseline of body temperature, heart rate and respiratory rate animals should be fitted with telemetric recording devices that measure these parameters at set intervals for long periods of time (Lopez-Olvera et al., 2006b). Some measurements of body temperature have now been acquired for Arabian oryx and show clear seasonal patterns (Hetem et al., 2010) (see Chapter 1, section 1.1.5.1), but telemetric devices were not available for the present study. The collection of respiratory and heart rates by auscultation is a common and widely used method by veterinarians (Gonzalez et al., 2008) but suffers from the immediate effects of proximity and contact with the animals. In future studies, telemetry devices should be utilised for Arabian oryx to obtain measurements close to baseline for comparison to published values.

Parameter	Units	ISIS, 2002				The present study		
		Mean	SEM	Samples	Animals	Mean	SEM	n
Chloride	mmol/L	104	0.368	118	69	105.08	1.46	36
Magnesium	mmol/L	0.971	0.057	14	12	1.69	0.50	20
Body temperature:	°C	38.8	0.197	37	17	38.39	0.15	16

Table 3.17 Comparison between the concentration of chloride, magnesium and body temperature reported by ISIS, 2002 data base and the present study.

For phosphorus, osmolality, urea, glucose and free triiodothyronine ( $T_3$ ) that differed significantly between males and females, each sex has its own reference value (Table 3.12, A). Reference values for total protein, albumin and free thyroxine ( $T_4$ ), differed significantly between oryx captured and blood sampled within 2 min and those sampled for which sampling took longer. However, these values are very close to the combined mean values presented in Table 3.11 and use of these data as reference values might be satisfactory.

The values of sodium and cortisol (Table 3.12, C) showed significant variability between males and females and in some cases between fast and slowly captured and blood sampled oryx. The concentration of sodium within female oryx had similar values regardless of the duration between capture and sampling (Table 3.12 C). Consequently, the combined value for female oryx (Table 3.8)  $140.83 \pm 1.55$  mmol/L ( $n = 12$ ) can be used as a reference value. For male Arabian oryx, the values differed significantly between fast ( $162.50 \pm 6.22$  mmol/L,  $n = 8$ ) and slowly ( $145.25 \pm 0.99$  mmol/L,  $n = 16$ ) captured and blood sampled oryx ( $P = 0.003$ ) (Table 3.12 C). It is not clear why the period between capture and blood sampling affected the concentration of sodium in male Arabian oryx, but not in females. Sodium is the major cation contributing to serum osmolality. In the present study, the osmolality was significantly higher in males than females as was the sodium concentration. Female Arabian oryx appear to be more stressed than males as indicated by the higher concentration of cortisol in females compared to males, regardless of the period between capture and blood sampling (Table 3.12 C). However, in both sexes the concentration of cortisol was lower after fast capture than after slow capture and blood sampling. The duration between capture and blood sampling was 122 seconds (2.03 min) in fast captured

oryx compared to 5-10 min in slowly captured ones. Romero and Reed (2005) found that serum glucocorticoids (corticosterone) in samples collected from reptiles and birds within 2-3 min are likely to reflect baseline concentrations or at worst are near baseline. For this reason, the cortisol concentrations obtained from fast captured oryx (within 2.03 min) are likely to provide a better indication of basal values for both sexes. Thus, these studies suggest that for physically captured oryx the reference baseline values of cortisol in males is  $73.25 \pm 15.41$  nmol/L (n = 8) and in females is  $100 \pm 15.41$  nmol/L (n = 8).

The speed of capture played a major role in obtaining a basal measurement for serum cortisol, but another important factor that might have played a role in lowering the serum cortisol measurement was familiarisation. In the present studies, 16 Arabian oryx (8 males and 8 females) were blood sampled on five occasions as detailed in Table 3.18. Before collection of blood samples no injections had been given to these animals.

Comparing the means cortisol in the repeated sampling (A, B, C, D and E, Table 3.18) for male and female oryx, by a two way repeated measures ANOVA, revealed no difference between males and females ( $P = 0.692$ ). Analysis of combined data for both sexes, showed a decrease in mean serum cortisol over time in the same animals housed in the same holding pen for an extended period of time (Table 3.18 and Figure 3.1). This suggests that animals are familiarised over time to the housing conditions and handling and therefore the response of the HPA axis to progressive handling significantly declined, as early as 2 weeks from the first capture (A versus C in Table 3.18 and Figure 3.1). After about 5 months and 3 months from the first capture, in males and females, respectively, the last capture of the 16 oryx (E, Figure 3.1) showed the lowest serum concentration of cortisol, which can be considered as the basal concentration. These data support the assertion that familiarisation of animals by frequent exposure to handling is likely to play a role in reducing the physiological response to handling over time and allow derivation of more valid reference data (Broom and Johnson, 1993a).

Sampling	Males	Females
A	09/09/2007	04/11/2007
B	16/09/2007	11/11/2007
C	24/09/2007	18/11/2007
D	12/01/2008	20/01/2008
E	03/02/2008	05/02/2008

Table 3.18 Dates of repeated sampling from 8 male and 8 female Arabian oryx.

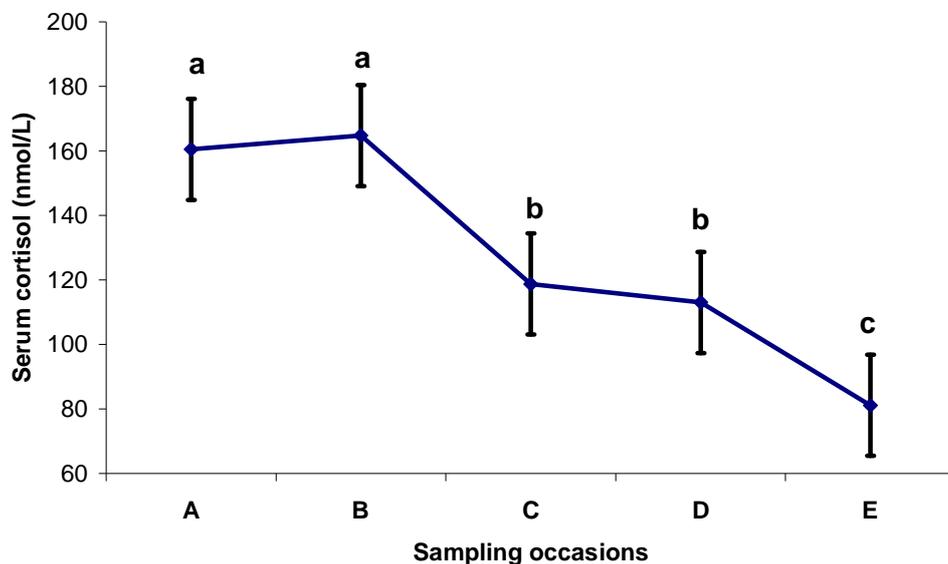


Figure 3.1 The serum cortisol concentration in repeated samplings from 16 Arabian oryx (8 males and 8 females) sampled as shown in Table 3.18. The cortisol values in different sampling occasions with similar small case letters are not significantly different from each other.  $P < 0.001$  except for occasion D versus E,  $P < 0.01$ , Two way repeated measured ANOVA, followed by Holm-Sidak multiple comparisons. Data are shown as mean  $\pm$  Standard error.

The rectal body temperature of Arabian oryx recorded in the present studies was  $38.39 \pm 0.15$  °C (mean  $\pm$  SEM,  $n = 16$ ), and was very close to the value  $38.4 \pm 1.3$  °C reported by Ostrowski et al. (2003) and close to  $38.9 \pm 0.6$  °C measured by Ancrenaz et al. (1996).

In the present study, there was significant difference between the serum glucose concentrations of male and female oryx ( $6.08 \pm 1.94$  mmol/L in males versus  $5.14 \pm 2.21$  mmol/L in females). A number of studies have reported the concentration of glucose in Arabian oryx, but for a mixed sex groups. Ostrowski et al. (2006) reported a lower glucose concentration of  $3.70 \pm 0.30$  mmol/L ( $n = 7$ ), while Kilgallon et al. (2008) and Vassart and Greth (1991) reported higher glucose concentrations of  $10.24 \pm 2.27$  mmol/L ( $n = 19$ ), and  $10.82 \pm 0.83$  mmol/L ( $n = 73$ , immobilised) respectively. Finally, the ISIS database gives a value of  $7.99 \pm 3.11$  mmol/L ( $n = 163$  samples from 98 animals). The concentration of glucose for merged sexes in the present study was  $5.76 \pm 2.05$  mmol/L. The concentration of circulating glucose is influenced by acute stress, and secretion of glucocorticoids and catecholamines increase the glucose in the blood (Steffens and de Boer, 1999). The animals in the present study and in the study of Ostrowski et al. (2006) were exposed to many occasions of handling and capture, before collection of the samples used in calculating reference values. In contrast, (Vassart and Greth, 1991; Kilgallon et al., 2008) used naïve animals, captured for the first time and this is likely to explain the higher concentrations of circulating glucose. Familiarisation of animals to handling might play a role in reducing the level of stress in oryx, as implied by the lower concentration of glucose in familiarised animals compared to unfamiliar ones.

The establishment of reference values for Arabian oryx that considers the differences between males and females has importance for future monitoring of the well-being of this endangered species. The effects of tranquillisation and immobilisation on physiological, biochemical and haematological parameters are discussed further in chapters 4 and 5, respectively.

### **3.5 Summary and conclusions**

In summary, in this chapter reference values and inter-percentile ranges (2.5 and 97.5 percentiles) were established for the first time in Arabian oryx captured without chemical

immobilisation and sex differences and the speed of capture were taken into consideration. The parameters that were included were haematological (WBC and percent neutrophils, lymphocytes, monocytes, eosinophils, basophils, RBC, haemoglobin, haematocrit, MCV, MCH, MCHC and platelet count), serum ions (concentrations of sodium, potassium, chloride, calcium, magnesium and phosphorus), serum osmolality, biochemical parameters (serum concentrations of urea, glucose, total protein, albumin and plasma lactate), serum hormone concentrations (cortisol, free thyroxine, free triiodothyronine and insulin) and clinical parameters (body temperature, heart and respiratory rates). The reference values and the percentile ranges established will help in diagnosis of health related abnormalities in populations of Arabian oryx.

The white blood cell count and percent neutrophils, lymphocytes and basophils differed significantly between males and females. The white blood cells and platelets of Arabian oryx are similar in shape to those of humans, but red blood cells are smaller in size in Arabian oryx with a similar biconcave shape to human erythrocytes. The serum concentrations of phosphorus, urea, glucose, free triiodothyronine and serum osmolality differed significantly between males and females.

The concentrations of total protein, albumin and free thyroxine differed significantly between oryx captured relatively quickly and sampled within 2 min, and those captured slowly and blood sampled within 5-10 min. The concentration of sodium was similar in females regardless of capture speed, but was higher in fast captured and sampled males than when capture and sampling took 5 to 10 min. The concentration of serum cortisol was higher in females than males. Importantly serum cortisol was affected by capture and sampling time in both sexes, and was higher when the total time was 5 to 10 min than when samples were obtained within a maximum of 2 min. Therefore, to obtain realistic measurements of circulating cortisol, samples need to be obtained rapidly.

## Chapter 4

### Investigations of the effects of the tranquilliser, perphenazine enanthate on Arabian oryx

#### 4.1 Introduction

Tranquillisation is a useful tool for wildlife management. The term tranquillisation is used to describe the state when animals have been given a chemical that renders them calm (quiet), indifferent to their surroundings and they do not mind being in a place that is strange and unfamiliar, but are still aware of what is going around them (Ebedes et al., 1996). The adoption of human long acting tranquillisers in wildlife management practices was a major advance in promoting welfare (Swan, 1993). These chemicals significantly reduced the number of animals lost during long-distance transportation, in captivity, and following introduction into new habitats (Diverio et al., 1996a).

Tranquillisers are used in wildlife to reduce stress for example in impala (*Aepyceros melampus*) (Gandini et al., 1989), cheetah (*Acinonyx jubatus*) (Huber et al., 2001) and Eurasian otter (*Lutra lutra lutra*) (Ventura et al., 2002), and promote welfare for example in roe deer (*Capreolus capreolus*) (Diverio et al., 1996b). Long acting tranquillisers have been used to induce calmness to enable easy handling, for example of impala (Gandini et al., 1989), horses (McCrinkle et al., 1989), roe deer (Dehnhard et al., 2001) and blue wildebeest (*Connochaetes taurinus*) (Fick et al., 2006). Tranquillisers are also used to eliminate fear and anxiety in captured animals and to prevent the occurrence of capture myopathy which in most cases is not treatable (Ebedes et al., 1996). Capture myopathy is characterised by recumbency or immobility when muscle masses disrupt during handling or violent exercise (Blood and Studdert, 1990).

There are different types of tranquillisers used for wildlife and these are categorised based on the similarities of their chemical structures and the duration of action (Read, 2002). These categories have been extensively reviewed by Ebedes (1993a) and Swan (1993). Examples of tranquillisers are phenothiazine derivatives (e.g. acepromazine, propionyl

promazine, chlorpromazine and promazine), butyrophenones (e.g. azaperone) and long acting tranquillisers (e.g. perphenazine enanthate, zuclopenthixol and pipothiazine).

Perphenazine enanthate is a long-acting tranquilliser that belongs to the phenothiazines (Swan, 1993). This chemical is widely used by psychiatrists to treat schizophrenia in humans (Kampman and Leppäluoto, 1970; Bohacek, 1986; David et al., 2005), but is also commonly used for wildlife tranquillisation (Read, 2002). Perphenazine enanthate has a long-acting effect, because it is prepared in an oily solution and forms a tissue depot after intramuscular administration, from which the chemical is slowly released into the blood circulation (Swan, 1993). The onset of action of perphenazine enanthate takes place about 10-12 h after injection, with a peak effect often reported on the third day, and effects that last up to 7 to 10 days after injection (Burroughs, 1993; Ebedes, 1993a; Portas, 2004; Pedernera et al., 2007).

The use of long-acting tranquillisers such as perphenazine enanthate has been recommended for safer restraint and handling of wild and captive wildlife alike (Huber et al., 2001; Portas, 2004). For example, perphenazine enanthate has been widely used to facilitate transport of animals (Ebedes, 1993b; Ancrenaz et al., 1995; Fowler, 2001; Fernandez-Moran et al., 2004; Portas, 2004), to facilitate assisted reproduction procedures (Gonzalez et al., 2008) and to ease the handling of animals in auctions after game capture, for example in South Africa (Ebedes et al., 1996).

Despite their advantages, there is a concern regarding the use of long-acting tranquillisers. An overdose of perphenazine enanthate might result in extra-pyramidal signs that should be monitored. Extra-pyramidal symptoms are a group of clinical disorders marked by abnormal involuntary movements, alterations in muscle tone and postural disturbances (Blood and Studdert, 1990). Fortunately, these symptoms can be treated successfully by various chemicals, such as biperiden (Akineton) and diazepam (Valium) as described by Ebedes (1993a) and Read (2002).

Tranquillisation by injection with perphenazine enanthate resulted in safer transport of Arabian oryx in Saudi Arabia compared to transportation without tranquillisation when some oryx were lost (Ancrenaz et al., 1995). However, the effects of this chemical have not been evaluated in Arabian oryx. Therefore, the studies described in this chapter aimed to

evaluate the effects of injection with perphenazine enanthate on haematological, biochemical and clinical parameters of the Arabian oryx.

## **4.2 Materials and methods**

### **4.2.1 Injection of perphenazine enanthate**

Adult Arabian oryx (8 males and 8 non-pregnant females) with weights ranging from 37 to 77 kg were used to evaluate the effects of perphenazine enanthate. The method of capture of the oryx and holding pens are described in Chapter 3 (section 3.2.2).

The Arabian oryx were tranquillised in the morning between 7 and 8 am by intramuscular injection of 100 mg of perphenazine enanthate, giving a mean ( $\pm$  SEM) dose of  $2.11 \pm 0.12$  mg/kg (see the list of animals in Table 4.1) (Decentan Depot, 100 mg/ml, Merck, Germany). Blood samples were collected (as described in section 3.2.4) immediately before injection (0 h) and then 3 h, 24 h, 72 h and 7 days after injection. After the collection of each blood sample, the respiratory rate, heart rate and body temperature were measured, as described in section 3.2.3. Faecal samples were also collected (as described in section 2.2.4) at time points 0 h, 24 h, 72 h, and 7 days. During the handling, some *ad hoc* notes about the behaviour of oryx were recorded at time points 0 h, 3 h, 24 h, 72 h and 7 days after perphenazine injection.

	<b>Sex</b>	<b>Weight (kg)</b>	<b>Dose (mg/kg)</b>
<b>1</b>	Male	77	1.30
<b>2</b>	Male	70.7	1.41
<b>3</b>	Male	65.8	1.52
<b>4</b>	Male	56.1	1.78
<b>5</b>	Male	54.6	1.83
<b>6</b>	Female	51.3	1.95
<b>7</b>	Male	50.9	1.96
<b>8</b>	Female	48.5	2.06
<b>9</b>	Male	48.4	2.07
<b>10</b>	Female	43.6	2.29
<b>11</b>	Female	42.3	2.36
<b>12</b>	Female	41.3	2.42
<b>13</b>	Female	40.6	2.46
<b>14</b>	Female	37	2.70
<b>15</b>	Female	35.4	2.82
<b>16</b>	Male	35.3	2.83

Table 4.1 List of the 16 Arabian oryx used for perphenazine enanthate experiment with body weight (kg) and dose (mg/kg), calculated retrospectively. The animals were weighed after the experiment due to unavailability of facilities during the experiment.

### 4.2.2 Injection of saline

For evaluation of the stress of handling and as a control for the injection treatment, the 8 male and 8 female oryx previously injected with perphenazine enanthate, were injected with 4 ml of saline (0.9 % NaCl, w/v), intra-muscularly. The Arabian oryx were injected with saline in the morning between 7 and 8 am. Blood samples were collected, as described in section 3.2.4, immediately before injection (0 h) and then 3 h, 24 h, 72 h and 7 days later. After the collection of each blood sample, the respiratory rate, heart rate and body temperature were measured, as described in section 3.2.3. Faecal samples were collected (as described in section 2.2.4) at time points 0 h, 24 h, 72 h, and 7 days. During the handling, some *ad hoc* notes about the behaviour of oryx were recorded at time points 0 h, 3 h, 24 h, 72 h and 7 days after saline injection.

The mean ambient temperatures during saline and perphenazine enanthate treatments are shown in Table 4.2. The oryx were injected with saline 2-3 months after tranquillisation treatment.

Treatment	Sex	0 h	3 h	24 h	72 h	7d	Start date	End date
Perphenazine	males	27	27	26.5	25	27	24/09/2007	01/10/2007
Perphenazine	females	22	22	22.5	22.5	21	18/11/2007	25/11/2007
<i>Mean</i>		<b>24.5</b>	<b>24.5</b>	<b>24.5</b>	<b>23.75</b>	<b>24</b>		
Saline	males	21.5	21.5	19.5	20.5	16	12/01/2008	19/01/2008
Saline	females	16.5	20.3	15.3	22	20	20/01/2008	27/01/2008
<i>Mean</i>		<b>19</b>	<b>20.9</b>	<b>17.4</b>	<b>21.25</b>	<b>18</b>		

Table 4.2 Mean ambient temperatures (°C) during the treatment of Arabian oryx with the tranquilliser, perphenazine enanthate or saline. The dates of these treatments are also shown.

### **4.2.3 Analysis of blood samples**

Blood samples were processed (section 0) and analysed for haematological, biochemical and hormonal parameters as described in Chapter 3 (sections 3.2.4, 3.2.7 and 3.2.8).

### **4.2.4 Analysis of faecal samples**

Faecal samples were extracted (section 2.2.5) and analysed with EIA I as described in Chapter 2 (section 2.2.8).

### **4.2.5 Statistics**

This chapter aims to evaluate the effects of perphenazine enanthate on haematological, biochemical, ions, hormones and clinical parameters compared to a control injection (saline). The data for the equal numbers of males and female oryx were combined for each parameter both in saline and perphenazine enanthate-injected oryx to avoid having a very small sample size.

Haematological, biochemical, hormonal and clinical data could not be successfully transformed to normality for a two-way repeated measures ANOVA with sampling time and procedure (saline or tranquiliser) as the two factors. Therefore, data obtained after saline-injection were analysed separately, from those after perphenazine enanthate injection by one-way repeated measures ANOVAs. These analyses examined differences between the time point 0 h in comparison to time points 3 h, 24 h, 72 h and 7 days, for each parameter, and where significant were followed multiple comparisons tests (Holm-Sidak method or Tukey tests after Friedman repeated measures ANOVA on ranks).

In each set of data, some parameters were transformed to achieve normality. For the data set linked to tranquillisation treatment, the following transformations were applied: natural log, ln, (albumin), rank (urea, glucose, monocytes and haematocrit), exponential (red blood cell count) and square (mean corpuscular haemoglobin concentration). Data for white blood cell count, percentage basophils, haemoglobin, mean corpuscular haemoglobin, serum cortisol, free T<sub>3</sub>, heart rate, body temperature and respiratory rate could not be transformed to normality and were tested with Friedman repeated measures ANOVA on ranks, followed by Tukey's test for multiple comparisons. For data linked to saline treatment, the following

transformations were applied: natural log, ln, (albumin and the percentage neutrophils), square root (percentage eosinophils) and rank (mean corpuscular volume and body temperature). Percentage monocytes, red blood cell count, serum cortisol and free T<sub>3</sub> data in saline-injection experiment could not be transformed to normality and were tested with Friedman repeated measures ANOVA on Ranks, followed by Tukey's test for multiple comparisons.

Data for faecal glucocorticoid metabolites after injection of saline or tranquilliser could not be successfully transformed to normality for a two-way repeated measures ANOVA. Consequently, each data set (saline-injection or tranquiliser injection) were transformed to normality using natural log (ln) and analysed with separate one-way repeated measures ANOVAs to investigate differences between time points 0 h, 24 h, 72 h and 7 days. When ANOVA showed significance differences were located using multiple comparisons by the Holm-Sidak method.

All oryx received 100 mg of perphenazine enanthate regardless of body weight, which was subsequently determined. Since the male oryx were generally heavier, they generally received a lower dose than the female oryx (see Table 4.1). Although the range of doses per kg of males oryx (1.30 to 2.83 mg/kg) and female oryx (1.95 to 2.82 mg/kg) overlapped in a few cases; the bias with respect to sex precluded comparison of dose related effects in males and females. Therefore, correlation analysis for combined sexes, between the dose received and the magnitude of effect for haematological, biochemical, hormonal and clinical parameters at different time points, was carried out using Pearson Product Moment Correlation test for normally distributed data and Spearman Rank Order Correlation for nonparametric data.

### 4.3 Results

Most haematological, biochemical, hormonal and clinical parameters (27 out of 31) showed no significant correlation or correlation at only one or two time points with the dose of perphenazine enanthate (mg/kg) injected (Table 4.3). Of these, eighteen parameters showed no correlation at all and six parameters showed correlation at only one time point (potassium, calcium, glucose, heart rate, respiratory rate and white blood cells count). Two parameters (basophils and free T<sub>3</sub>) showed correlation at two time points. However, haemoglobin, haematocrit, MCV and MCH showed a strong negative and significant correlation at all time points or all except one time point for MCH (Table 4.3).

Parameter	Unit	3 h	24 h	72 h	7 days
WBC	$\times 10^9/L$	-0.237	<b>-0.572*</b>	-0.346	-0.427 <sup>a</sup>
		0.376	<b>0.033</b>	0.189	0.096
		16	<b>14</b>	16	16
Neutrophils	%	0.00205	-0.161	-0.093	-0.251
		0.994	0.582	0.731	0.348
		16	14	16	16
Lymphocytes	%	-0.196	0.176	-0.181	0.208
		0.468	0.547	0.503	0.440
		16	14	16	16
Monocytes	%	0.162 <sup>a</sup>	-0.330 <sup>a</sup>	-0.036 <sup>a</sup>	0.157 <sup>a</sup>
		0.541	0.238	0.891	0.556
		16	14	16	16
Eosinophils	%	-0.312 <sup>a</sup>	-0.188	-0.125	-0.371 <sup>a</sup>
		0.233	0.519	0.645	0.153
		16	14	16	16
Basophils	%	<b>0.632</b> <sup>*,a</sup>	0.188	<b>0.591*</b>	0.235
		<b>0.008</b>	0.520	<b>0.016</b>	0.381
		<b>16</b>	14	<b>16</b>	16
RBC	$\times 10^{12}/L$	-0.169	-0.309	-0.341	-0.027 <sup>a</sup>
		0.532	0.282	0.197	0.917
		16	14	16	16
Haemoglobin	g/dL	<b>-0.542*</b>	<b>-0.566*</b>	<b>-0.628*</b>	<b>-0.519*</b>
		<b>0.030</b>	<b>0.035</b>	<b>0.009</b>	<b>0.039</b>
		<b>16</b>	<b>14</b>	<b>16</b>	<b>16</b>
Haematocrit	%	<b>-0.551*</b>	<b>-0.617*</b>	<b>-0.665*</b>	-0.477 <sup>a</sup>
		<b>0.027</b>	<b>0.019</b>	<b>0.005</b>	0.060
		<b>16</b>	<b>14</b>	<b>16</b>	16
MCV	fL	<b>-0.723*</b>	<b>-0.642*</b>	<b>-0.665*</b>	<b>-0.671*</b>
		<b>0.002</b>	<b>0.013</b>	<b>0.005</b>	<b>0.004</b>
		<b>16</b>	<b>14</b>	<b>16</b>	<b>16</b>
MCH	pg/cell	<b>-0.665*</b>	-0.532	<b>-0.518*</b>	<b>-0.536*</b> , <sup>a</sup>
		<b>0.005</b>	0.0501	<b>0.040</b>	<b>0.032</b>
		<b>16</b>	14	<b>16</b>	<b>16</b>
MCHC	g/dL	0.236	0.214	0.290	0.111 <sup>a</sup>
		0.380	0.462	0.277	0.672
		16	14	16	16
Platelets	$\times 10^9/L$	0.194 <sup>a</sup>	0.270 <sup>a</sup>	-0.206 <sup>a</sup>	-0.126 <sup>a</sup>
		0.463	0.340	0.436	0.632
		16	14	16	16
Sodium	mmol/L	0.0114	0.137	0.350	0.263
		0.968	0.614	0.201	0.325
		15	16	15	16
Potassium	mmol/L	0.372	<b>0.508*</b>	0.347	0.475
		0.172	<b>0.045</b>	0.206	0.063
		15	<b>16</b>	15	16
Chloride	mmol/L	0.0124	0.111	0.229	0.228
		0.965	0.683	0.412	0.397
		15	16	15	16
Calcium	mmol/L	0.102	0.169	<b>0.564*</b>	0.316
		0.719	0.531	<b>0.029</b>	0.233
		15	16	<b>15</b>	16

Parameter	Unit	3 h	24 h	72 h	7 days
<b>Phosphorus</b>	<i>mmol/L</i>	0.162	0.433	0.300	0.399
		0.564	0.094	0.277	0.126
		15	16	15	16
<b>Magnesium</b>	<i>mmol/L</i>	-0.442	-0.367	0.160	0.079
		0.099	0.162	0.569	0.771
		15	16	15	16
<b>Glucose</b>	<i>mmol/L</i>	0.315	0.296	<b>0.590*</b>	0.473
		0.252	0.265	<b>0.020</b>	0.064
		15	16	<b>15</b>	16
<b>Urea</b>	<i>mmol/L</i>	-0.150	-0.438	-0.162	-0.172
		0.594	0.090	0.565	0.524
		15	16	15	16
<b>Total protein</b>	<i>g/L</i>	-0.174	-0.138	0.224	-0.026
		0.534	0.610	0.422	0.925
		15	16	15	16
<b>Albumin</b>	<i>g/L</i>	0.010	0.003	0.336 <sup>a</sup>	0.187
		0.973	0.990	0.214	0.489
		15	16	15	16
<b>Lactate</b>	<i>mmol/L</i>	0.0209	0.007	0.119	-0.093
		0.961	0.988	0.799	0.827
		8	8	7	8
<b>Body temperature</b>	$^{\circ}\text{C}$	0.052 <sup>a</sup>	-0.088	0.496	0.268
		0.839	0.747	0.051	0.317
		16	16	16	16
<b>Heart rate</b>	<i>bpm</i>	<b>0.741*</b>	0.193	0.184	0.162
		<b>0.001</b>	0.474	0.495	0.550
		<b>16</b>	16	16	16
<b>Respiratory rate</b>	<i>bpm</i>	<b>0.510*</b>	-0.197 <sup>a</sup>	0.031	-0.103
		<b>0.044</b>	0.456	0.910	0.703
		<b>16</b>	16	16	16
<b>Cortisol</b>	<i>nmol/L</i>	-0.0481	0.136	0.129	0.260
		0.860	0.615	0.633	0.349
		16	16	16	15
<b>Free T<sub>4</sub></b>	<i>pmol/L</i>	0.285	0.338	0.306	-0.588 <sup>a</sup>
		0.370	0.217	0.250	0.067
		12	15	16	10
<b>Free T<sub>3</sub></b>	<i>pmol/L</i>	<b>0.509*</b> <sup>a</sup>	0.322	<b>0.532*</b>	0.111 <sup>a</sup>
		<b>0.043</b>	0.224	<b>0.034</b>	0.686
		<b>16</b>	16	<b>16</b>	15
<b>insulin</b>	<i>mIU/L</i>	-0.289	0.090 <sup>a</sup>	0.395	0.226
		0.278	0.738	0.130	0.418
		16	16	16	15

Table 4.3 The correlation between the dose of the tranquilliser perphenazine enanthate and the data for haematological, biochemical, hormonal and clinical parameters. All statistical analyses are shown as the correlation coefficient (Pearson's  $r$ , unless designated by <sup>a</sup> where Spearman's  $\rho$ ). Each cell shows, from top to bottom, (correlation coefficient, P value and  $n$  = number of samples). \* Asterisks indicate statistically significant correlations. Not that the number of samples,  $n$ , is sometimes less than 16 when samples could not be obtained from all animals. WBC, white blood cells; RBC, red blood cells; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration,  $T_4$ , thyroxine and  $T_3$ , triiodothyronine.

#### **4.3.1 Behavioural observations**

Although no systematic behavioural observations were made during this study, some differences in behaviour were clear from casual observations. Some behavioural effects of perphenazine enanthate on Arabian oryx were observed on *ad hoc* basis. Before the injection, the oryx were nervous and fearful and were observed pacing back and forth within the holding pens when the team approached the holding pen area. Shortly following the injection of perphenazine enanthate the oryx became excited with more pacing. Twenty-four hours later, the oryx were observed to be calm, indifferent to their surroundings, and showed no pacing movements. In contrast, saline injected oryx showed full active movements and were pacing back and forth, and were very nervous, excited during capture at all sampling time points. Tranquillised oryx could be captured directly by hand, without using the plywood board that was usually used for capturing non-tranquillised oryx in the holding pens. Seventy-two hours after the injection, in addition to what was observed 24 h after injection, tranquillised oryx seemed to have lost a considerable amount of hair, which was clearly seen on the floor of the holding pens. On the seventh day after tranquillisation with perphenazine enanthate, the oryx had started to recover from the effects of tranquillisation by showing moderate pacing. However, a few oryx could be captured without using the plywood board. No extra-pyramidal symptoms

were observed following the injection of perphenazine enanthate. Food and water consumption did not change after tranquillisation.

Perphenazine enanthate is long-acting tranquilliser, with a very slow rate of release from the site of injection. The onset of action starts at about 12 h and the peak effects take place about 3 days after intramuscular injection (Ebedes and Burroughs, 1992). The duration of action of perphenazine enanthate was reported to last up to 7-10 days (Ebedes, 1993a; Swan, 1993). In the present study, the 24 h and 72 h time points after injection with perphenazine enanthate are likely to be the time points when haematological, biochemical and physiological changes induced by the tranquilliser take place. The 3 h time point is probably too early to see changes and the 7 day time point too late to see significant changes due to the tranquilliser. Therefore, more focus in the discussion will be on the time points 24 and 72 h than 3 h and 7 day time points. In the present study, some oryx showed signs of recovery on the 7<sup>th</sup> day post-injection with perphenazine enanthate such as increased frequency of pacing.

#### **4.3.2 Body temperature, heart and respiratory rates**

In oryx injected with perphenazine enanthate, the body temperature was significantly lower at 24 and 72 h after injection in comparison to the time point 0 h ( $P < 0.05$ , Friedman repeated measures ANOVA, Tukey's test, Table 4.4). There was no apparent correlation between body temperature and the dose of perphenazine enanthate received (Table 4.3), which varied between 38.33 and 39.90. In oryx injected with saline, the body temperature increased significantly at 3 h but not at other time points (Table 4.4).

The heart rate showed no significant difference after injection of the tranquilliser or saline (Table 4.4). The respiratory rate was significantly lower at 72 h after injection of saline and tranquilliser, but not after 3 h, 24 h or 7 days compared to pre-injection control data at 0 h (Table 4.4).

The body temperature, heart and respiratory rates at time points 3 h, 24 h, 72 h and 7 days of oryx injected with saline were within the inter-percentile range established in Chapter 3 (Table 3.11) except for mean body temperature which was higher at time point 3 h (Table 4.4).

Parameter	Treatment	0 h		3 h		24 h		72 h		7 days	
		mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM
Body temperature	<i>saline</i>	38.91	0.17	<b>39.43**</b>	0.12	38.94	0.14	38.95	0.15	39.03	0.14
°C	<i>perphenazine</i>	39.46	0.24	39.90	0.17	<b>38.46*</b>	0.18	<b>38.33*</b>	0.15	38.84	0.14
Heart rate	<i>saline</i>	100.88	4.74	102.13	4.20	90.63	4.63	94.25	3.46	99.25	3.14
Bpm	<i>perphenazine</i>	101.25	3.35	104.63	4.62	105.38	4.75	102.94	5.17	102.00	4.53
Respiratory rate	<i>saline</i>	38.63	1.84	40.25	2.51	30.50	2.65	<b>30.19**</b>	2.27	37.38	3.04
Bpm	<i>perphenazine</i>	38.81	3.37	48.94	6.54	27.69	2.69	<b>25.81*</b>	2.09	31.63	1.86

Table 4.4 Clinical parameters of 16 Arabian oryx that were injected with saline and perphenazine enanthate at time point 0 h. SEM, standard error of mean. Asterisks and **bold font** indicate statistically significant different from time point 0 h, \* P < 0.05, Friedman repeated measures ANOVA, Tukey's test. \*\* P < 0.05, one way repeated measured ANOVA followed by Holm-Sidak multiple comparisons.

Parameter	Treatment	0 h		3 h		24 h		72 h		7 days	
		mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM
Cortisol	<i>saline</i>	113.00	8.72	113.13	7.03	104.27	10.88	99.81	7.82	99.80	9.27
nmol/L	<i>perphenazine</i>	118.75	10.28	134.69	15.97	109.81	8.71	<b>92.81*</b>	8.31	98.27	5.38
Free T <sub>4</sub>	<i>saline</i>	11.38	0.69	<b>14.50**</b>	0.69	11.95	0.71	10.34	0.44	13.24	0.43
pmol/L	<i>perphenazine</i>	9.39	0.41	<b>12.38**</b>	0.42	<b>11.38**</b>	0.43	10.94	0.51	10.33	0.64
Free T <sub>3</sub>	<i>saline</i>	4.78	0.35	4.54	0.25	4.70	0.29	4.52	0.55	4.78	0.33
pmol/L	<i>perphenazine</i>	3.49	0.20	3.38	0.19	3.40	0.22	3.54	0.25	3.47	0.27

Table 4.5 Hormonal parameters of 16 Arabian oryx that were injected with saline and perphenazine enanthate at time point 0 h. SEM, standard error of mean, T<sub>4</sub>, thyroxine and T<sub>3</sub>, triiodothyronine. Asterisks and bold font indicate statistically significant different from time point 0 h, \* P < 0.05, Friedman repeated measures ANOVA, Tukey's test, \*\* P < 0.05, One way repeated measures ANOVA followed by Holm-Sidak multiple comparisons.

### 4.3.3 Hormones

In all animals, serum cortisol did not change significantly after saline injection and remained within the inter-percentile range established in Chapter 3 (Table 3.11), but in oryx injected with perphenazine enanthate, serum cortisol was significantly lower 72 h after injection, but not at 3 h, 24 h and 7 days ( $P < 0.05$ , Friedman repeated measures ANOVA, Tukey's test, Figure 4.1 and Table 4.5). There was no apparent correlation between serum cortisol and the dose of perphenazine enanthate received (Table 4.3), which varied between 92.81 and 134.69.

The concentration of free thyroxine ( $T_4$ ) was significantly higher at time point 3h and 24 h but not at 72 h and 7 days than at time point 0 h in oryx injected with perphenazine enanthate ( $P = 0.001$ , one way repeated measures ANOVA, Table 4.5). The concentration of free thyroxine increased significantly at 3 h but not at 24 h, 72 h and 7 days compared to 0 h, for oryx injected with saline (Table 4.5).

The concentration of free triiodothyronine ( $T_3$ ) showed no statistical difference between 0 h and the time points 3 h, 24 h and 72 h after injection of perphenazine and saline (Table 4.5). The means of serum cortisol in oryx injected with saline at time points (0 h, 3 h, 24 h, 72 h and 7 days) (Table 4.5) were within the inter-percentile range established in Chapter 3 (Table 3.11). The thyroid hormones (free thyroxine and triiodothyronine) at all time points were within the inter-percentile range of Chapter 3 (Table 3.11) except that free thyroxine was higher at time points 3 h and 7 days.

Faecal glucocorticoid metabolites increased significantly one day after injection of either saline or perphenazine enanthate, but were not significantly elevated at day 3 or after 7 days (Figure 4.2).

### 4.3.4 Haematology

As the onset of behavioural effects of perphenazine enanthate, starts 10-12 h after injection and the peak effect, takes place about 72 h after injection, data at the time points 24 h and 72 h are most likely to reflect any effects of the injection of perphenazine enanthate, while data for blood samples collected 3 h after injection are likely to reflect the effects of

injection and handling. To investigate the effects of the long-acting tranquilliser, data for time points 24 h and 72 h were compared to the data collected at 0 h, by one way repeated measures ANOVAs, in oryx injected with perphenazine enanthate.

The haematological results for oryx injected with perphenazine enanthate and saline, are shown in Table 4.6. The white blood cell count increased significantly at time point 3 h and 24 h in saline injected oryx and at time point 3 h in perphenazine injected ones but not at other time points, compared to 0 h (Table 4.6). The percent neutrophils increased significantly at time point 3 h in saline injected oryx but not at other time points (Figure 4.3). In oryx injected with perphenazine enanthate, the percent neutrophils increased significantly at 3 h and 24 h and decreased significantly at 72 h and 7 days (Table 4.6). The percentage of lymphocytes decreased in both treatments at 3 h after injection (Table 4.6). The percent lymphocytes also increased significantly at 7 days after injection with perphenazine enanthate (Table 4.6). There were no changes in the percent monocytes in both saline and perphenazine injected oryx (Table 4.6). The percent of eosinophils did not differ in saline injected oryx at all time points in comparison to 0 h. However, there was a significant decrease at time points 3 h and a significant increase at 7 days after injection. The percentage of basophils did not change in perphenazine injected oryx at all time points compared to 0 h. While in those injected with saline, there was a significant decrease at 72 h and 7 days after injection.

In saline injected oryx, the red blood cell count decreased slightly but significantly at 7 days after injection, while in perphenazine injected oryx erythrocyte count declined significantly at 3 h, 24 h, 72 h and 7 days (Figure 4.3 and Table 4.6). There was no significant correlation between the red blood cell count and the perphenazine dose, over the dose range employed (1.30 to 2.83 mg/kg body weight, Table 4.1), at any time point (Table 4.3). For haemoglobin, there was a small but significant decrease in saline injected oryx at 72 h after injection, while in perphenazine injected oryx, the haemoglobin declined significantly at 24 h and 72 h but not 3 h and 7 days after injection (Figure 4.4 and Table 4.6). There were small but statistically significant decreases in haematocrit at 72 h and 7 days after saline injection, whereas in perphenazine injected oryx there were dramatic decreases at 3 h, 24 h, 72 h and 7 days after injection (Figure 4.5 and Table 4.6) and a

significant negative correlation between the injected dose of perphenazine and the haematocrit at all time points (Table 4.3).

The mean corpuscular volume (MCV) did not change in saline-injected oryx but significantly declined at 3 h, 24 h and 72 h and increased later at 7 days after perphenazine injection. There was a negative correlation between the dose of perphenazine and MCV at all time points after injection (Table 4.3). The mean corpuscular haemoglobin (MCH) and the mean corpuscular haemoglobin concentration (MCHC) did not change after saline or perphenazine injection except for a small but significant increase in MCHC at 3 h after the injection of perphenazine enanthate. However, MCH was negatively correlated with the dose of perphenazine 3 h, 72 h and 7 days after injection (Table 4.3).

All haematological parameters measured in oryx injected with saline were within the inter-percentile ranges established in Chapter 3 (see Table 3.7)

#### **4.3.5 Biochemistry and ions**

The serum ions (sodium, chloride, potassium, calcium and phosphorus) and the serum biochemical parameters (glucose, urea, total protein and albumin) did not show statistically significant differences in oryx injected with perphenazine enanthate or saline between the time point 0 h and the two points 24 and 72 h ( $P > 0.05$ , one way repeated measures ANOVA, Table 4.7). The exceptions are only small but statistically significant decrease in potassium, at 7 days in saline injected oryx, a small but significant decrease in glucose at 7 days and also a small but significant increase in urea at 3 h in perphenazine injected oryx.

All serum ions and biochemical parameters measured at all time points (0 h, 3 h, 24 h, 72 h and 7 days) in oryx injected with saline (Table 4.7), were within the inter-percentile range established in Chapter 3 (Table 3.11).

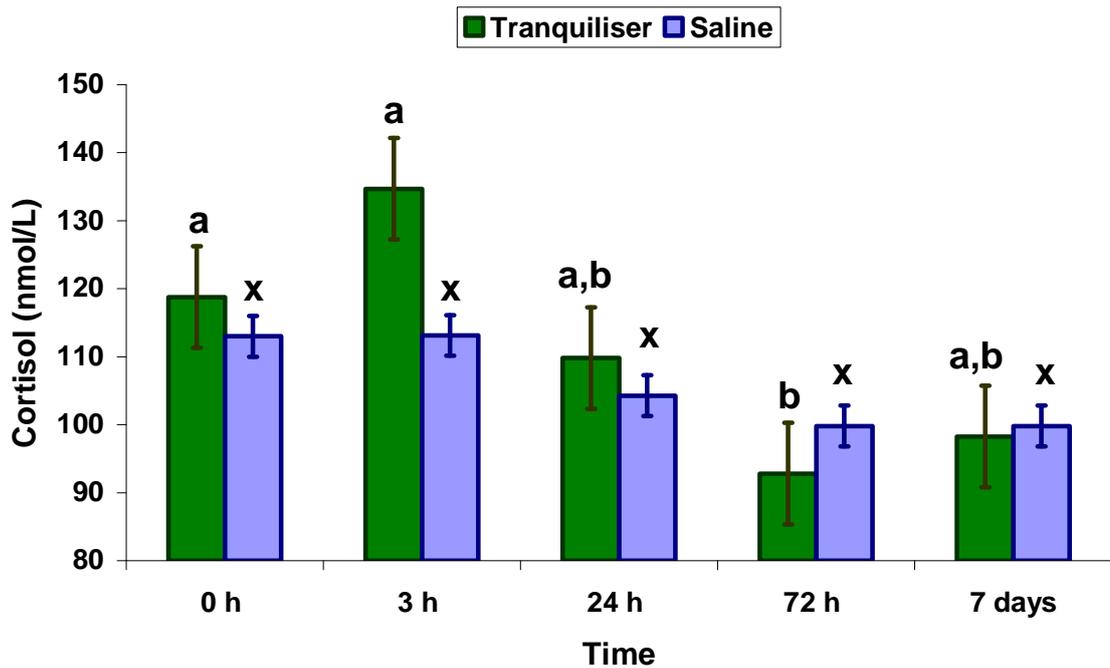


Figure 4.1 Serum cortisol measured in 16 Arabian oryx injected with the tranquilliser, perphenazine enanthate and saline. Data are presented as mean  $\pm$  standard errors. The time points that have similar small case letters are not significantly different from each other. Two separate (one way repeated measures ANOVA) tests were used for saline and perphenazine enanthate injections and each treatment has its own small case letters.

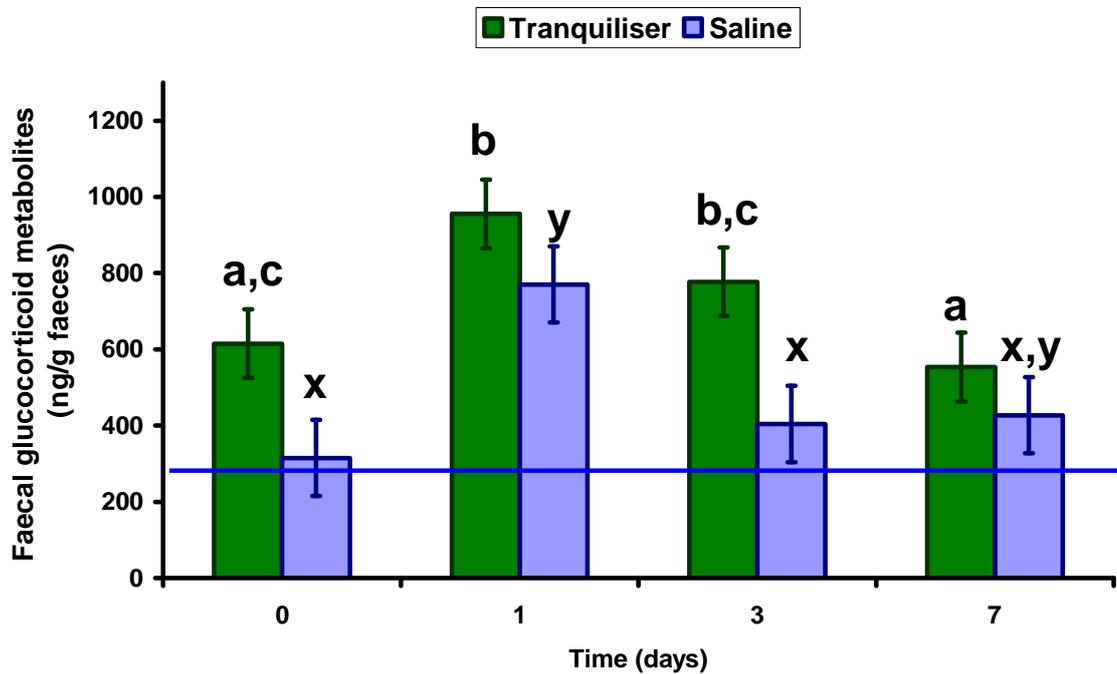


Figure 4.2 Faecal glucocorticoid metabolites in 16 Arabian oryx that were injected with the tranquilliser perphenazine enanthate or saline. Data are presented as mean  $\pm$  standard errors. The time points that have similar small case letters are not significantly different from each other. Two separate (one way repeated measures ANOVA) tests were used for saline and perphenazine enanthate injections and each treatment has its own small case letters. The blue line represents the baseline of faecal glucocorticoid metabolites (273 ng/g faeces), that was obtained from the ACTH experiment described in chapter 2 (section 2.3.1.1).

Parameter	Treatment	0 h		3 h		24 h		72 h		7 days	
		mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM
WBC x10 <sup>9</sup> /L	saline	7.58	0.30	<b>11.05**</b>	0.50	<b>9.55**</b>	0.41	7.26	0.55	8.37	0.52
	perphenazine	8.82	0.49	<b>11.18*</b>	0.44	9.61	0.49	7.76	0.38	7.02	0.51
Neutrophils %	saline	64.04	1.37	<b>71.93**</b>	1.16	70.13	0.67	68.74	2.28	69.83	2.05
	perphenazine	70.48	1.12	<b>76.95**</b>	1.16	<b>73.78**</b>	1.29	<b>67.09**</b>	1.17	<b>66.29**</b>	1.49
Lymphocytes %	saline	27.58	1.29	<b>21.38**</b>	1.18	21.91	1.09	23.10	1.88	22.79	1.95
	perphenazine	25.01	1.22	<b>19.10**</b>	1.06	22.16	1.29	27.18	1.13	<b>29.12**</b>	1.53
Monocytes %	saline	0.34	0.17	0.84	0.45	0.22	0.13	1.91	0.47	1.53	0.39
	perphenazine	0.08	0.05	0.13	0.06	0.23	0.09	0.02	0.01	0.18	0.14
Eosinophils %	saline	4.72	0.61	2.69	0.33	4.93	0.77	4.31	1.03	3.93	0.59
	perphenazine	0.98	0.17	<b>0.53**</b>	0.13	0.86	0.20	1.01	0.16	<b>1.52**</b>	0.24
Basophils %	saline	3.32	0.36	3.07	0.31	2.82	0.29	<b>1.95**</b>	0.46	<b>1.93**</b>	0.33
	perphenazine	3.43	0.34	3.30	0.39	3.00	0.33	4.74	0.54	2.89	0.41
RBC x10 <sup>12</sup> /L	saline	12.56	0.21	12.16	0.16	12.16	0.14	12.17	0.16	<b>12.03*</b>	0.21
	perphenazine	11.11	0.15	<b>10.70*</b>	0.18	<b>9.84*</b>	0.17	<b>9.84*</b>	0.15	<b>10.45*</b>	0.28
Haemoglobin g/dL	saline	18.30	0.23	17.67	0.23	17.76	0.23	<b>17.30**</b>	0.26	17.63	0.28
	perphenazine	15.81	0.31	15.39	0.34	<b>14.14*</b>	0.33	<b>14.19*</b>	0.27	15.51	0.33
Haematocrit %	saline	53.99	0.96	52.01	0.83	52.01	0.73	<b>50.19**</b>	0.95	<b>51.71**</b>	0.94
	perphenazine	47.66	0.90	<b>45.23**</b>	1.09	<b>41.69*</b>	0.99	<b>41.01*</b>	1.14	<b>46.11**</b>	0.93
MCV fL	saline	43.05	0.50	42.83	0.52	42.78	0.45	41.24	0.64	43.05	0.48
	perphenazine	42.91	0.58	<b>42.57**</b>	0.59	<b>41.94**</b>	0.57	<b>42.51**</b>	0.57	<b>43.20**</b>	0.59
MCH pg/cell	saline	14.60	0.17	14.58	0.14	14.63	0.18	14.21	0.16	14.68	0.17
	perphenazine	14.24	0.24	14.38	0.19	14.37	0.22	14.45	0.20	15.10	0.66
MCHC g/dL	saline	33.91	0.24	34.01	0.18	34.16	0.20	34.48	0.20	34.09	0.17
	perphenazine	33.20	0.50	<b>33.76*</b>	0.18	34.22	0.14	33.98	0.22	34.93	1.54

Table 4.6 Haematological parameters of 16 Arabian oryx injected with saline and perphenazine enanthate at time point 0 h. SEM, standard error of mean; WBC, white blood cells; RBC, red blood cells; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; and MCHC, mean corpuscular haemoglobin concentration. Asterisks and **bold** font style indicate significantly different than the time point 0 h, \*P < 0.05, Friedman repeated measures ANOVA, followed by Tukey's test. \*\* P < 0.05, one way repeated measured ANOVA followed by Holm-Sidak multiple comparisons.

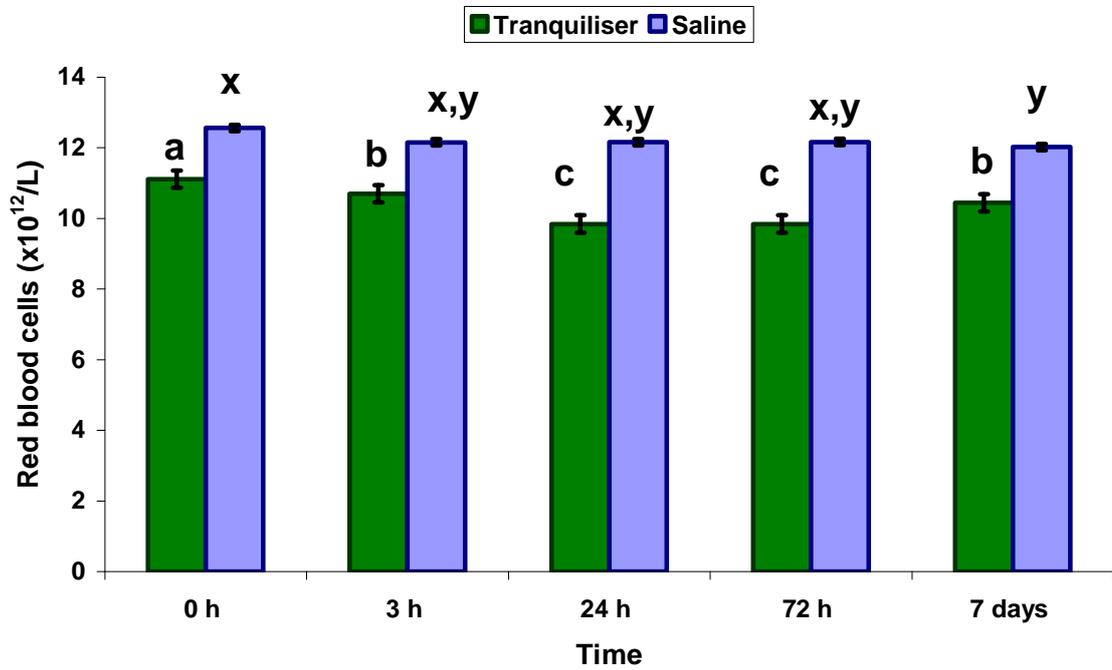


Figure 4.3 The red blood cell count in 16 Arabian oryx measured after injection of the tranquilliser, perphenazine enanthate and saline at time point 0 h. Data are presented as mean and standard errors. The time points that have similar small case letters are not significantly different from each other. Significant difference,  $P < 0.05$ , Friedman repeated measures ANOVA, Tukey's test.

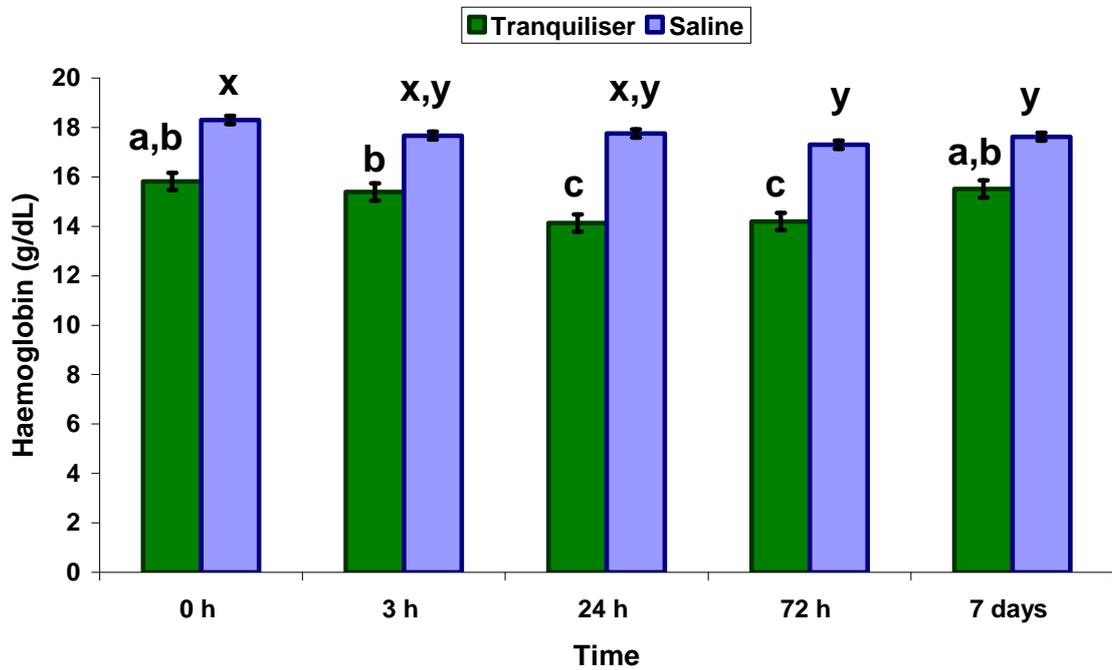


Figure 4.4 The concentration of haemoglobin in 16 Arabian oryx measured after injection of the tranquilliser, perphenazine enanthate and saline at time point 0 h. Data are presented as mean and standard errors. The time points that have similar small case letters are not significantly different from each other. Significant difference,  $P < 0.05$ , Friedman repeated measures ANOVA, Tukey's test.

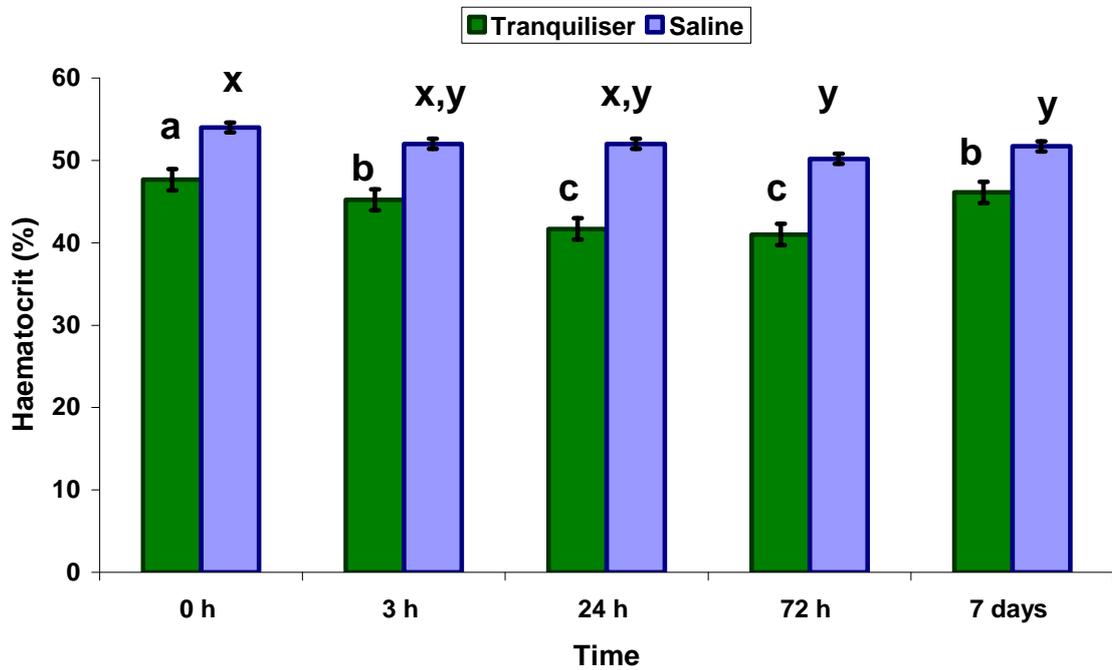


Figure 4.5 The haematocrit in 16 Arabian oryx measured after injection of the tranquilliser, perphenazine enanthate and saline at time point 0 h. Data are presented as mean and standard errors. The time points that have similar small case letters are not significantly different from each other. Significant difference,  $P < 0.05$ , Friedman repeated measures ANOVA, Tukey's test.

Parameter Unit	Treatment	0 h		3 h		24 h		72 h		7 days	
		mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM
Sodium Mmol/L	<i>saline</i>	164.00	11.55	158.80	8.43	157.14	8.90	179.44	8.56	159.60	8.37
	<i>perphenazine</i>	141.81	5.63	161.87	6.70	160.25	4.95	155.75	5.13	130.94	5.48
Potassium Mmol/L	<i>saline</i>	7.03	0.52	6.06	0.35	6.15	0.40	6.94	0.38	<b>6.21*</b>	0.31
	<i>perphenazine</i>	6.89	0.27	6.75	0.26	6.76	0.27	5.97	0.23	5.19	0.25
Chloride Mmol/L	<i>saline</i>	115.13	7.66	115.07	5.78	110.93	5.98	127.38	6.62	112.40	6.22
	<i>perphenazine</i>	103.56	3.76	119.87	4.96	118.38	3.46	113.25	3.59	96.06	3.91
Calcium Mmol/L	<i>saline</i>	2.64	0.23	2.47	0.15	2.40	0.13	2.80	0.14	2.49	0.13
	<i>perphenazine</i>	2.21	0.12	2.60	0.15	2.57	0.12	2.58	0.11	2.15	0.11
Phosphorus Mmol/L	<i>saline</i>	3.02	0.29	2.35	0.20	2.65	0.17	3.17	0.17	2.91	0.19
	<i>perphenazine</i>	2.33	0.12	2.37	0.16	2.47	0.14	2.57	0.15	2.12	0.14
Glucose Mmol/L	<i>saline</i>	5.61	0.48	5.39	0.40	5.50	0.44	5.55	0.28	5.51	0.35
	<i>perphenazine</i>	5.92	0.41	6.64	0.50	5.76	0.30	5.45	0.24	<b>4.57*</b>	0.33
Urea Mmol/L	<i>saline</i>	4.71	0.38	5.60	0.39	5.02	0.45	5.81	0.41	5.08	0.33
	<i>perphenazine</i>	5.45	0.25	<b>7.22*</b>	0.56	6.34	0.41	6.01	0.41	5.01	0.35
Total protein g/L	<i>saline</i>	74.67	5.75	69.53	3.22	70.07	4.36	79.31	3.54	72.53	3.61
	<i>perphenazine</i>	65.00	2.91	71.07	4.06	70.06	2.59	69.06	2.57	60.94	2.96
Albumin g/L	<i>saline</i>	52.27	5.01	48.80	3.75	49.21	3.54	57.75	4.02	50.00	2.58
	<i>perphenazine</i>	44.63	2.06	49.67	3.24	49.63	2.40	48.75	2.72	41.63	2.42

Table 4.7 Ions and biochemical parameters of 16 Arabian oryx that were injected with saline and perphenazine enanthate at time point 0 h. SEM, standard error of mean. Asterisks and **bold** font style indicate significantly different than the time point 0 h, \* P < 0.05, One way repeated measured ANOVA followed by Holm-Sidak multiple comparisons.

## 4.4 Discussion

### 4.4.1 Behavioural observations

The *ad hoc* collection of behavioural data showed some clear differences between the behaviour of oryx that received saline and those tranquillised with perphenazine enanthate. In future studies the use of systematic formal behavioural measurements would be desirable to obtain results that can be statistically validated.

The dose of perphenazine enanthate that was used initially by Ancrenaz et al. (1995) for Arabian oryx was 3 – 3.5 mg/kg, and resulted in dyskinesia (impairment of power of voluntary movement), akathisia and parkinsonism, which are extra-pyramidal symptoms. These were treated with 10 mg of diazepam (Valium) injected half intravenously and half intramuscularly and the symptoms halted immediately. Later, Ancrenaz et al. (1995) used a smaller dose of 2.5 – 3.5 mg/kg and just one male out of 17 showed some extra-pyramidal symptoms, but were treated with diazepam (Valium) and the symptoms were halted immediately. In the present study, a lower mean dose of 2.11 mg/kg than either of the earlier studies was used and no extra-pyramidal symptoms were observed. This dose was sufficient to make the oryx quiet and calm, and easy to handle. Calmness was also observed by many studies of other species that have used perphenazine enanthate at similar or lower doses. For example in impala (*Aepyceros melampus*) at 1.5-5.7 mg/kg (Gandini et al., 1989; Knox et al., 1990), horses at 0.5 mg/kg (McCrindle et al., 1989), gemsbok (*Oryx gazelle*) at 0.065 mg/kg (Burroughs et al., 2005) and roe deer (*Capreolus capreolus*) at 0.6 mg/kg (Dehnhard et al., 2001).

The loss of hair observed on the third day of the present study, after injecting oryx with perphenazine enanthate, was temporary. The animals recovered after about a week, and the hair loss would not be a major concern. Such an effect has not been previously reported in animals injected with perphenazine enanthate. However, in the present study, the holding pen was small (2.6 x 6.2 m) and therefore it was easy to notice the hair on the floor. When a larger area is used, it will not be as easy to notice this effect, so it is possible that the observation of hair loss was missed in previous studies.

The food and water consumption of the Arabian oryx was not affected by the tranquillisation with perphenazine enanthate, which agrees with the observations of Ancrenaz et al. (1995) for Arabian oryx and wildebeest (Fick et al., 2006). This is one of the reported advantages of using perphenazine enanthate as a long acting tranquilliser (Ebedes, 1993a).

#### **4.4.2 Body temperature, heart and respiratory rates**

There was no significant effect of perphenazine enanthate on heart rate of Arabian oryx. This observation agrees with the reported results in Mohor gazelles (*Gazella dama mhorr*) (Gonzalez et al., 2008). The respiratory rate decreased significantly at time point 72 h after the injection of perphenazine enanthate and saline by 33 % and 22 %, respectively (Table 4.4). As the decrease occurred in oryx that received saline, it cannot be an effect of the tranquilliser *per se*. This agrees with observations in Mohor gazelles in which perphenazine enanthate had no effect on the respiratory rate up to five days after injection (Gonzalez et al., 2008).

Although the ambient temperature during the perphenazine enanthate treatment was higher compared to the period during which the saline treatment took place (Table 4.2), there were significant decreases in body temperature at 24 h and 72 h after perphenazine enanthate injection. While, only a significant increase of body temperature occurred after saline injection at the 3 h time point, possibly due to handling. The reason that this effect was not seen in the oryx injected with the tranquilliser may be because the value at 0 h was already quite high and close to the value at 3 h after injection. Body temperature decreased significantly at the 24 and 72 h time points in comparison to the data collected at 0 h after perphenazine enanthate injection (Table 4.4). Although the decrease was small (1-1.3 °C), it was statistically significant. Some studies have reported the possible influence of perphenazine enanthate on the thermoregulatory mechanism of animals (Ebedes, 1993a; Swan, 1993). This occurs possibly through an interference with the set point of body temperature regulation at the hypothalamic level, resulting in thermal liability (Baldessarini, 1991), which could put animals at risk of hypo- or hyper-thermia at extreme cold or hot ambient temperatures, respectively (Swan, 1993). Possible interference of perphenazine enanthate with thermoregulation of animals during transportation was also

reported by Openshaw (1993). These reports about the effects of long acting tranquillisers on thermoregulation were unclear until recently, when Fick and others did extensive research on the effect of some tranquillisers (haloperidol, zuclopenthixol acetate, perphenazine enanthate and azaperone) on thermoregulation. Their studies found that tranquillisers including perphenazine enanthate do not impair thermoregulation of blue wildebeest (*Connochaetes taurinus*) (Fick et al., 2006), goats (*Capra hircus*) (Fick et al., 2007) and rats (Fick et al., 2005). Similarly, in Mohor gazelle (*Gazella dama mhorr*) (Gonzalez et al., 2008), no changes in body temperature were found in animals injected with perphenazine enanthate. Therefore, it can be concluded that the small decrease in body temperature seen in Arabian oryx in the present study does not reflect significant impairment of thermoregulation that can adversely put animals at risk of hyperthermia or hypothermia, depending on the ambient temperature. Despite this, to ensure good health, the body temperature should always be monitored when perphenazine enanthate is used.

In future studies the use of telemetric recording devices for recording body temperature, heart and respiratory rates would improve the results and allow more accurate conclusions to be obtained.

There were some differences in ambient temperature between saline and tranquilliser treatments and the most likely parameter to be influenced by the variation in ambient temperature is the body temperature, as Arabian oryx has been shown to use heterothermy (Hetem et al., 2010). In future studies, the use of a cross-over experimental design (see section 4.4.6) with some of the oryx receiving saline first and some receiving perphenazine first would eliminate the effect of variations such as ambient temperature from the statistical analysis of the results of the experiments.

#### **4.4.3 Hormones**

The decline in serum cortisol 72 h after injection of perphenazine enanthate (Figure 4.1) was probably an effect of the tranquilliser. This is in agreement with other studies that suggest a peak effect of perphenazine enanthate 3 days after injection (Ebedes, 1993a; Swan, 1993; Portas, 2004). The significant decline of cortisol 3 days after perphenazine enanthate injection indicates that the tranquilliser might have a possible role in reducing

cortisol secretion in Arabian oryx. However, 24 h after injection, there was no significant decline in the concentration of cortisol, which leaves this conclusion uncertain.

Previous studies have reported that in animals injected with long-acting tranquillisers, serum cortisol tends to return to basal level more rapidly compared to animals not injected with long-acting tranquillisers, for example perphenazine enanthate in red deer (Diverio et al., 1996b). Ebedes and Raath (1999) reported a reduction in plasma cortisol by 80 % in nyalas (*Tragelaphus angasii*) after administration of perphenazine enanthate. It has also been shown that combining perphenazine enanthate with a short-acting tranquilliser (zuclopenthixol acetate) in red deer results in significantly smaller increases in plasma cortisol than in non-tranquillised animals (1.3-3.4 times versus 1.7-5.4 times, respectively) (Diverio et al., 1996a, b). In contrast, Knox et al. (1989) found that the long-acting tranquilliser (perphenazine enanthate) was ineffective in reducing the cortisol response to capture stress in impala (*Aepyceros melampus*) but concluded that their sedative effects were beneficial in initial adaptation to confinement. In comparison, in the present study, the serum cortisol decreased 72 h after injection with perphenazine enanthate but not in saline-injected oryx.

If perphenazine enanthate has an effect of reducing the cortisol secretion, a decrease in faecal glucocorticoid metabolites measured on the third day after perphenazine injection might be expected but is not significantly lower than at 0 h (Figure 4.2). Faecal glucocorticoid metabolites increased significantly in the sample collected one day after injection of either saline or perphenazine enanthate. This increase was probably not due to tranquillisation because it also occurred after injection of saline (Figure 4.2). The increase of faecal glucocorticoid metabolites in the sample collected 24 h after injection of saline or perphenazine enanthate suggests that handling and injection on the previous day probably caused this increase. Handling and injection probably caused a sharp increase in cortisol in the circulation with concentrations remaining elevated for many hours after handling.

The faecal content of glucocorticoid metabolites at day 0 was significantly higher in oryx that received an injection of perphenazine enanthate than those receiving a saline injection (Figure 4.2). The saline injections took place about 2-3 months after injection of tranquilliser (Table 4.2) and the oryx might have been habituated to the housing and

handling procedures, leading to a reduction in faecal glucocorticoid metabolites. Familiarisation has already been discussed in Chapter 3 (see Figure 3.1). This idea is in agreement with the differences in the concentration of serum cortisol, which was also slightly, although not significantly higher in tranquillised oryx at time point 0 h than saline injected oryx. However, it is important to remember that the faecal glucocorticoids metabolites will reflect the circulating concentrations some 14 h earlier (Figure 4.1).

The administration of the long-acting tranquilliser, perphenazine enanthate before transportation of roe deer resulted in a significant decrease in the faecal glucocorticoid metabolites, by about 46 % compared to the group that were not injected with the tranquilliser (Dehnhard et al., 2001). Studies of the use of perphenazine enanthate in transportation of Arabian oryx are discussed in Chapter 6 of this thesis.

In summary, based on the serum cortisol decline on day 3 after perphenazine injection and the previous studies that reported a similar decline in cortisol, it can be concluded that perphenazine enanthate plays a considerable role in the reduction of the adrenocortical activity during stress.

In saline and perphenazine injected oryx, the significant increase at 3 h in free thyroxine might be attributed to handling and injection effects. In tranquillised oryx, there were significant but small increases in free thyroxine,  $T_4$ , at the 3 h and 24 h time points, but this may reflect the effects of the low free thyroxine concentration at 0 h, which was less than that in saline-injected oryx, rather than a specific action of perphenazine enanthate. Based on the variations in the concentration of thyroxine, it does not seem that perphenazine enanthate has an important effect on the concentration of free thyroxine in Arabian oryx.

The free triiodothyronine,  $T_3$ , concentration (Table 4.5), was consistently higher in saline-injected oryx than tranquillised ones. This might not be due to the injection of perphenazine enanthate because the  $T_3$  concentration was also low at time point 0 h even before the injection of the tranquilliser. Therefore, perphenazine enanthate has no apparent effect on this active thyroid hormone. Overall, thyroid hormones concentrations, free  $T_4$  and free  $T_3$ , were generally not affected by perphenazine enanthate.

#### 4.4.4 Haematology

The changes seen at time point 3 h in both saline- and perphenazine-injected oryx such as increase in white blood cell count, neutrophilia and lymphopenia were probably as a result of stress caused by handling and injection.

In the present study, significant decreases were found in red blood cell counts, haemoglobin concentration and haematocrit, each of which were lower in tranquillised oryx than in saline-injected oryx, almost at all time points (Table 4.6). The values for these parameters at 24 h and 72 h were their lowest compared to other time points in the tranquillised oryx. As the onset of action of perphenazine enanthate starts about 12 h after injection and peaks on the third day, the changes in these parameters correspond to the duration of action of perphenazine enanthate. These three parameters are closely related to each other. Other small variations such as in percent eosinophils, percent basophils and MCHC were erratic and do not reflect any effect of handling or tranquillisation.

Injection of chlorprothixene into roe deer resulted in erythropenia as reflected in the decrease of red blood cells, haematocrit and haemoglobin (Pujman and Hanusova, 1970). Chlorprothixene is a long-acting tranquilliser that belongs to thioxanthenes which have similar properties to phenothiazines such as perphenazine enanthate (Swan, 1993). In horses one of the most important toxic side effects of phenothiazines is haemolysis, leading to anaemia, jaundice and haemoglobinaemia (Jones, 1972; Ballard et al., 1982).

The first step in the stress response is the activation of the sympathetic nervous system, stimulating the adrenal medulla and releasing catecholamines. Increases in red blood cells, haemoglobin and haematocrit are associated with splenic contraction caused by the effect of catecholamines acting on  $\alpha$ -adrenergic receptors located in the splenic capsule (Stewart and McKenzie, 2002) and partly due to a reduction in plasma volume (Cross et al., 1988). In the present study, the lower red blood cell count, haemoglobin concentration and haematocrit in oryx injected with perphenazine enanthate can be explained by the  $\alpha$ -adrenergic blocking effects of tranquillisers of the phenothiazine family such as acepromazine and perphenazine enanthate (Swan, 1993). This results from the relaxation of the spleen and the subsequent splenic sequestration of erythrocytes (Jain, 1986). Haemodilution caused by perphenazine enanthate, due to lowering of blood pressure can be

ruled out because in that case total serum protein, serum albumin sodium concentration would also have decreased. Acepromazine (a phenothiazine tranquilliser with short acting effect) also had similar effects on red blood cells, haemoglobin and haematocrit in southern chamois (Lopez-Olvera et al., 2006b) and roe deer (Montane et al., 2003; 2007).

In the present studies, even though the dose range examined was small, there was a significant negative correlation of haematocrit and haemoglobin with the dose of perphenazine received. There might be some consequences of the decrease in the erythrocytic parameters. For example, haemoglobin is the circulating oxygen carrier to all cells of the body and a decrease in haemoglobin might influence the rapid response of free-ranging animals to dangers such as escaping predators, which requires maximum oxygen availability. Given the negative relationship between perphenazine dose and haemoglobin concentration and haematocrit seen in the present studies, it is probable that a low dose is desirable to achieve useful levels of tranquilisation, while reducing the effects on haematology. However, the tranquilliser will make animals less active and less aware of their surroundings and conservation managers should consider such effects when translocating tranquillised animals to the wild.

#### **4.4.5 Biochemistry and ions**

Perphenazine enanthate had no considerable effects on the serum ion balance or the serum concentrations of glucose, urea, total protein or albumin in Arabian oryx. This lack of disruption of metabolism or ion balance is an advantage in its application for management practices.

#### **4.4.6 Study design, conclusions and future studies**

All oryx were injected with 100 mg of perphenazine enanthate regardless of body weight, so heavier animals (mostly males) received lower doses than lighter ones (mostly females). The correlation analysis between the variable doses given to each animal and the various parameters measured showed no or only occasional correlations at one or two time points for most of the parameters. However, there were strong negative correlations of haemoglobin, haematocrit, MCV and MCH with the injected dose of perphenazine. Haematocrit and MCV showed a significant correlation with the dose of perphenazine even

prior to injection, at the time point 0 h (Haematocrit: Pearson's correlation coefficient,  $r$ , -0.661,  $P < 0.005$ ,  $n = 16$ ; MCV: Pearson's correlation coefficient,  $r$ , -0.706,  $P < 0.002$ ,  $n = 16$ ) (Table 4.6), which was not definitely as a result of tranquilisation and even though these parameters are linked to red blood cell count and haemoglobin concentration, there was no correlation between the dose of perphenazine and the red blood count which casts doubt on a dose-response relationship. However, for haemoglobin, haematocrit, MCV and MCH there were consistent strong negative correlations with the injected dose of perphenazine which does suggest dose-response relationships for these parameters.

For experiments that have a control group such as the saline (control) versus perphenazine enanthate, described in this chapter, a cross-over design is recommended whereby at one time half animals receive treatment and the others receive placebo or control. Although this design helps in reducing the effects of factors like ambient temperature, it has some reported limitations. The limitations include assumed lack of interactions between treatments, periods and subjects and the few degrees of freedom (df) for the residual, especially when carryover effects are separated out as a source of variation. Also, there are the usual difficulties of handling missing observations (Quinn and Keough, 2002). However, other studies stated that random cross-over design reduces bias associated with imbalance in known and unknown confounding variables (Mills et al., 2009). Therefore, such approaches could be used in future studies to improve the experimental design and compare the effects of treatment against control groups.

While recognising the importance of considering the difference in response between males and females to the perphenazine enanthate injection, this has not been achieved for several reasons. First, the sample size of 16 animals was quite small and considering gender differences sample size becomes 8 males and 8 females; combining data gave the larger sample size. Second, the statistical package employed (SigmaStat v3.5) cannot specify non-normal error structures in general linear models (GLM), and therefore requires transformation of non-normal data in 2-way repeat measures ANOVAS. Future studies should investigate gender differences using a larger number of animals and GLM with specified non-normal error structure.

The long acting tranquilliser, perphenazine enanthate had no significant adverse effects on haematological, physiological and biochemical parameters, except for a decrease in the red blood cell count, haemoglobin concentration, haematocrit and mean corpuscular volume. The decrease in these parameters was temporary and not severe enough to compromise well-being or jeopardise the life of animals. Animals should recover from such effects soon after the tranquilliser is cleared from the circulation at between 7 to 10 days post injection (Ebedes, 1993a), and the effects are not strong enough to prevent the utilisation of perphenazine enanthate for the tranquillisation of Arabian oryx.

It has been recommended that the long-acting tranquilliser, perphenazine enanthate should be combined with use of a short-acting one, in order to fill the gap from capture to the time when the action of the long-acting tranquilliser starts, usually 12-14 h after injection. Examples of short-acting tranquillisers that can be used with perphenazine enanthate are haloperidol and zuclopenthixol acetate (Clopixol – acuphase, Lundbeck) (Ebedes, 1993a). These two tranquillisers have been used in okapi during transportation and found to induce calmness and reduce stress associated with new environments (Redrobe, 2004). The actions of zuclopenthixol acetate starts within one hour after injection and lasts up to 3-4 days, while the actions of haloperidol start within 5-10 min after injection and its effects last 8 to 18 h (Swan, 1993). Zuclopenthixol acetate can be used alone for short duration of action. This tranquilliser used alone in wapiti (*Cervus elaphus*), a North American elk, was found to decrease handling stress as indicated by lowering of serum cortisol concentration (Read et al., 2000).

The application of long-acting tranquillisers for wildlife has proved to be useful for promoting welfare in management practices for many species and this study supports the literature encouraging the use of these tranquillisers to facilitate the handling of Arabian oryx and to induce calmness during transportation and housing in new environments.

## **Chapter 5**

### **Investigations of the effects of the immobilisation with xylazine on Arabian oryx**

#### **5.1 Introduction**

Immobilisation is a vital management tool for capture of wildlife for various purposes, including veterinary health checks, treatment of sick animals in captivity and translocation of animals from captivity to the wild or vice versa. There are many chemicals used for immobilisation. The types of immobilisation chemicals, their characteristics, pharmacological actions, dosages and antidotes are reviewed in detail by Swan (1993). The characteristics of an ideal immobilization drug for darting wild animals can be summarized as follows (Swan, 1993):

- An effective dose should not exceed the quantity that can be carried in an appropriately-sized, preferably less than 3 ml, dart
- Suitable stability.
- Rapid absorption into the systemic circulation.
- Rapid onset of action resulting in sufficient immobilization.
- Duration of effects should be long enough.
- A wide margin of safety.
- Availability of a reliable antidote.
- Rapid elimination from the body.
- No drastic effect on pregnant animals.
- No permanent damage to the animal.
- Should not cause tissue irritation.
- Minimum effects on cardio-respiratory function.
- Recovery and induction periods should be calm.

- Minimum risk to personnel handling the drug.
- Should reduce the animal's awareness of its surroundings to minimize fear, distress, and pain.

In the United Arab Emirates, different combinations of chemicals are used for immobilisation of Arabian oryx such as (1) etorphine, azaperone, reversed by diprenorphine; (2) xylazine, ketamine, reversed by atipamezole and (3) medetomidine, ketamine, butorphanol (torbugesic), reversed by atipamezole (Molnar et al., 2005; Molnarova et al., 2005). At high ambient temperatures, for example during summer when temperature reach 48 °C, ketamine and xylazine combination were found to be better than a combination of etorphine and azaperone (Molnar and Mckinney, 2002).

The common practice in Oman until recently was the immobilisation of Arabian oryx with etorphine which has many disadvantages such as a high toxicity to human and induction of a phase of excitement in the animal, characterised by running for long-distances until full ataxia is reached, during which it might crash into the fence or a tree and wound itself (personal observation). Xylazine has not been used in Oman although it has been reported to meet all the above mentioned characteristics of an ideal immobilising chemical, except for causing respiratory depression and a 2<sup>nd</sup>-3<sup>rd</sup> degree of atrioventricular (AV) block with overdosage (Swan, 1993). Therefore, for the present study, this chemical was chosen as a case example to further examine the effects of immobilising chemicals on haematological, biochemical, hormonal and clinical parameters.

Xylazine is commonly used for immobilisation at relatively high doses and as a sedative at lower doses (Swan, 1993). It is an alpha-2 adrenoreceptor agonist and its immobilising effect can be reversed by alpha-2 adrenoreceptor antagonists such as atipamezole (Swan, 1993). Alpha-2-adrenoceptors can be found in different locations, both in neuronal and non-neuronal tissues (Scheinin and MacDonald, 1989). These receptors have been classified into at least three alpha-2-isoreceptors ( $\alpha$ -2a, 2b and 2c) according to their affinity for  $\alpha$ -2-adrenoceptor ligands (Bylund, 1985; Murphy and Bylund, 1988; Bylund, 1992). These receptors act pre-synaptically by modulating the function of other neurons or by post-synaptic mediation of constriction of vascular smooth muscle in the periphery (Vähä-Vähä, 1991). Alpha-2-adrenoceptors play an important role in modulating sympathetic nerve functions, and in the regulation of vigilance, cognition, nociception, and

cardiovascular function. Pharmacological responses mediated by  $\alpha$ -2-adrenoceptors act on various systems. For example, in the central nervous system these responses cause sedation, analgesia and hypothermia, and also cause bradycardia, hypotension, a decrease in the respiratory rate and inhibition of insulin release (Vähä-Vähä, 1991).

These drugs have been used in wide range of non-domestic herbivores and carnivores. They are used mainly as synergists, mixed with either opioids (e.g. etorphine) or cyclohexylamines (e.g. ketamine), resulting in reduction of the doses required, improvements in induction times, and better relaxation. Xylazine was the first drug of alpha-2 adrenoceptor agonist group to be used. It was followed by detomidine, and then with medetomidine (Swan, 1993). Specific  $\alpha$ -2-adrenoceptor antagonists such as yohimbine, tolazoline, atipamezole, and idazoxan are used to reverse the effects of these drugs (Swan, 1993).

Xylazine has been used in Arabian oryx, either alone (Ancrenaz, 1994) or in combination with other immobilisation chemicals like etorphine (Greth and Vassart, 1989; Greth and Schwede, 1993; Ostrowski et al., 2002b; 2003). In scimitar horned oryx, xylazine was also used in combination with etorphine (Bush et al., 1983; Pearce and Kock, 1989).

Medetomidine (Domitor) is also an  $\alpha$ -adrenoceptor agonist that has been used in Arabian oryx and reversed by atipamezole (Antisedan) (Greth et al., 1993). Etorphine is an opioid that is used for wildlife immobilization and is reversed by diprenorphine (Revivon, M5050). Etorphine is usually given with the short acting tranquilliser, acepromazine in a commercially available combination (Large Animal Immobilin or M99). Etorphine is dangerous for humans as a small amount of it can cause dizziness, nausea, respiratory depression, cyanosis, hypotension, loss of consciousness and possibly death (Sterken et al., 2004; McMahon and Bradshaw, 2008). For safety reasons, etorphine must be handled extremely carefully or a safer alternative such as xylazine (Swan, 1993) or other chemicals should be used.

The effects of using xylazine alone for immobilisation, on the haematological, physiological and biochemical parameters have not been studied in depth in Arabian oryx or any other closely related species. Therefore, the aim of this study was to investigate the effects of xylazine on these parameters in Arabian oryx.

## **5.2 Materials and methods**

### **5.2.1 Immobilisation with xylazine**

The oryx that were used for the study of effects of xylazine in February 2008 had been exposed to several treatments before. The treatments were dexamethasone (described in Chapter 2) and tranquillisation with perphenazine enanthate (described in Chapter 4), which were carried out between September and November 2007 and injection with saline (described in Chapter 4), which was done in January 2008 (See Appendix I for details).

Sixteen adult Arabian oryx (8 males and 8 females) were captured in the holding pens at the Omani Mammals Breeding Centre and blood samples were collected immediately as described in Chapter 3 (Section 3.2.4). Heart rate, respiratory rate and body temperature were measured as described in Chapter 3 (Section 3.2.3) after collection of the first blood sample. Then the oryx were injected intramuscularly with a dose of 0.5 mg/kg of xylazine (Rompun, 20 mg/ml, Bayer). Minimal noise from the team of workers was maintained after injection, to ensure good immobilisation. Then at a mean time of  $28.1 \pm 3.0$  min after injection with xylazine, a second blood sample was collected and body temperature, respiratory and heart rates were measured again. The immobilisation effect was then reversed by intravenous injection of atipamezole at 0.091 mg/kg (Antisedan, 5 mg/ml, Novartis).

### **5.2.2 Analysis of blood samples**

The blood samples were analysed for haematological parameters (section 3.2.4). The blood samples were also processed (Section 0) and analysed for serum hormonal and biochemical parameters as described in Chapter 3 (Sections 3.2.7 and 3.2.8). For measurement of plasma lactate, blood samples were collected in glass vacutainer tubes containing the anticoagulant fluoride oxalate, and analysed as described in Chapter 3.

### **5.2.3 Statistical analysis**

The data for equal numbers of male and female oryx were combined to increase sample size for statistical analysis of the effects of xylazine on Arabian oryx.

Normality of data was tested using the Kolmogorov-Smirnov test. The parameters that did not achieve normality were transformed to normality with square (white blood cell count and the percentage of basophils), square root (the percentage of lymphocytes), exponential (body temperature) and with rank transformation (free triiodothyronine and phosphorus). The normally distributed or transformed data were tested for differences comparing data before injection of xylazine and after, using paired t-tests. All values are presented as mean  $\pm$  standard error unless stated otherwise. Significance was accepted at  $P < 0.05$ .

### **5.3 Results**

#### **5.3.1 General observations**

The time from injection of xylazine until the oryx fell down (ataxia) differed significantly between males and females. The males were immobilised within  $23.5 \pm 2.4$  min while females were immobilised within  $11.7 \pm 1.2$  min (t-test,  $P < 0.001$ ).

The immobilised oryx were easy to handle as they were incapable of standing and showed no resistance to handling. Xylazine had good myorelaxation as oryx had heavy sedation with the neck falling to one side. One male oryx, however did not reach complete immobilisation but was easily captured by hand. Some animals fell down and then stood again before final immobilisation.

The duration from injection with the reversal chemical, atipamezole until the oryx stood up, was  $74.5 \pm 13.6$  sec, which did not differ between males and females. The oryx started walking within few seconds after standing. However, one male and one female showed an exceptionally long duration between injection of the atipamezole and standing (16.0 min and 17.3 min, respectively). Therefore, these two animals were not included in the calculation of the mean duration between reversal and standing.

#### **5.3.2 Body temperature, heart and respiratory rates**

The rectal body temperature of Arabian oryx did not change significantly after immobilisation with xylazine (Figure 5.1,  $P = 0.299$ , paired t-test). The body temperature was  $38.20 \pm 0.19$  °C before immobilisation and  $37.86 \pm 0.24$  °C after 28.1 min post

injection of xylazine. However, heart rate dropped dramatically from  $107.63 \pm 5.77$  bpm to  $45.25 \pm 3.07$  bpm (Figure 5.1,  $P < 0.001$ , paired t-test). Similarly, there was a sharp significant decrease in the respiratory rate from  $39.25 \pm 2.60$  breaths per min to  $10.25 \pm 1.57$  (Figure 5.1,  $P < 0.001$ , paired t-test).

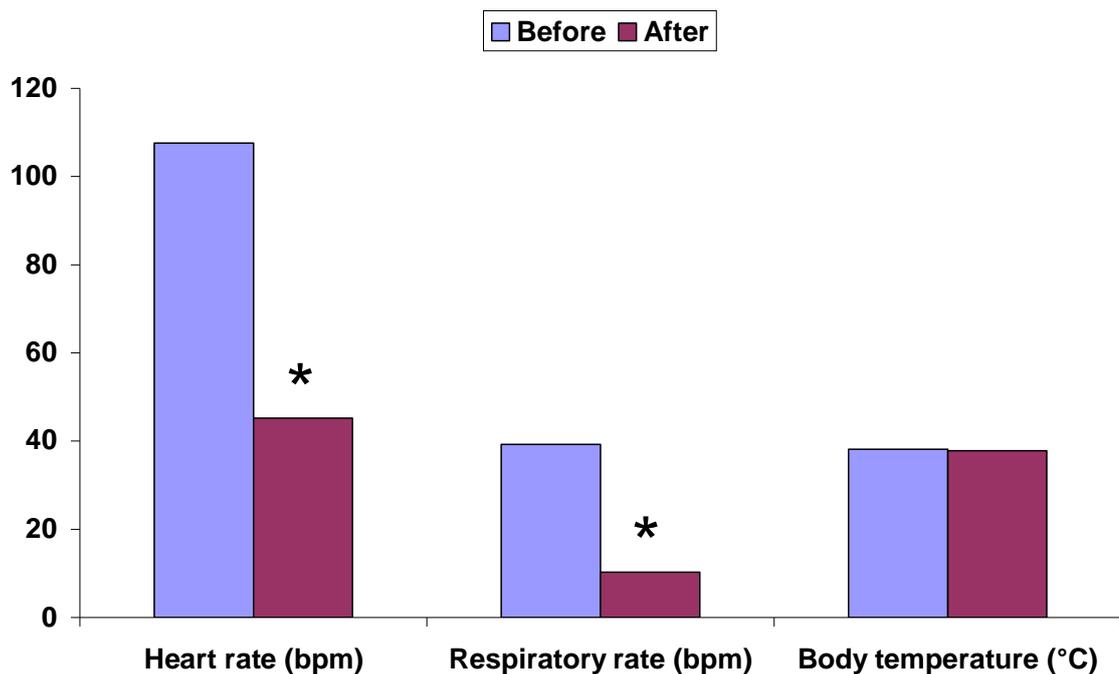


Figure 5.1 The effects of xylazine on heart rate, respiratory rate and body temperature. \*  $P < 0.001$ , paired t-test.

### 5.3.3 Hormones

After the immobilisation of Arabian oryx with xylazine, the concentration of serum cortisol increased significantly by 85 % from  $81.13 \pm 8.94$  nmol/L to  $149.94 \pm 10.71$  nmol/L ( $P < 0.001$ ). The thyroid hormones also increased significantly. The free thyroxine ( $T_4$ ) increased slightly but significantly by 7 % from  $10.08 \pm 0.29$  pmol/L to  $10.80 \pm 0.54$  pmol/L ( $P = 0.018$ ). The concentration of free triiodothyronine ( $T_3$ ) increased significantly by 53 % from  $3.56 \pm 0.25$  pmol/L to  $5.43 \pm 0.46$  pmol/L ( $P < 0.001$ , paired t-test). In

contrast, the concentration of insulin decreased significantly from  $0.74 \pm 0.09$  mIU/L to  $0.19 \pm 0.03$  mIU/L ( $P < 0.001$ , paired t-test).

#### **5.3.4 Haematology**

The effects of xylazine on haematological parameters of Arabian oryx are shown in (Table 5.1). There were significant decreases in the white blood cell count, percent lymphocytes, red blood cell counts, haemoglobin concentration, haematocrit and mean corpuscular volume (Table 5.1). On the other hand, there was significant increase in the percentages of neutrophils and mean corpuscular haemoglobin concentration (Table 5.1). There was no significant change in the monocytes %, eosinophils %, basophils % and mean corpuscular haemoglobin (Table 5.1).

#### **5.3.5 Biochemistry and ions**

The effects of xylazine on serum ions and biochemical parameters in Arabian oryx are shown in (Table 5.2). After the injection of xylazine, there was significant decrease in the concentration of potassium, calcium, phosphorus, total protein, albumin and lactate (Table 5.2). In contrast, there was a significant increase in the concentration of glucose after immobilisation (Table 5.2). The concentration of sodium, chloride and urea did not change significantly after the injection of Arabian oryx with xylazine.

Parameter	Unit	Before xylazine injection		After xylazine injection		P value	Mean difference	95 % C.I. for difference
		mean	SEM	mean	SEM			
<b>WBC*</b>	$\times 10^9/L$	8.07	0.34	6.88	0.43	$P < 0.001$	4.09	3.05 to 4.92
<b>Neutrophils*</b>	%	62.71	1.84	67.19	1.55	$P < 0.001$	- 4.48	-5.76 to -3.195
<b>Lymphocytes*</b>	%	30.41	1.82	25.76	1.52	$P < 0.001$	4.65	3.20 to 6.10
Monocytes	%	1.06	0.36	0.61	0.23	$P = 0.486$	0.02	-0.09 to 0.35
Eosinophils	%	2.84	0.37	2.84	0.42	$P = 0.987$	- 0.003	-0.44 to 0.43
Basophils	%	2.99	0.32	3.60	0.19	$P = 0.070$	- 1.75	-2.54 to 0.54
<b>RBC*</b>	$\times 10^{12}/L$	12.18	0.14	9.45	0.19	$P < 0.001$	2.73	2.41 to 3.05
<b>Haemoglobin*</b>	g/dL	18.08	0.19	13.93	0.25	$P < 0.001$	4.14	3.64 to 4.65
<b>Haematocrit*</b>	%	53.11	0.73	39.65	0.79	$P < 0.001$	0.14	0.12 to 0.15
<b>MCV*</b>	fL	43.65	0.47	41.99	0.50	$P < 0.001$	1.66	1.39 to 1.93
MCH	pg/cell	14.84	0.15	14.77	0.18	$P = 0.499$	0.08	-0.16 to 0.31
<b>MCHC*</b>	g/dL	34.04	0.19	35.14	0.16	$P < 0.001$	- 1.11	-1.64 to -0.57

Table 5.1 Haematological parameters in 16 Arabian oryx before and after injection of xylazine. SEM, standard error of mean; C.I. confidence interval, WBC, white blood cells; RBC, red blood cells (erythrocytes); MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; and MCHC, mean corpuscular haemoglobin concentration. Asterisks (\*) and bold font indicate significant difference.

Parameter	Unit	Before		After		P value	Mean difference	95 % C.I. for difference
		mean	SEM	mean	SEM			
Sodium	mmol/L	152.40	4.47	138.44	6.22	P = 0.158	13.80	-6.02 to 33.62
<b>Potassium</b>	mmol/L	6.51	0.19	4.94	0.24	P < 0.001	1.55	0.79 to 2.31
Chloride	mmol/L	108.67	3.17	100.50	4.50	P = 0.226	8.27	-5.72 to 22.26
<b>Calcium*</b>	mmol/L	2.39	0.07	2.03	0.10	P = 0.030	0.35	0.04 to 0.67
<b>Phosphorus*</b>	mmol/L	2.59	0.16	2.08	0.14	P = 0.003	NA	NA
<b>Glucose*</b>	mmol/L	4.47	0.17	8.87	0.66	P < 0.001	-4.59	-5.84 to -3.33
Urea	mmol/L	4.49	0.21	4.30	0.18	P = 0.499	0.20	-0.42 to 0.82
<b>Total protein*</b>	g/L	68.60	3.06	54.31	2.23	P = 0.009	14.13	4.16 to 24.11
<b>Albumin*</b>	g/L	49.27	2.84	37.38	1.67	P = 0.009	11.80	3.45 to 20.15
<b>Lactate*</b>	mmol/L	10.80	1.08	5.87	0.72	P < 0.001	4.93	3.51 to 6.34

Table 5.2 Ions and biochemical parameters in 16 Arabian oryx before and after administration of xylazine. SEM, standard error of mean. Asterisks (\*) and bold font indicate significant difference. NA, not available because the data were rank transformed for statistical analysis.

## 5.4 Discussion

### 5.4.1 Effectiveness of xylazine and reversal in Arabian oryx

The dosages of xylazine (0.5 mg/kg) and the antagonist, atipamezole (0.091 mg/kg) administered to Arabian oryx were similar to those used by Ancrenaz (1994) which were 0.5 and 0.087 mg/kg, respectively. Although all oryx were completely immobilized with this dosage of xylazine, one male was not immobilized. A similar observation was reported by Ancrenaz (1994) who found that three males out of twenty-seven, never became recumbent after xylazine administration. It is not clear why some oryx are not immobilised. This might be because the given dosage was not enough for some individuals. Another factor that might be relevant is that the noise of the working team could provoke oryx to secrete catecholamines. These hormones were reported to interfere with immobilisation (Short, 1992), probably through overriding the effects of the immobilising agent. For this reason, the capture team was encouraged to keep noise to minimum after injection of xylazine.

The time from injection of xylazine to recumbency of male oryx was higher in this study  $23.5 \pm 2.2$  min ( $n = 8$ ) than the interval of  $9.4 \pm 1.1$  min ( $n = 27$ ) reported for male oryx by Ancrenaz (1994). The reason for the delay in recumbency in the present study is unclear. The females in the present study became recumbent within  $11.7 \pm 1.2$  min ( $n = 8$ ), which was close to the duration reported for male oryx by Ancrenaz (1994).

The duration from injection of the reversal, atipamezole, until the oryx stood up was  $74.5 \pm 13.1$  seconds ( $n = 14$ ). This duration was comparable with that observed by Ancrenaz (1994) which was  $87.1 \pm 8.3$  seconds ( $n = 24$ ). However, one male and one female oryx in the present study took an exceptionally long time to stand after injection of atipamezole injection: 16.0 and 17.3 min, respectively, even though they received a similar dosage as other oryx. In the study of Ancrenaz (1994), all immobilized oryx were completely reversed within 87.1 seconds, after a similar dosage to that used in the present study. It is concluded that the dosage of atipamezole was not enough for some oryx in the present study, although the reasons for this are unclear.

Ancreanz (1994) reported that a re-sedation period occurred between two and five hours after the injection of atipamezole. Re-sedation was characterized by lowering of the ears and sometimes recumbency that lasted up to two hours. In the present study, re-sedation was not monitored. However, if xylazine or other alpha-2 agonists such as medetomidine are used for immobilization of Arabian oryx, or any other wildlife, shortly before they are released to the wild, the occurrence of re-sedation after release might put the animals at risk of poaching or predation. Therefore, in future application of xylazine in Arabian oryx, re-sedation should be monitored, and finding a solution to avoid its occurrence would be useful. Injection of one third of the dosage of atipamezole sub-cutaneously and two thirds intra-venously was reported to reduce the chance of re-sedation occurrence (Swan, 1993). This was not possible in Arabian oryx at the time of these experiments, because the total volume of atipamezole was about 1 ml, which was found too small to divide effectively into intravenous and subcutaneous injections by the veterinarian. In future immobilisation events, this can be done by using appropriate syringes to divide the dose effectively, or by dilution of the solution so that small volumes can be handled more easily. Re-sedation was also reported after immobilization with medetomidine (an alpha-2 agonist like xylazine) followed by reversal with atipamezole in reindeer, and was explained by the longer elimination half-life of medetomidine compared to atipamezole (Ranheim et al., 1997). This explanation might also be applicable in the case of xylazine in the Arabian oryx or other species.

#### **5.4.2 Body temperature, heart and respiratory rates**

The respiratory rate dropped sharply by 74 % after immobilisation of Arabian oryx with xylazine to  $10.25 \pm 1.57$  bpm, which was below the reference inter-percentile range of 20.00 to 54.00 bpm reported in Chapter 3. A decrease in the respiratory rate after xylazine administration was also reported in horses (Lavoie et al., 1992; Klein et al., 2006), goats (Sanhoury et al., 1992) and sheep (Doherty et al., 1986). In the present study, breathing of immobilised oryx was characterised by prolonged exhalation and apnoeic periods which was also observed in sheep (Doherty et al., 1986) and horses (England and Clarke, 1996). In sheep, the respiratory depression caused a significant decrease in the arterial oxygen partial pressure ( $\text{PaO}_2$ ), which can be life threatening (Doherty et al., 1986). Therefore, the

respiratory rate should always be monitored during immobilisation with xylazine and the effects of immobilisation should be reversed if necessary.

The heart rate decreased remarkably, by 58 % after immobilisation with xylazine, reaching  $45.25 \pm 3.07$  bpm, which was far below the reference inter-percentile range (80.00 – 150.00 bpm) that was established in Chapter 3. The slowness of the heart rate (bradycardia) was also reported after immobilisation with xylazine in scimitar horned oryx (Pearce and Kock, 1989), goats (Kinjavdekar et al., 1999), sheep (Doherty et al., 1986), dogs (Ilback and Stalhandske, 2003) and horses (England and Clarke, 1996). Bradycardia is caused through the inhibition of nor-adrenaline release from peripheral pre-synapses by the alpha-2 receptor agonists (Dejonge et al., 1981; Hayashi and Maze, 1993). The decrease in the heart rate causes a decrease in blood pressure (hypotension). The drop in blood pressure will alter the balance in the hydrostatic forces on the capillary beds and reduces fluid efflux from the capillaries and may increase influx of water from the interstitium into the lumen of the capillaries, leading to dilution of the blood (Levick, 1999). The blood pressure also decreases with decreased inspiration (Levick, 1999). Thus, oryx that had low respiration rate after xylazine injection and experienced low blood pressure, and therefore, the possibility of blood dilution was greater.

The body temperature did not change in Arabian oryx after 28.1 min of injection with xylazine. However, if immobilisation is prolonged to more than 30 min it might decrease. In scimitar-horned oryx (*Oryx dammah*), the body temperature dropped gradually after immobilisation with xylazine from 38.0 °C at 15 min to 37.1 °C at 30 min, 36.6 °C at 60 min, 36.3 °C at 90 min, 35.9 °C at 120 min, and the lowest recorded body temperature was 33 °C (Pearce and Kock, 1989). A similar gradual decline in body temperature was observed in impala injected with xylazine, reaching 36.3 °C from an initial value of 39.1 °C after 75 min (Drevemo and Karstad, 1974). The immobilisation of Arabian oryx in the present study was reversed by atipamezole after a mean time of 28.1 min from the injection of xylazine. At this time, it was probably early for body temperature to have decreased as happened in the above mentioned studies. If xylazine can cause hypothermia, as shown by these studies, then animals should be reversed with the antagonist as soon as the handling finishes to avoid a drop in body temperature that can compromise an animal's health.

### 5.4.3 Hormones

After 28.1 min from injection of Arabian oryx with xylazine, the concentration of serum cortisol increased by 85 % which indicates that the oryx were stressed by the procedures. In white-tailed deer, the plasma cortisol concentration increased by 164 %, 80 min after injection with xylazine (Chao et al., 1984).

The secretion of cortisol might be affected by xylazine. In sheep and goats, plasma cortisol increased by 25 % and 50 %, respectively 30 min after xylazine injection (Ali et al., 2006). On the other hand, the increase of cortisol was higher in sheep and goats that were injected with saline 123 % and 157 %, respectively, than those injected with xylazine (Ali et al., 2006). The cortisol concentration remained elevated in saline injected goats and sheep, whereas the concentration of cortisol decreased with time after xylazine injection (Ali et al., 2006). In elk, xylazine was used to reduce stress by administration through an intranasal route (Cattet et al., 2004). Cattet et al. (2004) found that cortisol differed significantly between saline and xylazine injected elk with concentrations of  $93 \pm 5.9$  mmol/L and  $75 \pm 4.8$  mmol/L, respectively. Cortisol concentrations were also suppressed by xylazine in goats (Sanhoury et al., 1992). Sanhoury et al. (1992) found that xylazine pre-treatment does not alter the cortisol response to CRH, suggesting that the hypothalamus is under  $\alpha$ -2 adrenergic suppression. Therefore, stimulation of cortisol secretion can be inhibited by  $\alpha$ -2 adrenergic agonists. This suggests that the increase in cortisol seen in Arabian oryx and other studies was probably due to the stress of handling (see Chapter 4, Section 4.4.3) that overrides the direct effects of the xylazine at the hypothalamic level.

The concentration of free thyroxine increased slightly but significantly, by 7 %, from 10.08 to 10.80 pmol/L, while that of the free triiodothyronine increased considerably, by 53 %, from 3.56 to 5.43 pmol/L, after immobilisation with xylazine. However, the concentrations of both thyroid hormones remained within the reference inter-percentile ranges (established in Chapter 3), which were 6.06 – 12.79 pmol/L for free thyroxine and 0.83 – 8.57 pmol/L for free triiodothyronine. There were no previous reports of free thyroid hormones after xylazine injection in Arabian oryx. The increase in thyroid hormones might be linked to the increase in oxygen consumption and carbohydrate, protein and fat metabolism (Norman and Litwack, 1990). The significant decrease in respiratory rate seen in Arabian oryx

following xylazine injection might lower the amount of oxygen inhaled by oryx and therefore triiodothyronine hormone increased as a response to enhance oxygen consumption in order to meet oxygen requirements.

The concentration of insulin decreased by 75 % in Arabian oryx 28.1 min after injection with xylazine. Hypoinsulinemia after xylazine administration was reported in horses (Thurmon et al., 1982), rats (Saha et al., 2005) and cattle (Hsu and Hummel, 1981), which agrees with the results of the present study on Arabian oryx. Hsu and Hummel (1981) suggested that the decrease in insulin was mediated by the action of xylazine on  $\alpha$ -2 adrenergic receptors in the  $\beta$  cells of pancreatic islets to inhibit the release of insulin.

#### **5.4.4 Haematology**

Although the white blood cell count decreased significantly, by 15 % in immobilised Arabian oryx, from 8.07 to 6.88 x 10<sup>9</sup>/L, they remained within the reference inter-percentile range (4.45 – 11.11 x10<sup>9</sup>/L) established in Chapter 3. The percentage of neutrophils increased significantly by 7 % after xylazine injection, but again was within the reference established inter-percentile range of 46.19 – 78.20 %. The significant decrease in the percentage of lymphocytes, by 15 %, from 30.41 to 25.76 %, was also within the established reference range (17.25 – 47.91 %), and percentage of monocytes, eosinophils and basophils did not change. Therefore, it can be concluded that although some redistribution of white blood cell types occurred, it does not indicate pathological abnormalities as all values were within the established reference ranges discussed in Chapter 3.

The mean red blood cell count, haemoglobin concentration and haematocrit decreased by 22 %, 23 % and 25 % respectively after immobilisation with xylazine, indicating similar percentage decreases in these three related parameters. The red blood cell count decreased from 12.18 to 9.45 x 10<sup>12</sup>/L, which was below the established reference inter-percentile range (10.74 – 13.57 x 10<sup>12</sup>/L) established in Chapter 3. Similarly, the concentration of haemoglobin decreased from 18.08 to 13.93 g/dL, and the latter value was below the reference inter-percentile range for haemoglobin (15.59 -20.56 g/dL). The haematocrit also fell from 53.11 to 39.65 %, to a value below the reference inter-percentile range (47.20 -

68.20 %). A similar decrease in haematocrit was reported in scimitar-horned oryx (*Oryx dammah*) from 35 to 30 %, compared to 40 % in non-immobilised oryx under manual restraint (Pearce and Kock, 1989). The decrease in these parameters might be caused by the dilution of blood, due to a decrease in the cardiac output following immobilisation with xylazine, as discussed in section 5.4.2. The similarity in the percentage decrease of these parameters supports this explanation. In addition the stress of handling and injection would be expected to cause splenic contraction and release of red blood cells into the blood stream (Stewart and McKenzie, 2002), that could partially offset the effects of dilution. The mean corpuscular volume of red blood cells, decreased significantly after xylazine injection, but only to a small extent (3.8 %), and mean corpuscular haemoglobin did not change, while the decrease of mean corpuscular haemoglobin concentration, even though significant, was also minute (3.3 %) and not of significant concern.

#### **5.4.5 Biochemistry and ions**

The concentration of glucose doubled after administration of xylazine from a mean value of 4.47 to 8.87 mmol/L. Although it was within the reference inter-percentile range (3.52 – 10.44 mmol/L), the increase by 98 % percent was a dramatic change. There are several possible explanations for the increase of glucose concentration after the injection with xylazine. First, the decrease in insulin concentration after immobilisation of Arabian oryx with xylazine may have played a major role in the increase of serum glucose. Similar findings of concurrent hyperglycemia and hypoinsulinemia after xylazine administration were reported in cattle (Hsu and Hummel, 1981), horses (Thurmon et al., 1982; Tranquilli et al., 1984) and rats (Saha et al., 2005). In addition to this explanation that a decrease in insulin caused elevated glucose, an increase in the hormone, glucagon was observed in sheep after xylazine injection and suggested to have an important role in the increase of blood glucose (Brockman, 1981). The increase of glucose after xylazine injection may also have occurred due to gluconeogenesis, which may be triggered by increased concentrations of cortisol and triiodothyronine. Both hormones are well known to induce gluconeogenesis pathways which increase the production of glucose (Norman and Litwack, 1990). Cortisol also induces mobilisation of amino acids in muscles and therefore increases the concentration of blood glucose (Norman and Litwack, 1990).

The decrease in plasma lactate was considerable as it declined by 46 % from 10.80 to 5.87 mmol/L. The latter value was still close to the reference inter-percentile range (5.03 – 21.80 mmol/L) shown in Chapter 3. The concentration of blood lactate increases in relation to the increased level of muscular activity (Hughson et al., 1987; Raymer et al., 2009). In immobilised oryx, muscular activity was very low and this may explain the lower concentration of lactate.

The serum concentrations of total protein and albumin decreased significantly after injection of xylazine, by 21 and 24 %, respectively. These percentages were similar to those for the decrease in red blood cell count, haemoglobin and haematocrit which were 22, 23 and 25 %, respectively. These similarities suggest that blood dilution caused a decline in the concentration of blood proteins of similar proportion.

The dilution of the blood serum by interstitial fluid should not have a major effect on the serum ions as interstitial fluid and blood serum are fairly similar in composition, although can be affected to a small extent by Gibbs Donnan effects on charged ions. This agrees with the lack of change in the concentrations of the major ions, sodium and chloride in oryx immobilised with xylazine. However, serum concentrations of potassium, calcium, and phosphorus decreased by small amounts that cannot be explained.

#### **5.4.6 Study design, conclusions and future studies**

In the experiment described in the previous chapter, a control group was used to evaluate the effects of saline for comparison with perphenazine enanthate. However, due to unavailability of qualified veterinarians after the experiments on xylazine treatment, a control experiment could not be carried out. The veterinarians of the Royal Court Affairs were involved in duties in a different part of the country at that time. Therefore, the data for parameters measured after injection of xylazine were compared to measurements made on blood samples collected before injection. Future studies should employ a control group injected with normal saline and exposed to the injection, blood sampling and handling procedures alone to allow the effects of xylazine administration to be confidently discerned (Quinn and Keough, 2002).

While recognising the importance of considering the difference in response between males and females to xylazine injection, this was not achieved for several reasons. First, the sample size of 16 animals was quite small and considering gender differences sample size becomes 8 males and 8 females; combining data gave the larger sample size. Second, the statistical package employed (SigmaStat v3.5), cannot specify non-normal error structures in general linear models (GLM), and therefore requires transformation of non-normal data. Future studies should investigate gender differences of the effects of xylazine using a larger number of animals and GLM with specified non-normal error structure.

Overall, xylazine produced good immobilisation in Arabian oryx with the dosage (0.5 mg/kg) used in this study that was well reversed with a dosage of (0.091 mg/kg) of atipamezole. Xylazine caused decrease in the heart and respiratory rates, which led to blood dilution in the Arabian oryx. Because of blood dilution, there were decreases in red blood cells, haemoglobin, haematocrit, serum albumin and total protein. Therefore, the respiratory and heart rates should be monitored during immobilisation with xylazine. The duration of handling should always be minimised to avoid any adverse effects of xylazine. Xylazine, acting on  $\alpha$ -adrenoreceptors of the  $\beta$ -islets of the spleen, reduced the secretion of insulin, which resulted in doubling of the serum glucose concentration. As xylazine induced good myorelaxation and subsequent lowering of muscular activity, the plasma lactate declined significantly.

In conclusion, although xylazine produced a good state of immobilisation, it had a wide range of changes in haematological, biochemical and clinical parameters. Therefore, it is difficult to recommend the use of xylazine for immobilisation of Arabian oryx when safer alternatives are available. In addition, the biochemical, haematological and clinical parameters previously reported in immobilised Arabian oryx were substantially affected by immobilisation. Further comparative research is needed on the effects of other immobilising agents that are widely used in Arabian oryx such as etorphine, medetomidine and ketamine. Such research will allow the selection of the immobilising chemical with good immobilisation state and the least adverse effects on Arabian oryx.

In future studies the use of telemetric recording devices for recording body temperature, heart and respiratory rates could improve the results and more accurate conclusions could be reached.

The possible alternative to using xylazine alone is its use in combination with medetomidine, ketamine and butorphanol (torbugesic), which has been seen to give good results in Arabian oryx even during extremely hot climates. This combination was found to be better than ketamine-xylazine and etorphine-azaperone combinations in terms of effects on acid-base, electrolytes, haematology and biochemical parameters (Molnar and Mckinney, 2002; Molnarova et al., 2005). The effects of these recommended combinations should be further investigated in the Arabian oryx.

## **Chapter 6**

### **Monitoring the well-being of Arabian oryx during translocation and after release to the wild**

#### **6.1 Introduction**

Translocation is an important tool for conservation of wildlife. The term translocation is used to mean the introduction or re-introduction of a species to an area. For example, Griffith et al. (1989) defined translocation as the intentional release of animals to the wild in an attempt to establish, re-establish or augment a population. The Re-introduction Specialist Group defined translocation as “deliberate and mediated movement of wild individuals or populations from one part of their range to another” (IUCN, 1998). Sometimes the term translocation is used interchangeably to mean transportation of animals. However, transportation is one of many stages of translocation that include capture, confinement and release of animals.

After extinction of Arabian oryx in the wild in 1972 (Henderson, 1974), they were re-introduced in 1982 (Stanley Price, 1989). After thriving in the wild of Oman for a decade and a half until 1996, the population sharply declined, within three years, because of poaching (Spalton et al., 1999), and then gradually decreased to less than 30 males and no females nowadays (S. AlMahdhoury, Personal Communication, 2008). In 1998, a captive breeding programme was resumed and continues until now (2010). The re-establishment of a viable wild population depends on a solution to wildlife poaching, and the government of Oman has decided to fence the Arabian Oryx Sanctuary to protect the oryx from poachers. For future establishment of a viable free-ranging population of Arabian oryx, translocation of animals from captivity to the fenced area will be an essential process.

Most oryx nowadays live in captivity (Strauss, 2008) and their numbers continue to increase worldwide (Section 1.1.1 in Chapter 1). Arabian oryx is a social species, having a social hierarchy, where only one male is the dominant one and the rest of males are subordinates (Tear and Ables, 1999). There is continual fighting for dominance between the dominant and some subordinate adult males. The dominant male does not allow other males

to mate with females in the herd (Spalton, 1993; Tear and Ables, 1999; van Heezik et al., 2003). The fighting between males in captivity can lead to serious injuries or even death of some individuals in the herd (Personal observation). So, translocation of surplus males is necessary to maintain the well-being of the herd. Small scale translocation of oryx used to be applied for management purposes which involved release of males from captivity to the wild. In Oman, surplus males were regularly released from captivity to the wild as a measure to reduce the aggression and fighting between the dominant male and the subordinate males and also as a part in the breeding control programme (Al-Kharousi, 2003).

The process of translocation involves capture, temporary confinement in a small place, transport and then release or introduction of animals from one location to another. Capture has been reported to cause stress to animals (Harper and Austad, 2001; Montan, et al., 2002; Suleman et al., 2004). The most common method of capture in wildlife involves immobilisation. Chemical restraint has been reported to be less stressful than physical restraint in 712 individual animals of 18 wildlife species, as indicated by lower plasma cortisol (Morton et al., 1995). Transport is also stressful for wild and farm animals and can lead to injuries or mortality (Openshaw, 1993; Fazio and Ferlazzo, 2003). Confinement in a moving vehicle is one of the most stressful aspects of transportation as indicated by the increase in faecal glucocorticoid metabolites (Palme et al., 2000). Animals that are transported for the first time, particularly those that have never been handled before, may show a greater stress response, due to fear induced by the novelty of the situation (Grandin, 1997). Fear is a psychological stress that is governed by novelty (lack of previous experience) and genetic factors (Grandin, 1997). For example, transportation of naïve tigers resulted in much higher concentrations of plasma cortisol than in tigers that had previously experienced transport (Dembiec et al., 2004).

Before transportation of animals to another location, it has been recommended that wild animals should be held in temporary confinement for 4 to 8 weeks, until they become accustomed to the sight, sound and smells associated with human activity (Openshaw, 1993). This familiarisation process is often referred to as “boma training” and was found to help the animals become accustomed to handling over time (Openshaw, 1993).

Tranquillisers have been reported to play a role in ameliorating the effects of stress during transport. Perphenazine enanthate has a potential role in reducing stress for example in impala (Gandini et al., 1989), cheetah (Huber et al., 2001) and Eurasian otter (Ventura et al., 2002). Short- or long-acting tranquilisers have been used to facilitate transportation of wild animals (Burroughs, 1993; Ebedes, 1993a; Swan, 1993; Read and McCorkell, 2002). The long-acting tranquiliser, perphenazine enanthate, helped in reduction of stress in transported roe deer as implied by the decrease in faecal content of glucocorticoid metabolites compared to that in non-tranquilised deer (Dehnhard et al., 2001). In Arabian oryx in Saudi Arabia, a combination of familiarisation (“boma training”) for several weeks and use of perphenazine enanthate resulted in transport without mortality or adverse effects (Ancrenaz et al., 1995; Chassot et al., 2005; Strauss and Anagariyah, 2007), whereas previous transportations that did not use these methods (Ancrenaz et al., 1995) resulted in some mortality of the transported oryx. In Oman, for first re-introduction, Arabian oryx were transported by air from San Diego, USA to Oman and then by road with no report of using tranquilisers and no mortality occurred (Stanley Price, 1989). Further road transport of many oryx from Jaaluni to Muscat or Salalah and vice versa, without tranquilisers also resulted in no mortality cases (Spalton J.A., Personal Communication). However, the transport-associated mortalities reported in Saudi Arabia and subsequent regular use of tranquilisers to avoid mortalities during transport, encourage the assessment of physiological effects of these tranquilisers during transport.

There are two types of release of animals to the wild (soft versus hard). A soft release does not involve capture or handling of animals before release, but instead opening the gate of the enclosure to let the animals out. This type of release was used for the first re-introduction of Arabian oryx in Oman in 1982 (Stanley Price, 1989). Arabian oryx had been housed in a 1 km<sup>2</sup> enclosure in Jaaluni (Head Quarters of the Arabian Oryx Sanctuary) for two years and on the day of release to the desert the food and water troughs were moved 20 m away from the gate and the gate was open 1 h after feeding time. The oryx quietly left the enclosure (Stanley Price, 1989). Supplementary feeding was provided after release until rain fell and there was new grazing (Stanley Price, 1989). In contrast, hard release of animals is done by capturing them physically or chemically and then transporting them to the release site. For example, mountain sheep were transported either by helicopter or by

road to the release site after being held in holding pens (Thompson et al., 2001). Hard release has been used for releasing surplus adult male Arabian oryx from the enclosure at Jaaluni (Arabian Oryx Sanctuary) to avoid the fighting between them and aggression towards other oryx in the captive herd. Hard release is mainly used when the conditions around the enclosure are not good enough to provide sufficient vegetative food requirements for the herd. Therefore, a farther site with good vegetation cover is selected and oryx are transported to the site.

Releasing of animals from captivity to the wild has many consequences. The animals in the wild must become independent in searching for food and looking for water, because obviously food and water are not provided after release, as was the case before release, except for soft release. In arid and hot areas like Oman this is a concern as the animals might face malnutrition, starvation or dehydration and possible death (Spalton, 1999). Therefore, the release site must be carefully selected, based on the availability of food, water and shade.

This chapter describes studies that aimed to evaluate the effects of transport process on the haematology, serum ions, serum biochemical parameters and serum cortisol that were evaluated before and after transport. The studies also employed the newly validated enzyme immuno-assay that measures faecal glucocorticoid metabolites in oryx (described in Chapter 2) to assess acclimatisation of the Arabian oryx to a new environment after transport to a new captive enclosure, and after release from captivity to the wild.

## **6.2 Materials and methods**

### **6.2.1 Arabian oryx**

The Royal Court Affairs in Muscat planned translocation of some surplus male Arabian oryx from the Omani Mammals Breeding Centre to Jaaluni (Arabian Oryx Project) and 15 male oryx were employed in the studies described in this chapter. Three females were also donated by the Omani Mammals Breeding Centre for translocation to Jaaluni.

Adult Arabian oryx to be transported were captured initially from the large enclosures at the Omani Mammals Breeding Centre and moved to individual small holding pens (2.6 x

6.2 m) (Figure 2.4, Chapter 2). A total of 18 oryx were transported in three batches of six (Table 6.1).

<b>Batch</b>	<b>Animals</b>	<b>Date of capture</b>	<b>Date of transport</b>
A	Three males (1A, 5A and 10A) and three females (995, 996 and 997)	between August and September 2006	25 December 2006
B	six male oryx (1B, 10B, 16B, 17B, 19B and 20B)	January 2007	20 February 2007
C	six male oryx (1C, 10C, 16C, 17C, 19C and 20C)	March 2007	18 April 2007

Table 6.1 The Arabian oryx transported from the Omani Mammals Breeding Center to the Arabian Oryx Sanctuary.

While oryx were held in the pens before transport, they had health check-ups by the veterinarians of the Directorate General of Veterinary Services. Oryx received de-worming treatments against endo-parasites either orally (albendazole: Zentel suspension, Smith Kline & Beecham) or subcutaneously (0.5 ml, ivomic). The oryx of batch A were vaccinated against rinderpest and enterotoxemia. Oryx also had regular close contact with people during feeding, water provision and cleaning of the floor of the holding pens. During this period they became more familiar with people. For batch A, the oryx spent about 3-4 months in the holding pens, while oryx of batches B and C each spent about 1 month in the holding pens before transport.

The oryx of batches A and B were transported without tranquilization, while those of batch C were tranquillised. Two days before transport, the oryx in batch (C) were injected intramuscularly with the tranquilliser, perphenazine enanthate (Decentan Depot, 100 mg/ml; Merck, Germany) at a dose of 2 mg/kg. Blood samples were collected just before injection of the tranquilliser. A veterinarian from the Directorate General of Veterinary Services Royal Court Affairs accompanied the oryx to treat any adverse effects developing during transport.

## **6.2.2 Logistics and organisation of transport**

The Royal Court Affairs provided a 10-ton truck with a hydraulic winch, wooden crates and workers to help with loading of the Arabian oryx before transport and unloading afterwards. The truck was also used in releasing Arabian oryx to the wild.

Prior to transport, the Office for Conservation of the Environment and Arabian Oryx Project organised the preparation of holding pens at Jaaluni to allow receipt and care of the oryx while they were held in the pens. Accommodation and hospitality services were provided by the Arabian Oryx Project at Jaaluni camp for the team accompanying the transported oryx (veterinarian and assistant, drivers, workers). The Arabian Oryx Project also supplied ear tags and subcutaneous microchips for identification of Arabian oryx after transport and release.

## **6.2.3 Collection of blood and faecal samples**

Prior to loading for transport, oryx were captured, and blood samples were collected (as described in section 3.2.4). When available, a faecal sample was collected (Section 2.2.4). Blood and faecal samples were placed in a cool-box containing ice and transferred to a  $-20^{\circ}\text{C}$  freezer as soon as possible (within 3 hours maximum). An identification number (e.g. 1A, 1B, 1C..etc) was written with a stain (gentian violet) on the flank of each oryx for easy identification after transport.

## **6.2.4 Transport**

The oryx were placed in closely-fitting wooden crates and loaded with a winch onto the truck. The distance between Muscat and Jaaluni was 630 km and the journey took 9 h 45 min for batch A, 8 h 35 min for batch B and 8 h 20 min for batch C. The maximum ambient temperatures during transport of batches A, B and C, were 26, 30 and 42  $^{\circ}\text{C}$  (measured at 2 pm), respectively. After arrival, the crates were unloaded with a winch one at a time inside the holding pens and the oryx were captured from the crate for blood sampling. The unloading order of all batches was recorded except for batch A. The unloading order for batch B was 19B, 1B, 17B, 20B, 10B and 16B and for batch C was 1C, 10C, 16C, 17C, 19C and 20C.

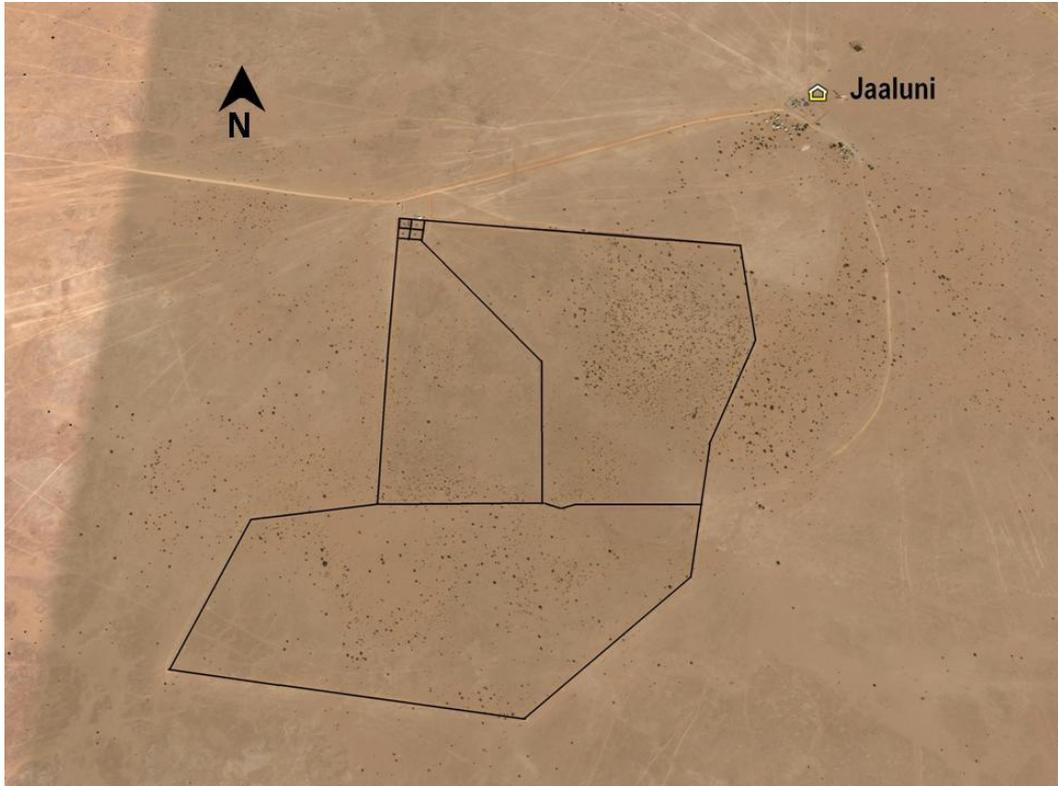


Figure 6.1 The Arabian oryx enclosure at Jaaluni with total area of 1.898 km<sup>2</sup>. The areas of the sub-enclosures are 0.734 km<sup>2</sup> (top right), 0.383 km<sup>2</sup> (top left) and 0.756 km<sup>2</sup> (the south enclosure). Holding pens are at the top left corner.

(A)



(B)

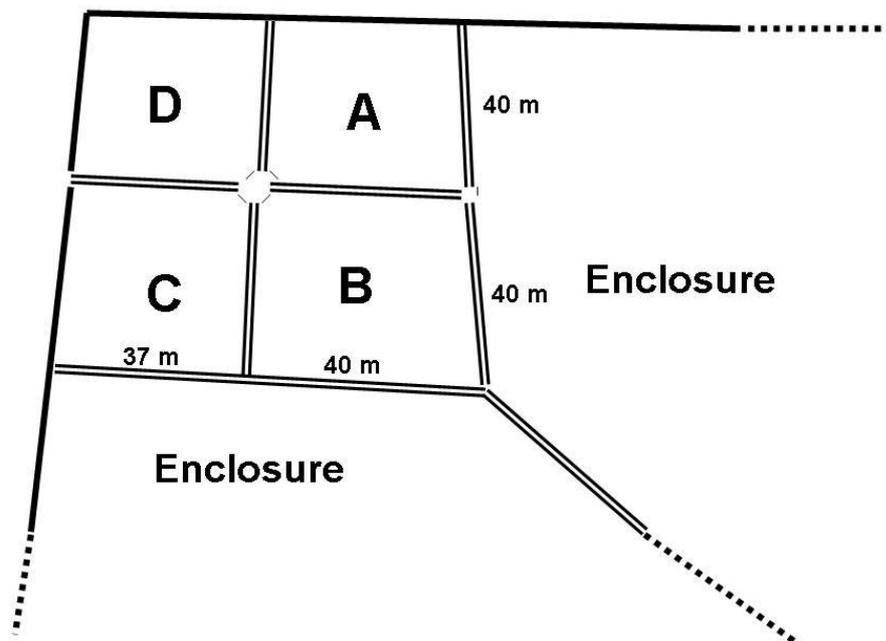


Figure 6.2 A satellite image (A) and a diagram (B) of the holding pens (A, B, C and D) in Jaaluni at the corner of the main oryx enclosure. Note the artificial shade within the holding pens in the satellite image.

### **6.2.5 Post-translocation monitoring**

Blood samples were collected (as described in Chapter 3, section 3.2.4) within 1 to 2 h after arrival at Jaaluni. The first batch (A) arrived after sunset and took about 2 h to unload and collect blood samples, but this time was reduced to about 1 h for later batches of oryx (B and C), which arrived before sunset.

For identification, the oryx were tagged on each ear with plastic-tags (Allflex, Australia) that had a combination of a unique number, shape and colour. Microchips (Trovan) were implanted under the skin of the oryx for identification and security purposes.

The enclosures at Jaaluni (Figure 6.1) have four holding pens, which are larger (about 40 m x 40 m) and fewer (four pens) (Figure 6.2) than those at the Omani Mammals Breeding Centre (2.6 x 6.2 m, 20 pens) (Figure 2.4). The six oryx in each transported batch were divided into two vacant pens, three in each pen. Food (hay and dried lucerne) and water were provided in the holding pens before arrival of oryx.

In December, 2006, oryx of batch A were housed in holding pen A (1A, 5A and 10 A) and pen B (995, 996 and 997). In February 2007, oryx of batch B were housed in pen A (1B, 17B and 19B) and pen C (10B, 16B and 20B). In April 2007, oryx of batch C were housed in pen A (16C, 17C and 1C) and in pen C (10C, 19C and 20C).

For 11 days after transport, fresh faecal samples were collected each morning (between 7-9 am) from the transported oryx in the holding pens. Within 1 h of collection the faecal samples were stored in a -20 °C freezer at Jaaluni. In order to collect fresh faecal samples from individual animals, the animals in relevant pens were observed for several hours, until defecation occurred. However, animals from batch A showed stereotypic behaviour in the first few days after transport (pacing along the fence of the pen back and forth) and this made the collection of faecal samples difficult. Some animals were aggressive and attempted to attack during the collection of faecal samples.

Before and after transport of oryx, some *ad hoc* notes about the behaviour of oryx were recorded for both tranquillised and non-tranquillised groups.

### **6.2.6 Release of oryx to the wild**

The male oryx from batches (A, B and C) were held in the holding pens in Jaaluni until release to the wild. These oryx were released to the wild either by “soft” or “hard” release. The three male oryx from batch A (1A, 5A and 10 A) were soft-released on the 20<sup>th</sup> of January, 2007, approximately one month after arrival in Jaaluni. Two of these animals rapidly moved away from Jaaluni and disappeared, and faecal samples could not be collected after release. One male (5A) remained around the Jaaluni enclosure and was housed later with the oryx of batch B. The three female oryx from batch A were not released to the wild, where there was a great risk of poaching of females, but were introduced to the captive herd of oryx in the larger Jaaluni enclosure before the transport of batch B.

The hard release method uses capture (by immobilisation or net) followed by transport of animals to a selected release site far away from where they have been housed. The immobilisation involved darting oryx using a blowpipe with a combination of 1.5 ml ketamine and 1.5 ml medetomidine and reversed by 3 ml atipamezole. For hard release, a site was selected which had a good availability of suitable food, mainly grass *Stipagrostis spp.* which is favoured by oryx (Stanley Price, 1989; Tear, 1992; Spalton, 1999) and trees e.g. *Acacia tortilis* or *Prosopis cineraria* that are used for shade (Seddon and Ismail, 2002). The oryx of batch B plus male 5A from batch A and oryx from batch C plus male 10B were transported to release sites about 50 and 70 km away from Jaaluni, respectively (Figure 6.3). The two sites were 24 km apart. The ambient temperature at the point of release for batch B was 31 °C, at 9 am, and increased in the afternoon to 42 °C, at 3 pm. The ambient temperature at the point of release of batch C was 42.5 °C, at 11 am.

The oryx in batch B together with oryx 5A from batch A were hard-released on 19 April, 2007. One oryx (10 B) returned to Jaaluni two days after hard release, crossing a distance of more than 50 km. This male oryx was herded later with batch C in pen C on 21 April 2007. The oryx in batch C and oryx 10B were hard-released on 16 May 2007.

On the day of release to the wild from the Jaaluni holding pens, the oryx were captured by immobilization. They were injected intramuscularly, from a distance, using a blowpipe to deliver a dart containing a combination of 1.5 ml medetomidine (a dosage of about 30

µg/kg) (Domitor, 1 mg/ml, Novartis) and 1.5 ml ketamine (a dosage of about 3 mg/kg) (Ketavet, 100 mg/ml; Parke-Davis GmbH, Berlin-Germany). When the oryx became totally or partially immobilized, they were taken close to the crate and the effect of immobilization was reversed with 3 ml of atipamezole (a dosage of about 0.3 mg /kg) (Antisedan, 5 mg/ml; Novartis) and then placed into the crate.

After the “hard release” of batch B (plus 5A) and batch C (plus 10B), the oryx were tracked for 11 days to attempt to collect faecal samples. The white colour of Arabian oryx makes them conspicuous on the open desert from a long distance. The tracking involved several patrol rangers from the Arabian Oryx Project, use of binoculars, a Global Positioning System (GPS) (12XL, Garmin) and information from local people. The GPS was used to record the coordinates of the sites of release and the places where the oryx were sighted. The recorded mapping data were processed using PC software (MapSource, Garmin). The animals were located either by following their distinctive footprints, or by using a binocular from a high place like a hill or standing on the roof of the vehicle.

Whenever released oryx were found, fresh faecal samples were collected. Fresh faecal specimens were placed immediately in a cool-box containing a solid block of ice and were transferred to the – 20 °C freezer immediately on return to Jaaluni. All collected faecal samples were stored in the freezer within 24 h from collection.

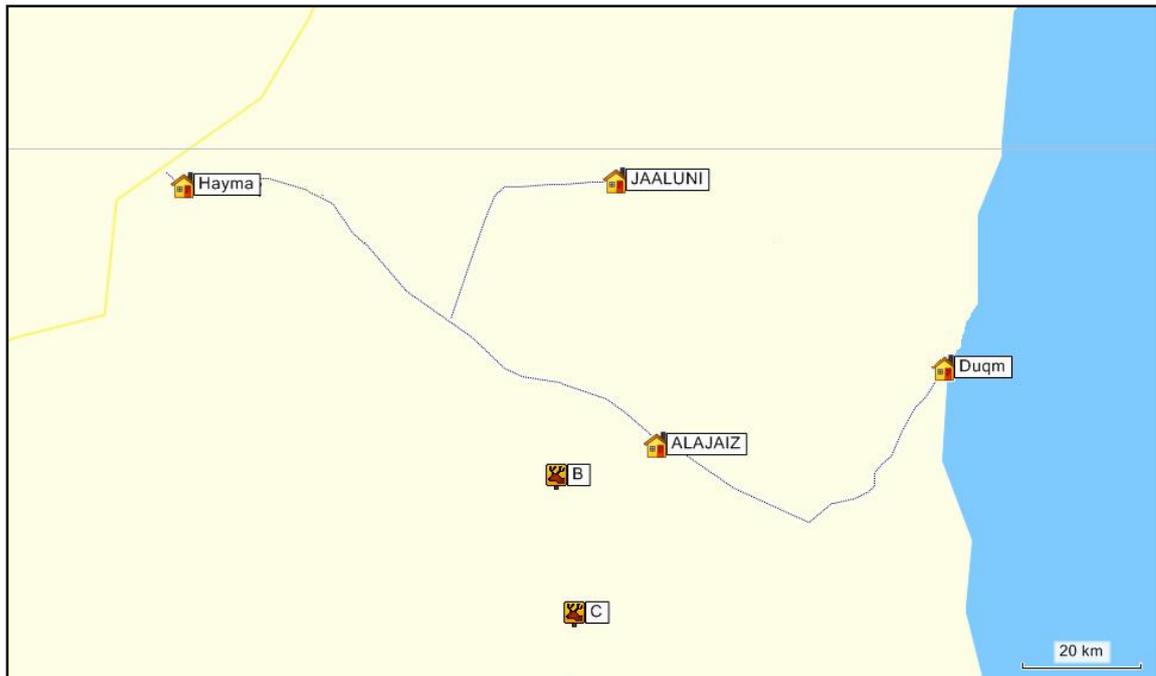


Figure 6.3 Arabian oryx were released from Jaaluni ( $19^{\circ} 56' 45.7''$  N,  $57^{\circ} 06' 11.8''$  E) to the wild at the sites ( $19^{\circ} 29' 48.8''$  N,  $56^{\circ} 59' 38.4''$  E) for group B and ( $19^{\circ} 17' 21.0''$  N,  $57^{\circ} 01' 32.4''$  E) for group C. The map was produced using (MapSource, Garmin).

### 6.2.7 Analysis of faecal samples

Faecal glucocorticoid metabolites were extracted (section 2.2.5), measured by enzyme immuno-assay (EIA I) for all samples and (EIA II) for some samples, as described in Chapter 2, section 2.2.8. A baseline reference for faecal glucocorticoid metabolites of 273 ng/g faeces, obtained in Chapter 2, (section 2.3.1.1) prior to ACTH injection, was used as a reference value for evaluating the magnitude of stress resulting from transport and release of the Arabian oryx.

### 6.2.8 Analysis of blood samples

The haematological analysis of blood samples collected before transport was carried out within about 2 h of collection. The blood was centrifuged at 2500 g for 15 min (Mistral

3000). The serum and plasma was transferred to polypropylene microcentrifuge tubes, which were stored in a – 80 °C freezer.

Blood samples collected after translocation and used for haematological analysis were stored in a refrigerator overnight at Jaaluni. The following morning these samples were taken to the Department of Haematology at the Sultan Qaboos University Hospital in Muscat, for analyses (as described in section 3.2.4).

Samples for the analysis of serum osmolality (Section 3.2.8), serum ions, biochemical parameters (urea, total protein, albumin and glucose) and serum cortisol (Section 3.2.7) were centrifuged at 2500 g for 15 min (Hawksley, England). The serum was placed in polypropylene microcentrifuge tubes, which were stored in a container of liquid nitrogen. The following day, these samples were taken to Sultan Qaboos University and transferred to a – 80 °C freezer until analysis.

For three oryx in batch A (females 995, 996 and the male 10A), no haematological data could be obtained after transport because either the collected samples clotted (10A and 995) or no sample could be collected (996).

### **6.2.9 Statistics**

Normality of data was measured using the Kolmogorov-Smirnov test. The haematological data of oryx transported under tranquillisation (batch C) were normally distributed, except for two parameters that had to be transformed to achieve normality using square transformation (% neutrophils) and rank transformation (% basophils). Haematological data were compared between the three sampling time points i.e., 2 days before transport (before injection of the tranquilliser, perphenazine enanthate), before departure, and just after arrival, using one way repeated measures ANOVA.

For the oryx transported without tranquilliser, haematological data were normally distributed, except for % neutrophils and % lymphocytes, which were transformed to normality using rank and reciprocal transformations, respectively. The haematological data for these oryx were analysed using paired t-tests for differences between data obtained for samples collected before and after transport. The difference between haematological data

after transport for both tranquillised and non-tranquillised oryx were tested using t-tests or Mann-Whitney U tests, as appropriate.

The serum ions, biochemical parameters and serum cortisol concentration, for animals transported under tranquilization, were normally distributed except for serum urea, which did not achieve normality, despite several attempted transformations. The normal data were analysed using one way repeated measures ANOVA for the 3 sampling times, while the urea data were analysed using Freidman's repeated measures ANOVA on ranks.

The serum ions, biochemical parameters and serum cortisol of animals transported without tranquilization were normally distributed except albumin and phosphorus that did not achieve normality by transformation. Data before and after transport were analysed using either paired t-tests (for normally distributed data) or Wilcoxon tests (for non-parametric data).

Collection of faecal samples from each oryx every day after transport or after release was not feasible because some oryx excreted no faeces during the observation time (7-9 am). Therefore, there were missing values for faecal glucocorticoid metabolites in different animals on different days, which impeded robust statistical analysis of data, for example by one way repeated measures ANOVA. The data, therefore, were analysed using a Kruskal-Wallis one-way ANOVAs comparing the faecal content of glucocorticoid metabolites from the samples that were obtained each day. A basal value for faecal content of glucocorticoid metabolites obtained in Chapter 2 (section 2.3.1.1) prior to ACTH challenge was used as a reference value to which the overall changes after transport or release were compared. Data throughout this chapter are presented as mean  $\pm$  standard error unless stated otherwise.

## 6.3 Results

### 6.3.1 Transport

#### 6.3.1.1 *General behavioural observations during transport*

##### *Batches A and B (without tranquillisation)*

The oryx transported in batches A and B did not show any evidence of muscular weakness or exhaustion after transport, in contrast to the tranquillised animals in batch C (see below). Oryx of batches A and B moved about actively within the holding pen on arrival to Jaaluni and for several days after. Some oryx showed stereotypic pacing along the fence of the holding pen lasting several days. However, the oryx fed and drank water normally. They showed no muscular weakness or fatigue after transport, unlike the oryx of batch C.

##### *Batch C (with tranquillisation)*

Two days after injection of the oryx in batch C with perphenazine enanthate and just before transport, these oryx were calm and not active. After translocation, oryx 16C, 17 C and 20C started eating the supplied food shortly after download. Oryx numbered 1C, 10C and 19C showed signs of exhaustion and fatigue. After transport, oryx 19C showed stiffness of muscles especially with neck bent backwards (torticollis) and was treated with a multi-vitamin injection. Torticollis is a sign of capture myopathy beside other signs like poor appetite, exhaustion, and depression. Because of capture myopathy symptoms, the animal probably did not drink or feed after arrival. Oryx 10C was very weak and lay down on the ground shortly after download. This oryx was treated by subcutaneous administration of glucose and saline after blood sampling. Oryx 1C was also weak and lay down shortly after download (within 1 h after arrival).

One day after transport, not all the oryx were active. Oryx (19C) was still showing symptoms of capture myopathy and dehydration as indicated by stiff skin and therefore about 1L of saline was administered subcutaneously followed by an injection of 8 ml of flunixin (Finadyne) as a painkiller. None of the oryx were as active as those in batches A and B that were transported without tranquilization. The weakness and fatigue of 1C and

10C started to subside on the second day after transport. On the third day after transport, the animals were still not active and none of the oryx were seen running or moving actively. The symptoms of capture myopathy in the oryx 19C had improved by the third day as the neck was not bent anymore, and it was eating, walking and standing. Six days after transport (which was 8 days after the injection of the perphenazine enanthate), all of the oryx were active and some were seen fighting (10C, 16C and 1C), running (17C), or attempting an attack when approached (20C), while 19C had recovered from its capture myopathy and showed full activity of running, walking and eating normally.

#### *6.3.1.2 Haematology*

All haematological values measured before transport for batches A and B and just before the injection of batch C with perphenazine enanthate (Table 6.4), were within the established reference ranges derived in Chapter 3 (Table 3.7).

##### *Batches A and B (without tranquillisation)*

For non-tranquillised oryx, the values for haematological parameters of blood samples collected before and after transport were compared (Table 6.2 and Figure 6.4). Male oryx in batches A and B, transported without tranquillisation, showed significant increases in the white blood cell (leukocytes) count ( $P = 0.020$ ) and the percentage of neutrophils ( $P = 0.006$ ) and significant decreases in the percentages of lymphocytes ( $P = 0.007$ ) and eosinophils ( $P = 0.010$ ) after transport (Table 6.2). No other significant changes in haematological parameters were found after transport in the absence of tranquillisation (Table 6.2).

Haematological data was only available for one translocated female (997) as explained in (Section 6.2.8) (Table 6.3). The haematological values of female 997, like the males, showed increases in the white blood cell count and the percentage of neutrophils and decreases in the percentages of lymphocytes and eosinophils (Table 6.3). But unlike males (Table 6.2), the haematocrit and the mean corpuscular volume were noticeably lower after transport.

*Batch C (with tranquillisation)*

To evaluate the effect of transportation during tranquillisation on the haematological parameters, the values for blood samples collected immediately prior to injection of perphenazine enanthate (2 days before transport) and immediately before transport were compared to those after transport (Table 6.4). There were significant increases in the white blood cell count ( $P < 0.001$ ), reflecting increases in the percent neutrophils ( $P < 0.001$ ) and percent monocytes ( $P < 0.01$ ). On the other hand, there were significant decreases in the percentage of lymphocytes ( $P < 0.01$ ), eosinophils ( $P < 0.01$ ) and basophils ( $P < 0.01$ ) (Figure 6.5).

Transportation had no effect on the red blood cell count, haematocrit, blood haemoglobin concentration and mean corpuscular haemoglobin concentration. Values for corpuscular volume and corpuscular haemoglobin (Table 6.4) were slightly reduced after transport compared with values before injection of perphenazine. However, these changes had already begun before transportation (Table 6.3). There were no other significant changes in haematological parameters relating to the erythrocytes between injection of tranquilliser and blood re-sampling immediately before transport.

There were no significant differences between values for haematological parameters after transport in tranquillised and non-tranquillised oryx (Figure 6.4, Figure 6.5; Table 6.2 and Table 6.3; t-tests,  $P > 0.05$ ).

*6.3.1.3 Serum biochemistry and ions*

The mean concentrations of ions in serum, and serum osmolality measured in blood samples collected before transport of non-tranquillised oryx (batches A and B) (Table 6.5) and those collected immediately before injection of perphenazine enanthate and immediately before transport for tranquillised oryx from batch C (Table 6.6) were within the reference inter-percentile ranges established in chapter 3 (Table 3.11).

For oryx transported without tranquillisation i.e. batches A and B, there were no changes in the serum osmolality, or the serum concentrations of sodium, potassium, chloride, calcium,

and phosphorus, however a slight, but significant decrease in serum magnesium (Table 6.5).

Table 6.6 shows the data for serum ions and serum osmolality for oryx transported under tranquillisation. There was no difference between the serum concentrations of sodium, chloride, magnesium, calcium, and blood osmolality in samples collected immediately before injection of perphenazine enanthate and samples collected 1-2 h after transport. However, there were significant decreases in the concentration of potassium ( $P < 0.05$ ) and phosphorus ( $P < 0.01$ ) after transport compared to data immediately before injection with perphenazine enanthate (Table 6.6).

The serum concentration of total protein increased slightly (4 %) but significantly after transport of batches A and B without tranquillisation (Table 6.7). The serum concentration of urea, albumin and glucose in batch A and B did not change after transport (Table 6.7).

Table 6.7 gives data for serum glucose, urea, albumin and total protein in batch C oryx transported under tranquillisation with perphenazine enanthate. The serum concentration of glucose increased significantly after transport in comparison to the values measured in the blood samples collected immediately prior to injection of the tranquilliser ( $P < 0.05$ , Table 6.8), but was not significantly elevated compared to the glucose concentration immediately prior to transport. The serum concentration of urea, total protein and albumin did not differ after transport in tranquillised oryx from those prior to transport or immediately prior to injection of the tranquilliser (Table 6.8).

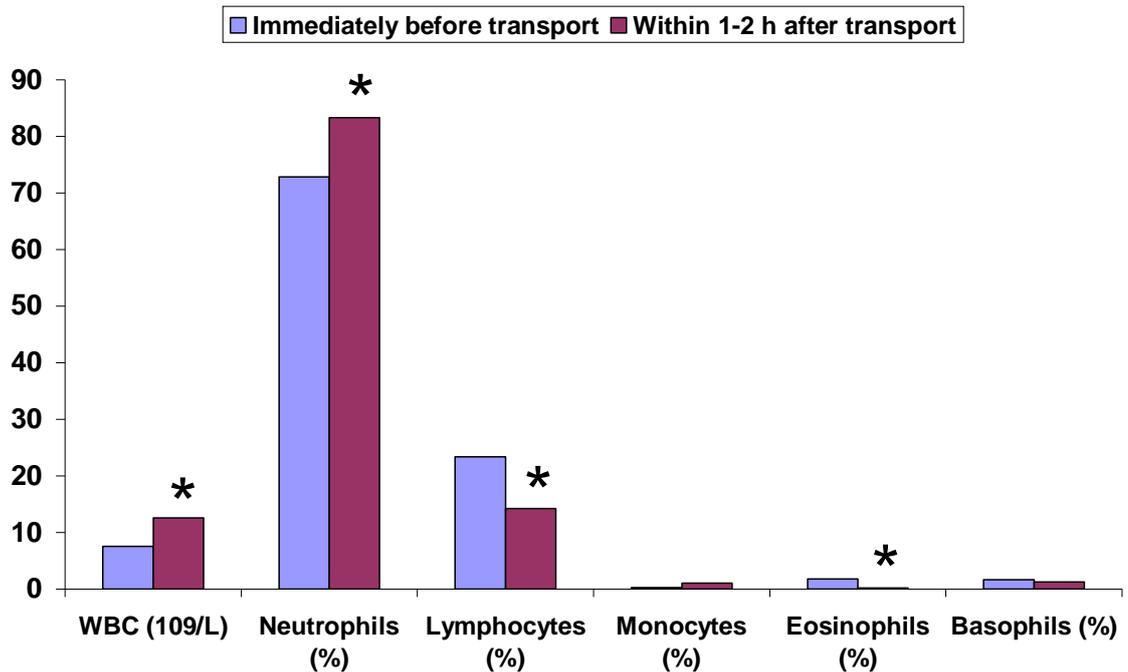


Figure 6.4 White blood cell count (WBC,  $\times 10^9/\text{L}$ ) and the percentages of differentials of seven male Arabian oryx (from batches A and B) measured immediately before transport and within 1-2 h after transport. \*  $P < 0.05$ , paired t-test.

<b>Parameters</b>	<b>Immediately before transport</b>	<b>Within 1-2 h after transport</b>
RBC ( $10^{12}/\text{L}$ )	12.10 (0.37)	11.91 (0.38)
Haemoglobin (g/dL)	17.87 (0.70)	17.91 (0.66)
Haematocrit (%)	54.40 (1.89)	53.00 (2.15)
MCV (fL)	44.96 (0.60)	44.39 (0.74)
MCH (pg/cell)	14.79 (0.21)	15.01 (0.22)
MCHC (g/dL)	32.86 (0.57)	33.87 (0.44)

Table 6.2 Haematological parameters of seven male Arabian oryx (from batches A and B) measured immediately before transport and within 1-2 h after transport. Data are shown as mean  $\pm$  standard error of mean. RBC, red blood cells (erythrocytes); MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; and MCHC, mean corpuscular haemoglobin concentration.

<b>Parameters</b>	<b>Immediately before transport</b>	<b>Within 1-2 h after transport</b>
WBC ( $10^9/L$ )	5.81	12.60
Neutrophils (%)	63.20	86.70
Lymphocytes (%)	28.80	12.80
Monocytes (%)	1.38	0.12
Eosinophils (%)	3.72	0.37
Basophils (%)	2.92	0.00
RBC ( $10^{12}/L$ )	12.70	12.00
Haemoglobin (g/dL)	19.10	18.00
Haematocrit (%)	56.10	49.50
MCV (fL)	44.30	41.40
MCH (pg/cell)	15.10	15.00
MCHC (g/dL)	34.00	35.30

Table 6.3 Haematological parameters in one female (997) from batch A transported without tranquillisation. WBC, white blood cells (leukocytes); RBC, red blood cells (erythrocytes); MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; and MCHC, mean corpuscular haemoglobin concentration.

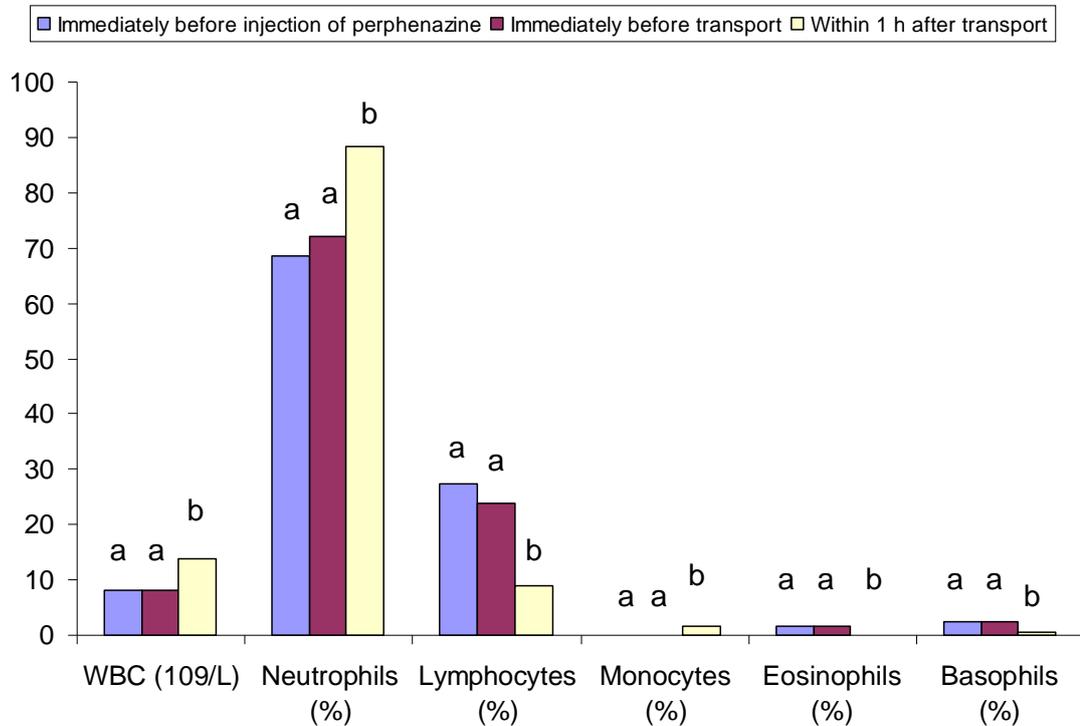


Figure 6.5 White blood cell (WBC,  $\times 10^9/\text{L}$ ) count and differentials of six male Arabian oryx (batch C) measured immediately before injection of tranquilliser perphenazine enanthate (two days before transport), immediately before transport and within 1h of translocation. Data with different letters are significantly different from each other (One way repeated measures ANOVA and post hoc multiple comparisons by Holm-Sidak method).

<b>Parameters</b>	<b>Immediately before injection of perphenazine</b>	<b>Immediately before transport</b>	<b>Within 1 h after transport</b>
RBC ( $10^{12}/L$ )	12.40 (0.26) <sup>a</sup>	12.73 (0.30) <sup>a</sup>	12.37 (0.33) <sup>a</sup>
Haemoglobin (g/dL)	18.72 (0.37) <sup>a</sup>	18.13 (0.56) <sup>a</sup>	17.93 (0.35) <sup>a</sup>
Haematocrit (%)	54.80 (0.01) <sup>a</sup>	55.30 (1.82) <sup>a</sup>	53.40 (1.35) <sup>a</sup>
MCV (fL)	44.15 (0.67) <sup>a</sup>	43.37 (0.65) <sup>a,b</sup>	43.08 (0.48) <sup>b</sup>
MCH (pg/cell)	15.10 (0.13) <sup>a</sup>	14.22 (0.28) <sup>b</sup>	14.50 (0.17) <sup>b</sup>
MCHC(g/dL)	34.22 (0.32) <sup>a</sup>	32.82 (0.41) <sup>a</sup>	33.65 (0.23) <sup>a</sup>

Table 6.4 Haematological parameters of six male Arabian oryx (batch C) measured immediately before injection of tranquilliser perphenazine enanthate (two days before transport), immediately before transport and within 1h of translocation. Data are shown as mean (standard error). Data with different letters are significantly different from each other (One way repeated measures ANOVA and post hoc multiple comparisons by Holm-Sidak method. RBC, red blood cells (erythrocytes); MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; and MCHC, mean corpuscular haemoglobin concentration.

<b>Parameters</b>	<b>Immediately before transport</b>	<b>Within 1-2 h after transport</b>	<b>P-Value</b>
Sodium (mmol/L)	145.89 (0.94)	143.33 (1.82)	P = 0.098
Potassium (mmol/L)	5.14 (0.26)	5.18 (0.17)	P = 0.889
Chloride (mmol/L)	102.33 (0.90)	103.00 (1.35)	P = 0.567
Calcium (mmol/L)	2.21 (0.02)	2.20 (0.04)	P = 0.664
Magnesium (mmol/L)	1.28 (0.04)	1.19 (0.03)	P = 0.044
Phosphorus (mmol/L)	2.59 (0.13)	3.94 (2.26)	P = 0.129
Osmolality (mosmo/L)	308.14 (2.35)	306.00 (3.55)	P = 0.560

Table 6.5 Serum ions and osmolality measured in nine male Arabian oryx (batches A and B) measured immediately before transport and within 1-2 h after transport (paired t-test). Data are shown as mean  $\pm$  standard error.

<b>Parameters</b>	<b>Immediately before injection of perphenazine</b>	<b>Immediately before transport</b>	<b>Within 1 h after transport</b>
Sodium (mmol/L)	143.83 (2.21) <sup>a</sup>	141.17 (2.21) <sup>a</sup>	144.50 (0.43) <sup>a</sup>
Potassium (mmol/L)	7.23 (0.76) <sup>a</sup>	6.15 (0.37) <sup>a,b</sup>	4.95 (0.25) <sup>b</sup>
Chloride (mmol/L)	104.33 (1.52) <sup>a</sup>	101.17 (0.87) <sup>b</sup>	107.67 (1.36) <sup>a</sup>
Calcium (mmol/L)	2.22 (0.08) <sup>a</sup>	2.26 (0.03) <sup>a</sup>	2.21 (0.04) <sup>a</sup>
Magnesium (mmol/L)	1.10 (0.05) <sup>a</sup>	1.07 (0.04) <sup>a</sup>	1.01 (0.03) <sup>a</sup>
Phosphorus (mmol/L)	2.70 (0.27) <sup>a</sup>	2.21 (0.32) <sup>a,b</sup>	1.71 (0.13) <sup>b</sup>
Osmolality (mosmo/L)	307.67 (6.31) <sup>a</sup>	298.92 (5.17) <sup>a</sup>	308.08 (2.84) <sup>a</sup>

Table 6.6 Serum ions and osmolality of six male Arabian oryx measured immediately before injection of perphenazine enanthate (2 days before transport), immediately before transport and within 1 h after transport. Data are shown as mean  $\pm$  standard error. Data with different letters are significantly different from each other (one way repeated measures ANOVA).

<b>Parameters</b>	<b>Immediately before transport</b>	<b>Within 1-2 h after transport</b>	<b>P-Value</b>
Urea (mmol/L)	6.71 (0.45)	7.16 (0.49)	P = 0.082
Total Protein (g/L)	70.22 (1.06)	73.00 (0.99)	P = 0.027
Albumin (g/L)	52.33 (1.39)	48.39 (5.84)	P = 0.195
Glucose (mmol/L)	7.54 (0.57)	7.00 (0.31)	P = 0.479

Table 6.7 Biochemical parameters of nine male Arabian oryx measured just before transport and shortly after transport (paired t-test). Data are shown as mean  $\pm$  standard error.

<b>Parameters</b>	<b>Immediately before injection of perphenazine</b>	<b>Immediately before transport</b>	<b>Within 1 h after transport</b>
Urea (mmol/L)	7.97 (0.83) <sup>a</sup>	5.07 (0.63) <sup>b</sup>	6.33 (0.77) <sup>a,b</sup>
Total Protein (g/L)	70.50 (2.55) <sup>a</sup>	69.67 (1.99) <sup>a</sup>	68.00 (1.27) <sup>a</sup>
Albumin (g/L)	50.67 (1.15) <sup>a</sup>	51.67 (1.56) <sup>a</sup>	51.50 (1.73) <sup>a</sup>
Glucose (mmol/L)	5.76 (0.78) <sup>a</sup>	5.64 (0.32) <sup>a,b</sup>	8.89 (1.27) <sup>b</sup>

Table 6.8 Biochemical parameters of six male Arabian oryx measured at injection of tranquilliser (2 days before transport), just before transport and shortly after transport. Data are shown as mean  $\pm$  standard error. Data with different letters are significantly different from each other (one way repeated measures ANOVA).

#### 6.3.1.4 *Serum cortisol*

Individual data obtained for concentration of serum cortisol in oryx before and after transport are shown in Table 6.9. The concentration of cortisol in samples collected from non-tranquillised oryx before transport ( $125.38 \pm 16.21$  nmol/L) and after transport ( $111.57 \pm 15.66$  nmol/L) did not differ significantly (paired t-test,  $P = 0.740$ ). In tranquillised oryx, the concentration of cortisol after transport ( $217.18 \pm 49.81$  nmol/L) did not differ significantly from the values measured immediately before injection of perphenazine enanthate ( $141.00 \pm 26.90$  nmol/L) (one way repeated measures ANOVA,  $P > 0.05$ ), but was significantly higher than the values measured in blood samples collected just before transport ( $73.83 \pm 18.59$ ) (one way repeated measures ANOVA,  $P < 0.05$ ). There were variations between individual oryx in the concentration of cortisol before and after transport, in both tranquillised and non-tranquillised oryx (Table 6.9).

#### 6.3.1.5 *Faecal glucocorticoid metabolites*

A scatter plot for the immuno-reactive faecal glucocorticoid metabolites measured in faecal samples collected before and after transport for each oryx is shown in Figure 6.6. Faecal samples collected one day before transport showed values close to or above the baseline, with the highest value for oryx 16C (Figure 6.6). The samples collected on the day of transport were generally close to the baseline. One day after transport, no faecal samples were collected due to travel from Jaaluni to Muscat (630 km) to carry out haematological analyses of blood samples. The values for faecal content of glucocorticoid metabolites on day two after transport were generally higher than those of other days but showed considerable scatter. Table 6.10 shows that oryx of batch B had a much higher mean faecal glucocorticoid content 2 days after transport than before transport. From day 3 to 11 after transport, most values for the glucocorticoid metabolite content of faecal samples were close to the baseline (Figure 6.6). Figure 6.7 shows the median faecal content of glucocorticoid metabolites before and after transport. On most days, the faecal content of glucocorticoid metabolites did not differ significantly from each other. However, on day 2 after transport there was a significantly higher content than at day 8 after transport (Figure 6.7; Kruskal-Wallis one way ANOVA,  $P < 0.05$ ).

(A)

<b>Animal</b>	<b>Immediately before transport</b>	<b>Within 1-2 h after transport</b>
5A	153.00	NA
10A	152.00	100.00
1B	130.00	76.00
10B	170.00	160.00
16B	76.00	121.00
17B	79.00	103.00
19B	179.00	54.00
20B	64.00	167.00

(B)

<b>Animal</b>	<b>Immediately before injection with perphenazine</b>	<b>Immediately before transport</b>	<b>Within 1 h after transport</b>
1C	139.00	47.00	380.00
10C	213.00	46.00	139.00
16C	NA	42.00	109.00
17C	NA	109.00	161.00
19C	129.00	151.00	366.00
20C	83.00	48.00	148.00

Table 6.9 The concentration of serum cortisol (nmol/l) in individual non-tranquillised (A) and tranquillised Arabian oryx (B). NA, not available.

<b>Batch</b>	<b>1 day before transport</b>	<b>immediately prior to transport</b>	<b>2 days after transport</b>
A	NA	101 (1)	NA
B	384.25 (4)	415.25 (4)	1155.4 (5)
C	652.75 (4)	NA	NA

Table 6.10 The mean immuno-reactive faecal glucocorticoid metabolites (ng/g faeces) for the three transported batches at one day before transport, immediately before transport and 2 days after transport to Jaaluni. Number of samples given in brackets. NA, not available.

### **6.3.2 Release of oryx to the wild**

After hard release of batch B plus male (5A) and batch C plus 10 B to the wild, the oryx were tracked for collection of faecal samples in attempt to monitor their stress status, using faecal glucocorticoid metabolites. The tracking of oryx after release is summarised in Figure 6.8 for batch B and in Figure 6.9 for batch C.

The immuno-reactive faecal glucocorticoid metabolites measured in the faecal samples of the released oryx, collected on the day of release and one day after the release, were generally low and close to baseline, except for 1C, but later samples showed a high faecal content of glucocorticoid metabolites (Figure 6.10). Most values of faecal glucocorticoid metabolites measured in samples collected on days 2-6 after release were very high compared to the baseline (Figure 6.11). The male oryx, 19B showed the highest value of 3924 ng/g faeces on day three after release (Figure 6.10). This male, as shown in (Figure 6.8), was found at site 3 with two other males, 16B and 17B that also showed very high levels of faecal glucocorticoid metabolites. Between day 7 and 10 days after release, no faecal samples could be collected because the oryx were not found during attempted tracking. On day 11, one faecal sample was collected from the male oryx 19B and had a glucocorticoid metabolite content that was close to the baseline, which might indicate recovery.

The male 10B that returned to Jaaluni within 2 days having travelled a distance of more than 50 km from the site of release, had very high values of immuno-reactive faecal glucocorticoid metabolites in the first sample collected after return to Jaaluni (2301 ng/g faeces by EIA I) and (4175 ng/g faeces by EIA II). Subsequently, the faecal content of glucocorticoid metabolites collected from this oryx on the six following days decreased dramatically to below the basal line (Figure 6.12).

Sadly, oryx, 19B was found dead by the rangers of the Arabian Oryx Project about six months after release (October 2007). It is believed that it had been killed by poachers.



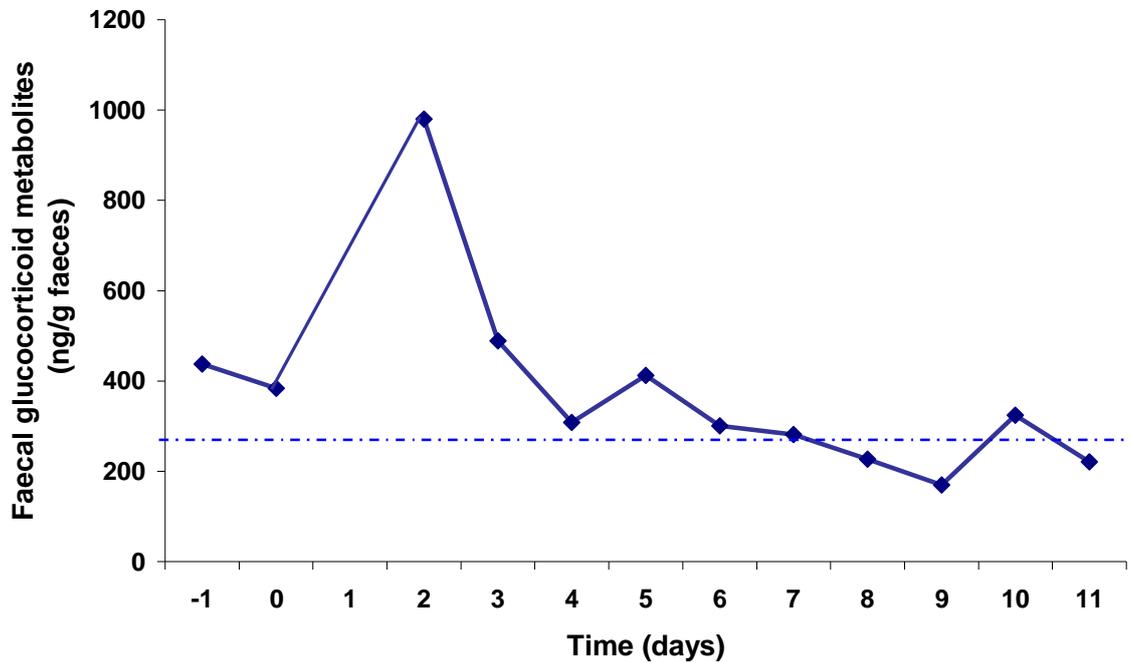


Figure 6.7 Median of faecal glucocorticoid metabolites one day before and 11 days after transport from Muscat to Jaaluni holding pens. The blue horizontal line represents the baseline value (273 ng/g faeces) obtained in Chapter 2, (see section 2.3.1.1).

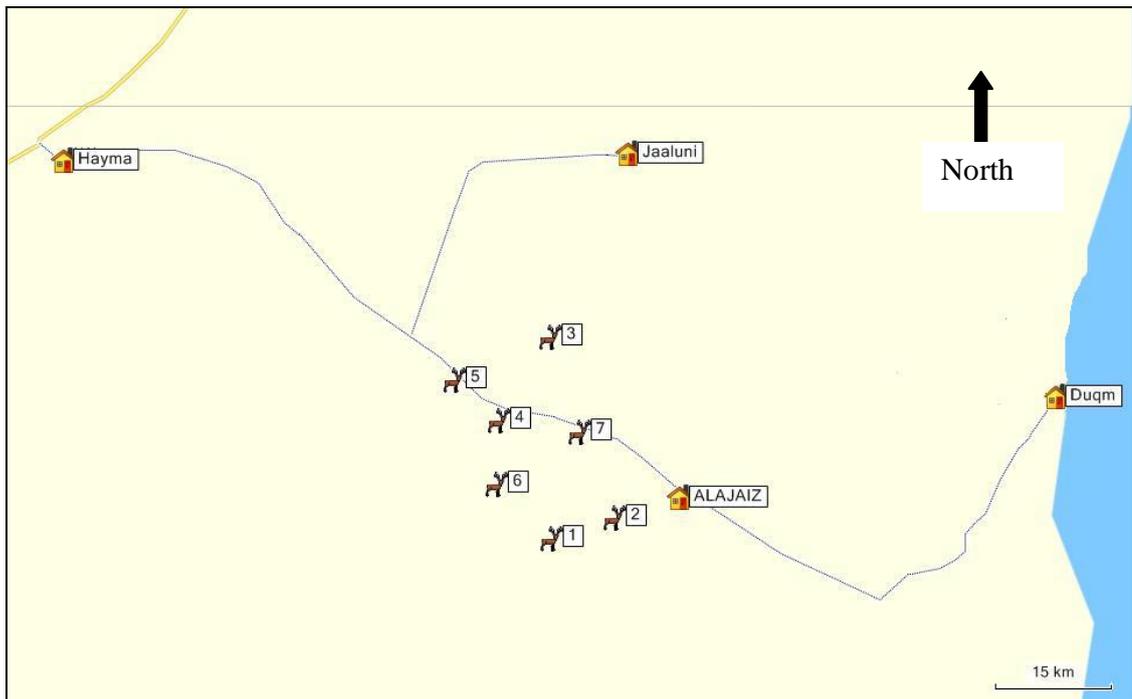


Figure 6.8 Release of batch B on 19 April 2007 was at site 1. The male oryx, 1B was found at site 2, one day after release at 3 pm (temperature 45 °C). The male oryx, 10B returned to Jaaluni (50 km away from site 1, within two days from release and was housed in Jaaluni holding pens with oryx of batch C and faecal samples were collected for up to seven days. On day 3 after release, three oryx (16B, 17B and 19B) were found at site 3, walking towards Jaaluni, at 6 pm (37 °C) but they were herded back to the south away from Jaaluni. They were observed foraging for food while walking at that day and water was offered to these three oryx but they refused to drink, suggesting a lack of thirst. On day 4 after release, a male oryx, 20B was found close to the tarmac road at site 4, at 9 am (35 °C). On day 5, the three male oryx (16B, 17B and 19B), were found again together at site 5 at 6 pm (34 °C), which was also close to the road. On day 6, oryx 20B was seen at site 6, at 10 am (37 °C), which is about 9 km away from where the oryx was last seen at site 4. At site 7, oryx 19B was found alone on day 11, indicating that it had separated from 16B and 17B.

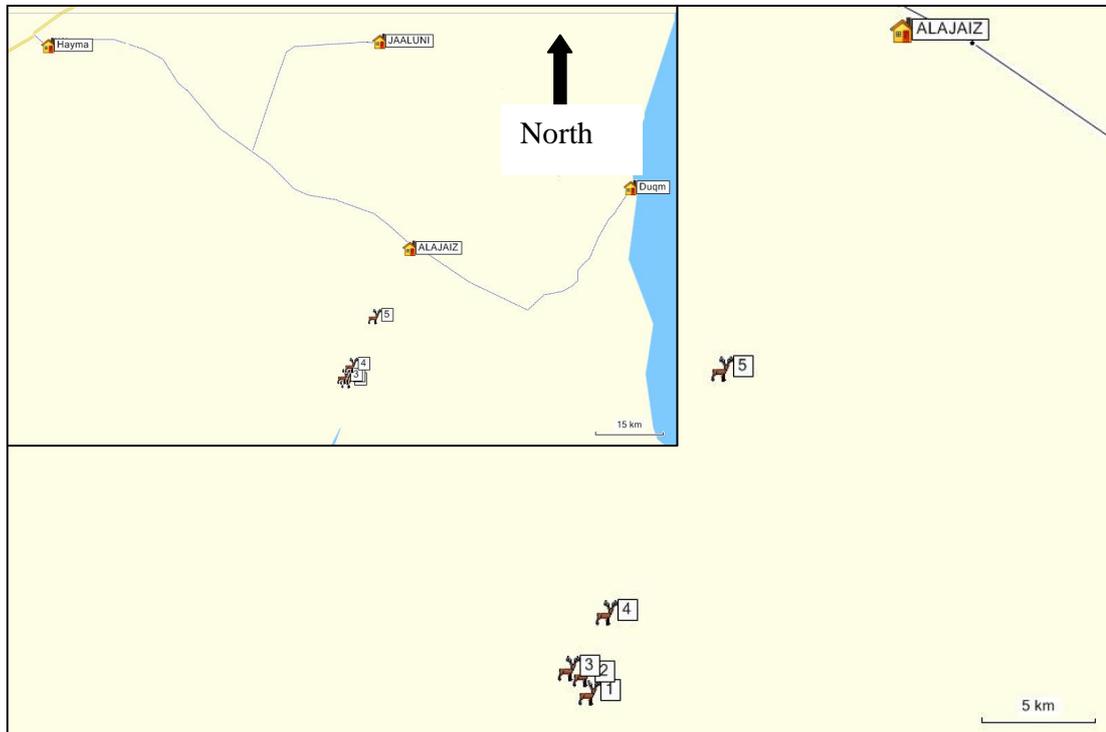


Figure 6.9 The release of oryx in batch C on 16 May 2007 at site 1. The inset map shows Jaaluni and the tarmac road from Hayma to Duqm. The oryx were released at site 1 which is 73 km from Jaaluni. One day later, 10C and 16C were seen foraging in a very good vegetated area at site 2 at 4 pm (39 °C). The male 16 C had an abscess on the skin below the lower jaw. On day 2 after release, 17C and 20C were found first at site 3 at 7 am (27 °C) and 10C and 16C were seen later at site 4 at 8 am (27 °C). On the fourth day, 17C and 20C were found together lying down under an *Acacia sp.* tree at site 5 at 4 pm (38 °C). The search for released oryx continued for about 10 days after release, but none of the released oryx were seen after the fourth day.

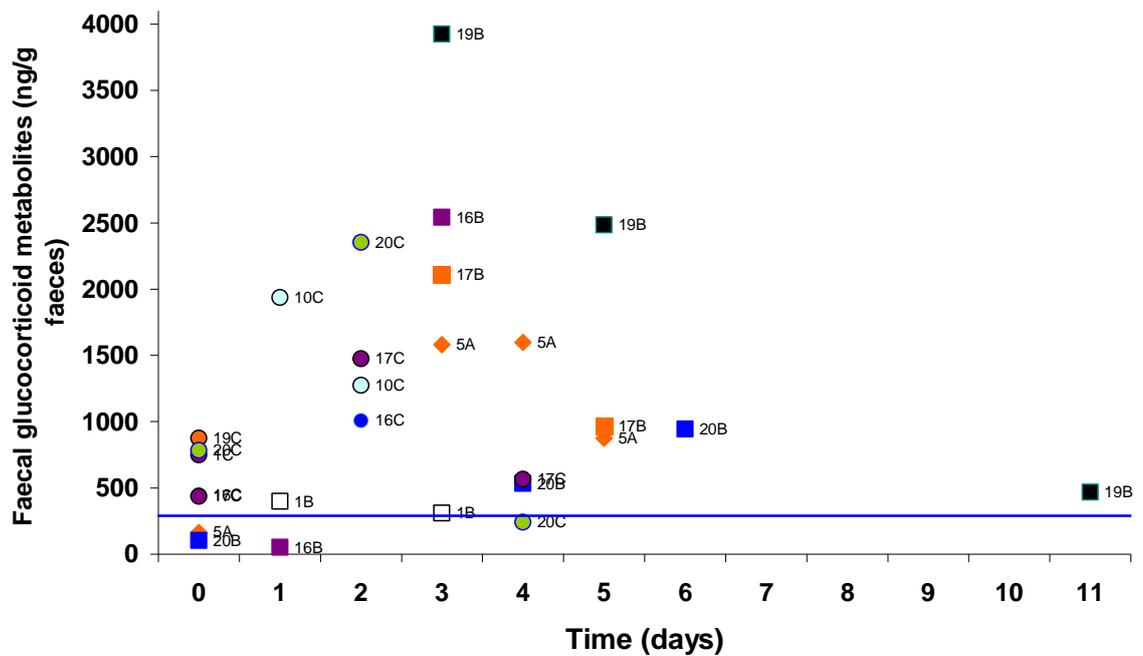


Figure 6.10 A scatter plot of the faecal content of immuno-reactive glucocorticoid metabolites at and after hard release of 13 male Arabian oryx from the holding pens in Jaaluni to the wild. The blue horizontal line at value 273 ng/g faeces represents the baseline value obtained in Chapter 2, (section 2.3.1.1). The shapes represent batches as follows, diamond (batch A), square (batch B) and circle (batch C).

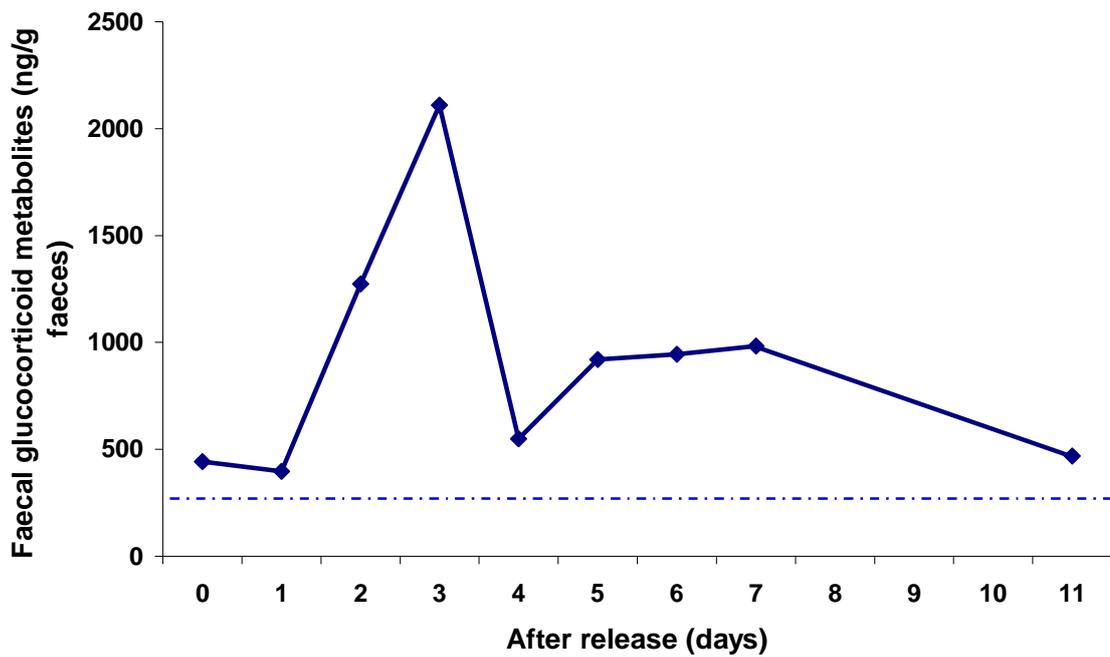


Figure 6.11 Median of faecal glucocorticoid metabolites after hard release of Arabian oryx from the holding pens of Jaaluni to the wild. The blue horizontal line represents the baseline value (273 ng/g faeces) obtained in chapter 2: see (section 2.3.1.1).

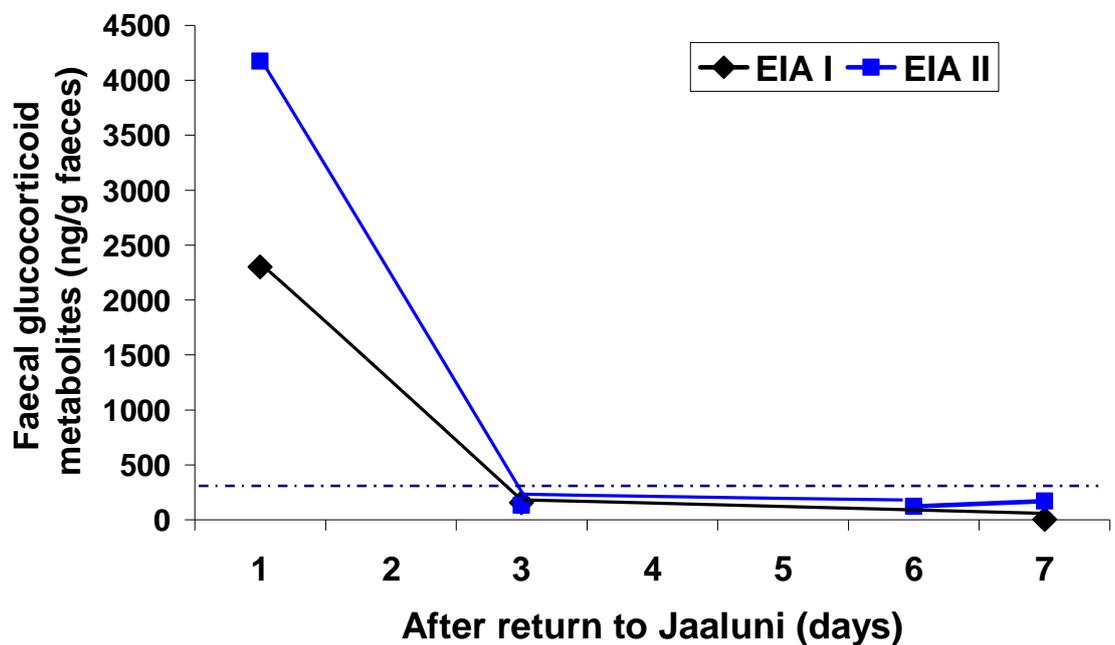


Figure 6.12 Immuno-reactive faecal glucocorticoid metabolites measured by EIA I and EIA II (as described in chapter 2) in the male oryx, 10B. This male returned to Jaaluni after two days from hard release crossing more than 50 km and faecal samples were collected just before herding in the holding pen (day 1) and for six days later. The dashed line represents baseline value obtained in Chapter 2, section 2.3.1.1.

## **6.4 Discussion**

### **6.4.1 Transport**

In the present studies, several Arabian oryx transported under tranquillisation (batch C: 1C, 10C and 19 C) showed signs of fatigue after translocation, but this was not apparent in batches A and B transported without tranquillisation. These results are similar to those of Ancrenaz et al. (1995) who also reported apparent tiredness of Arabian oryx transported under tranquillisation with perphenazine enanthate. The causes are unclear, particularly since the non-tranquillised oryx would be more active during transport. However, the maximum ambient temperature during the transport of the tranquillised batch C reached 42 °C, which was much higher than that during the transport of batches A and B (26 and 30 °C, respectively). Therefore, it is possible that the high ambient temperature during the transport of batch C caused the fatigue and tiredness. Perphenazine enanthate might interfere with the thermoregulation of Arabian oryx, as others have suggested for other species (Ebedes, 1993a; Swan, 1993). Creatine kinase was not measured in the present study and it is recommended to include it in future studies as an indicator of muscle damage and capture myopathy.

The exhaustion seen in oryx of batch C might also be attributed to inactive state produced by the tranquilliser as the animals did not respond adequately to the movement of the vehicle. Tranquillised animals are not fully alert, so would not be able to control their position inside the crates and this might have led to the exhaustion and fatigue observed in the present study as in previous translocation of Arabian oryx in Saudi Arabia (Ancrenaz et al., 1995). In Chapter 4, injection of perphenazine enanthate in non-transported oryx did not cause any fatigue of animals and therefore the combination of tranquilisation and transport probably caused exhaustion of the animals. However, this idea requires further investigation since other factors such as the high temperature during the transportation could also have been as important. The tranquilliser perphenazine enanthate was only used for batch C. Random assignment of animals in a transported group to a sub-set receiving tranquilliser and a sub-set receiving saline would potentially eliminate the confounding effects of factors differences such as weather and duration of transport from the analysis (Quinn and Keough, 2002). For the translocation of oryx, the tranquilliser was ordered

(from Germany) through a local company well before transportation of the first group of oryx, but was not received until several months later, just before the transport of batch C. The planned translocation of batches A and B had to go ahead given the advance planning that was required and the agreements in place. Once received, tranquiliser was used for the entire group of 6 animals translocated in batch C in an effort to gain useful information on the potential benefits of tranquiliser administration. At the time, translocation of further batches of oryx was envisaged (a total of 30 oryx according to original plan), which would have increased the information gained, but this did not occur because of poaching issues and the increasing issue of crowding of animals at Jaaluni enclosure. So, the need for further investigations remains to clarify the potential benefits of tranquilisation and effects on fatigue or high temperatures.

The veterinary treatment of some of the oryx transported under tranquillisation and that showed fatigue may have helped in their later recovery, and all oryx recovered by the third day after transport. Capture myopathy has been reported as probably the most important cause of death following the capture of ungulates and is caused by a combination of excessive muscular exertion and stress (Fivaz, 2005). The chance of treating capture myopathy in wild animals was considered poor and the use of tranquilisers to prevent capture myopathy was recommended (Ebedes et al., 1996). In the case of Arabian oryx number 19C, which showed some symptoms of capture myopathy, the fact that it was tranquillised with perphenazine enanthate probably played a major role in its recovery.

There was no mortality of any of the transported oryx in the present study. Pre-transport familiarisation, by housing the oryx in holding pens for several weeks to months, probably played a major role in achieving mortality-free transport. A combination of familiarisation, sometimes called “boma training”, and tranquillisation with perphenazine enanthate also resulted in no deaths after transport of Arabian oryx in Saudi Arabia (Ancrenaz et al., 1995; 1996; Strauss and Anagariyah, 2007). However, 20 % mortality (8 out of 40) of Arabian oryx was reported related to capture myopathy during transport, when the oryx were not familiarised before transport (Ancrenaz et al., 1995). Capture myopathy also resulted in 9 % mortality of transported Arabian oryx in Saudi Arabia (Greth and Schwede, 1993).

All haematological parameters measured before transport with (Table 6.4) or without (Table 6.3) tranquillisation with perphenazine enanthate, were within the reference inter-percentile ranges of haematological parameters that were established in Chapter 3 (Table 3.7). There are no previous reports in literature of haematological parameters in Arabian oryx after transport. The present studies showed significant increases in white blood cell count and percent neutrophils and a decrease in the percent lymphocytes and eosinophils after transport of both tranquillised and non-tranquillised oryx, and resulted in values that were outside the reference inter-percentile ranges established in Chapter 3. The white blood cell count increased significantly after transport of tranquillised oryx (mean  $13.87 \times 10^9/L$ ) and non-tranquillised oryx (mean  $12.61 \times 10^9/L$ ) to levels above the established reference inter-percentile range ( $4.45 - 11.11 \times 10^9/L$ ). Similarly, the percentages of neutrophils increased significantly after transport in tranquillised (mean 88 %) and non-tranquillised oryx (mean 83 %) to levels exceeding the established reference inter-percentile range of 46.19 – 78.20 %. The percentages of lymphocytes dropped significantly below the established range to a mean value of 9.05 % in tranquillised oryx and 14.24 % in non-tranquillised oryx. Both values were below the established reference inter-percentile range of 17.25 to 47.91 %. The percentages of eosinophils also were lower than the reference range of 0.40 to 5.90 % for both tranquillised (mean 0.13 %) and non-tranquillised oryx (mean 0.21 %).

The increases in white blood cell count and the percentage of neutrophils and the decrease in the percentage of lymphocytes after transport of Arabian oryx agrees with what was reported in Southern chamois (*Rupicapra pyrenaica*) after transport with or without using the tranquilliser, acepromazine (Lopez-Olvera et al., 2006b). Acepromazine belongs to the same category of tranquillisers as perphenazine enanthate; both are phenothiazines, but acepromazine has a short acting effect that only lasts up to 8 h (Swan, 1993). An increase in white blood cells and neutrophils and a decrease in lymphocytes after transportation were also reported in wild fallow deer (*Dama dama*) (English and Lopherd, 1981), and a decrease in lymphocytes occurred after road transport of calves (Riondato et al., 2008). In humans, administration of cortisol and adrenaline induced leukocytosis, neutrophilia and lymphopenia (Tonnesen et al., 1987). Therefore, the acute stress of transport of the Arabian oryx in the present study, which can be expected to have increased the serum

concentrations of cortisol and adrenaline, would probably have been the cause of the leukocytosis, neutrophilia and lymphopenia.

Several haematological parameters changed significantly in transported and tranquillised Arabian oryx compared to values before injection of the tranquilliser, but were unaffected in non-tranquillised oryx. These were percentages of basophils and monocytes, mean corpuscular volume and mean corpuscular haemoglobin. However, in all cases the values for these parameters remained within the reference inter-percentile ranges established in Chapter 3 (Table 3.7). After transport of tranquillised and non-tranquillised oryx, the rest of haematological parameters (red blood cell count, haemoglobin, haematocrit, and mean corpuscular haemoglobin concentration) were also within the inter-percentile ranges established in Chapter 3 (Table 3.7). Comparing the values for haematological parameters after transport of tranquillised and non-tranquillised oryx showed no significant differences, which suggests that the tranquilliser *per se* had no specific effects on these parameters and the changes observed were mainly caused by the transport itself.

In contrast to the changes in white blood cells and differentiation of cell types, the red blood cell count, blood haemoglobin concentration and haematocrit did not change after transport of either tranquillised or non-tranquillised oryx. In Chapter 4 (Table 4.6), these parameters decreased significantly at the 24 h and 72 h time points after administration of perphenazine enanthate while animals remained in the holding pens.

Response to a stressor typically causes activation of the sympathetic nervous system, stimulating the adrenal medulla to release catecholamines. Increases in circulating red blood cells, haemoglobin and haematocrit are associated with splenic contraction caused by the effect of catecholamines on  $\alpha$ -adrenergic receptors located in the splenic capsule (Stewart and McKenzie, 2002) and partly due to a reduction in plasma volume (Cross et al., 1988). In the present studies (Chapter 4), the lower values for red blood cell count, haemoglobin and haematocrit 24 h and 72 h after injection of oryx with perphenazine enanthate (Table 4.6) can be explained by the  $\alpha$ -adrenergic blocking effects of the phenothiazine family of compounds such as acepromazine and perphenazine enanthate (Swan, 1993). The  $\alpha$ -adrenergic blocking causes relaxation of the spleen and subsequent

splenic sequestration of erythrocytes (Jain, 1986), which lead to the decrease in the red blood cells, haemoglobin and haematocrit.

In the studies reported in this chapter, the stress of capture before transport and during transport probably caused splenic contraction and increased the red blood cell count, haematocrit and haemoglobin concentration prior to transport to a level that remained high throughout the translocation process.

Haemodilution caused by perphenazine enanthate due to a lowering of blood pressure (see Chapter 4, Section 0) can probably be ruled out as significantly influencing red blood cell parameters after transport because in that case, a simultaneous decrease in total protein, albumin and possibly sodium concentration would also have occurred, but did not occur in tranquillised oryx after transport. Acepromazine (a phenothiazine tranquilliser with short acting effect) also had similar effects of unchanged red blood cells, haemoglobin and haematocrit in transported southern chamois (Lopez-Olvera et al., 2006b).

So, it appears that in the present studies the stress of transport overrides the antagonistic effects of the tranquillisers and causes splenic contraction, resulting in no net decrease in the red blood cell count, haemoglobin concentration or haematocrit.

Haematocrit, total protein and albumin are often considered convenient and simple measures of dehydration (Knowles and Warriss, 2000). Osmolality can be used as a further, simple measure of plasma water content as osmolality is a colligative property and therefore includes all solute species (Knowles and Warriss, 2000). Serum osmolality might therefore, reflect the hydration state of animals (Knowles and Warriss, 2000; Armstrong, 2007). The stable osmolality during transportation in both groups indicated good fluid balance in Arabian oryx. This is in line with the known exceptional capability of Arabian oryx in maintaining plasma osmolality (as discussed in Chapter 1) with a long term deprivation of water and food (Ostrowski et al., 2006). The unchanged concentration of the major ions, sodium and chloride, seen after transport of both tranquillised and non-tranquillised oryx agrees with the stable serum osmolality.

The concentration of magnesium did not change significantly after transport of tranquillised oryx and the mean concentration (1.01 mmol/L), was within the reference inter-percentile

range (0.98 – 1.39 mmol/L). There was a small decrease in the concentration of magnesium after transport of non-tranquillised oryx (Table 6.5), by 7 %, but the mean value after transport (1.19 mmol/l) was within the reference inter-percentile range. This decrease is in line with previous studies in transported sheep that reported a decrease of 21 % of plasma magnesium in sheep after road transport (Ali et al., 2006). Hypomagnesemia has been related to low intestinal or ruminal absorption of magnesium due to anorexia or poor food intake (Stockham and Scott, 2002), so food deprivation during transport might be the cause of the small decrease in magnesium concentration seen in transported Arabian oryx.

For oryx transported under tranquillisation, the serum concentrations of potassium and phosphorus decreased significantly after transport in comparison to the values measured immediately before injection of the tranquilliser (Table 6.6). However, the mean concentration of potassium and phosphorus after transport (4.95 and 1.71 mmol/L, respectively) were within the reference inter-percentile ranges (4.20 – 9.77 and 1.85 – 4.11 mmol/L, respectively), although closer to the lower value of the range. As the serum potassium and phosphorus concentration after transport did not differ significantly from those immediately prior to transport, the decline in potassium and phosphorus cannot be considered to be a result of transport *per se*.

The tranquillised oryx (batch C) showed no changes in total protein during transport. However, oryx transported without tranquillisation (batches A and B), showed a slight but significant increase in serum total protein after transport (to mean of 73.00 g/L) compared to the concentration before transport although total protein remained within the established reference inter-percentile range (56.22 – 96.46 g/L). In three breeds of Omani goats, total serum protein increased significantly after 2 h of road transport (Kadim et al., 2006). In long distance (30 h) transport of horses total protein increased significantly in non-watered horses, but not in the watered group (Friend, 2000). This supports the use of total protein as a good indicator of dehydration (Knowles and Warriss, 2000). Other physiological indicators of dehydration during transport are increases in serum osmolality, albumin and blood haematocrit (Knowles and Warriss, 2000). Although none of these parameters were significantly altered by transport in Arabian oryx, the small increase in total protein in Arabian oryx transported without tranquillisation might reflect a small amount of dehydration in this group.

Albumin is the dominant protein in the plasma (Putnam, 1960). The concentration of albumin did not change after transport in either tranquillised or non-tranquillised oryx. The increase in total protein in oryx transported without tranquillisation might therefore be attributed mainly to an increase in globulins and other plasma proteins. However, future studies are needed to measure different proteins of the plasma to confirm this idea.

In oryx transported under tranquillisation (Batch C), the concentration of glucose increased significantly after transport, to 8.89 mmol/L, but was within the established reference range of 3.52 – 10.44 mmol/L, though close to the upper value in the range. Plasma glucose was not significantly elevated in oryx transported without tranquillisation, suggesting lower levels of stress (Knowles and Warriss, 2000). It is not clear why the non-tranquillised animals were apparently less stressed by the transportation than the tranquillised animals. However, the high ambient temperature during the transport of tranquillised oryx might cause more stress. The increase of glucose in tranquillised oryx was probably due to increase of cortisol stimulating the gluconeogenic pathway and glycogenolysis to increase the production of glucose (Norman and Litwack, 1990; Randall et al., 1997).

Blood cortisol is a key indicator of stress and well-being in mammals and other animals (Morton et al., 1995; Lindfors and Lundberg, 2002; Möstl and Palme, 2002). Transportation is a well known cause of stress in animals (Nyberg et al., 1988; Smith et al., 1996; Broom, 2003; Dembiec et al., 2004) and hence increases blood cortisol. For example, the transport of calves or adult cattle for 14 h or 16 h respectively was shown to cause a significant increase in serum cortisol (Odore et al., 2004; Fazio et al., 2005). Shorter periods of transport have also been shown to result in significant increases of serum cortisol in stallions (Fazio et al., 2008) and Omani sheep (Al-Kindi et al., 2005).

The baseline concentration of cortisol in Arabian oryx was established in Chapter 3 (Table 3.12 C) as  $73.25 \pm 15.41$  nmol/L for male oryx captured and sampled quickly. The concentration of cortisol in non-tranquillised oryx both before and after transport were higher than this baseline, at  $125.38 \pm 16.21$  nmol/L and  $111.57 \pm 15.66$  nmol/L. This suggests that these oryx were stressed by the capture procedures before transport, and throughout transport.

In tranquillised oryx of batch C, the animals had a high cortisol concentration before the injection of perphenazine enanthate ( $141.00 \pm 26.90$  nmol/L), which indicates the effects of the stress of capture and venipuncture. The concentration of cortisol decreased to a similar value as the baseline (Chapter 3, Table 3.12C), 2 days after tranquillisation to  $73.83 \pm 18.59$  nmol/L. The significant decrease of the serum concentration of cortisol in the 2 days after injection of perphenazine enanthate is similar to that reported in Chapter 4, Table 4.5 and Figure 4.1 at three days after injection. This suggests that perphenazine enanthate played a major role in reducing the activation of the HPA axis. However, after transport of the tranquillised oryx, serum cortisol increased to  $217.18 \pm 49.81$  nmol/L. This indicates an overriding effect of the stress of confinement and transportation probably exacerbated by the high ambient temperature. In the present study, the transport of tranquillised oryx took place during a period when high ambient temperatures were reached, and this prevents reaching a clear conclusion about the usefulness of perphenazine enanthate in potentially reducing stress and a HPA response during transport. Further studies are needed to examine the responses of Arabian oryx translocated during comparable conditions to those used for the non-tranquillised oryx, i.e. not during the hot summer months.

Comparison of the serum cortisol values after transport of non-tranquillised Arabian oryx with those before transport showed no significant changes. However, comparison of both values with the reference baseline for serum cortisol, established in Chapter 3 (Table 3.12 C), shows clearly that Arabian oryx were stressed both before and after transport. The baseline value also helps in assessing the lowering of serum cortisol after injection of perphenazine enanthate. This emphasises the importance of reference values that help in assessment of deviation of parameters from normality.

The enzyme immuno-assay (EIA I) (see also Chapter 2) that was used to measure the faecal content of glucocorticoid metabolites in Arabian oryx prior to and after transport and release of oryx to the wild, to assess the stress of transportation. Significant increases in faecal glucocorticoid metabolites have been seen in many species, including cattle 12 h after the start of a 2 h period of transport (Palme et al., 2000) and okapi (*Okapia johnstoni*), in which faecal content of glucocorticoid metabolites measured by EIA I increased 5 to 10 fold (Schwarzenberger et al., 1998). In the present study, faecal samples, unfortunately, could not be collected in the first 24 h after arrival at Jaaluni, because blood samples had to

be taken to Muscat for haematological analyses. The faecal content of glucocorticoid metabolites was relatively high two days after transport (Figure 6.6) compared to later values. Thereafter, the faecal content of glucocorticoid metabolites declined to levels after the third day of transport that were close to the baseline value reported in Chapter 2 (section 2.3.1.1), suggesting recovery from transport stress.

#### **6.4.2 Release to the wild**

Although Arabian oryx are white in colour and conspicuous from a long-distance in the open desert, tracking of them after hard release proved very difficult in this study, particularly as the animals in the released group scattered in different directions after release. The use of tracking technology such as VHF-radio collars or satellite collars that send updated information through a mobile network (Wikelski et al., 2007) would help in monitoring and locating animals after release and allow frequent collection of faecal samples for investigation of their welfare.

It can be concluded from Figure 6.10, that hard release of captive oryx to the wild is a very stressful event, increasing plasma cortisol, as implied by the large increase of the faecal content of glucocorticoid metabolites. This conclusion is confirmed by the effect seen in male 10B that returned to Jaaluni after two days in the wild (Figure 6.12). The first faecal sample, collected shortly after the animal's return, contained an extremely high amount of glucocorticoid metabolites (2301 ng/g faeces), which was close to the peak after ACTH injection (2515 ng/g faeces) reported in section 2.3.1.1. However, after re-housing the oryx in the holding pen, the faecal glucocorticoid metabolites returned to below the baseline suggesting rapid recovery (Figure 6.12). The faecal content of glucocorticoid metabolites measured in this oryx in particular validated the usefulness of the immuno-assays established by Palme and Möstl (1997); EIA I and Möstl et al. (2002); EIA II for assessment of stress in Arabian oryx.

It is unclear how male 10B navigated its way back to Jaaluni. The male oryx, 16B, 17B and 20B were also found heading towards Jaaluni at site 3 (Figure 6.8). For release to be effective, solutions need to be sought to avoid the return of released oryx to the place from which they were released. The use of long or short-acting tranquillisers might help in keeping the oryx at the release site for at least several days in order to adapt to the site and

avoid them leaving a good site and moving to a poorly vegetated area lacking shade or returning to the place from where they were released. Leaving the release site might have major consequences such as potential dehydration and starvation, and makes post-release monitoring by the reserve rangers difficult. The animals get dehydrated because of walking for long distance; possibly becoming starved and suffering from hyperthermia as a result of leaving the well vegetated and shaded area to enter an area with few grasses or trees (Seddon and Ismail, 2002; Mesochina et al., 2003b).

In the present study, Arabian oryx were released by “soft” and “hard” release, but no data could be collected after soft release of oryx (males in batch A). Therefore it is not possible to compare the relative stress attributable to the two types of release measured by the levels of faecal glucocorticoid metabolites. The strategy of release, and whether hard or soft release is adopted, largely depends on the habitat quality (Tenhumberg et al., 2004). Unpublished data on survival after release of Arabian oryx released in Oman between 1999 and 2005 (Arabian Oryx Project, Office of Conservation of Environment, Diwan of Royal Court, Oman) indicates that of the 52 animals released to the wild, seven (13 %) died. Five of these mortalities occurred after hard release of captive-born oryx. It was reported that oryx died after release because of maladaptation to desert environment followed by deterioration of body condition, stress, drought [average rainfall was 33 mm from 1990 to 2008, (Figure 1.7) compared to a mean rainfall of 37 mm at Jaaluni between 1987 and 1993 (Spalton, 1999)] or capture myopathy (AlKharousi, Personal Communication, 2006).

Releasing oryx to the hyper-arid desert that has low rainfall and high ambient temperatures is a serious issue especially for captive-born oryx that have not learned the skills of surviving in the wild. Some 22 wild-born oryx were rescued from poaching at the Arabian oryx sanctuary and moved from the wild to captivity in 1998 (Spalton et al., 1999). Since then the ratio of captive-born to wild-born oryx in captivity is increasing (Al-Kharousi, 2003, 2006). Therefore, careful planning is necessary for future release of captive-born oryx from captivity to the wild, which was the main objective of the re-introduction programme that started in Oman in 1980 (Stanley Price, 1986, 1989). The continued threat of poaching delays the plans of releasing oryx to the wild in Oman in a ‘second re-introduction’. Current fencing of the Arabian Oryx Sanctuary may help in protecting

animals from poaching but it increases the risk of oryx struggling to face possible prolonged droughts and subsequent stress. The well-being of oryx released in the future should be monitored using faecal glucocorticoid metabolites and complementary measures to improve the management strategies for Arabian oryx in the wild (See Chapter 7, section 7.4).

## **6.5 Summary and conclusions**

In summary, Arabian oryx were stressed by handling and transport as they showed higher serum cortisol than the reference basal values of both tranquillised and non-tranquillised oryx. The faecal glucocorticoid metabolites increased remarkably two days after transporting oryx and then returned to basal values up to 11 days after transport, indicating recovery.

The transport stress in both tranquillised and non-tranquillised Arabian oryx caused significant increase in the count of white blood cells and neutrophils and decrease in lymphocytes, which are probably due to the increase in cortisol levels. The erythrocytic parameters and indices (red blood cell count, haemoglobin, haematocrit and MCH) did not change in either group.

There were no major changes in biochemical parameters in either tranquillised or non-tranquillised oryx after transport, except for small erratic changes in some serum ions. There were no significant changes in the concentration of serum sodium, chloride, calcium, urea, albumin and osmolality in either tranquillised or non-tranquillised oryx after transport. There were small but significant decrease in the serum magnesium and increase in total protein in non-tranquillised oryx but not in tranquillised oryx after transport. There were also significant decreases in serum potassium and phosphorus and significant increase in glucose in tranquillised but not in non-tranquillised oryx after transport.

Transport is a stressful process for Arabian oryx and the use of previous familiarisation for several weeks in combination with the use of long-acting tranquillisers should provide safe transport, if ambient temperature is not too high during the process. The transport during

high ambient temperatures caused fatigue to all Arabian oryx. Therefore, transport of oryx during high ambient temperatures should always be avoided.

In future, consideration should be given to using short-acting tranquillisers such as acepromazine. The advantage of using short- rather than long-acting tranquillisers is that the onset of action is quicker and it can be injected just before transport, unlike long-acting tranquillisers that have to be injected a long time before transport e.g. perphenazine enanthate is given 2 days before transport. The disadvantage of short-acting ones is that their action does not last for long enough after transport to calm animals during the post-release acclimatisation period, but using a combination of short- and long-acting tranquillisers should solve this.

The hard release of oryx from captivity to the wild caused an increase to the highest levels seen in these studies in faecal glucocorticoid metabolites that reflect the magnitude of stress experienced by released oryx. Using this non-invasive method is invaluable for post-release monitoring of stress in Arabian oryx.

## Chapter 7

### General discussion

#### 7.1 Overview

Conservation biology is a relatively young science, and considering animal welfare in the conservation of animals is only just beginning. Animal welfare and conservation biology developed as separate fields for a long time and therefore communication between animal welfare scientists and conservation biologists has been meagre (Fraser, 2010). As a result, there is a growing call for a synthesis of conservation and animal welfare science based on the overlap between the two fields (Fraser, 2010).

Conservation biology focuses on wild animals and addresses concerns at the population level, ecological systems and on particular taxa. In contrast, animal welfare science is focused on captive animals mostly domesticated (e.g. pets and farm animals) and addresses concerns at the level of individuals and groups and it is concerned about the health of animals, their quality of life and their effective states (Rawles, 1997). Animal welfarists have to date paid little attention to the welfare of free-ranging wildlife. Therefore, there is a need for more consideration of the welfare of wild (non-domesticated) animals particularly those in captivity and free-ranging wildlife. Obviously some human activities such as forestry (Blumstein, 2010), agriculture (Mathews, 2010), and pest control (Littin, 2010) have severe impacts on the welfare of wild animals. Other practices related to development like road construction, mining, quarrying and hydrocarbon explorations have also caused direct or indirect negative impacts on the welfare and conservation of species, particularly in arid areas (Swaisgood, 2010a).

Conservation practices sometimes contradict animal welfare, for example culling of animals when there is over-population, such as in red deer populations (Bradshaw and Bateson, 2000) and pest control of unwanted or overabundant wildlife (Littin, 2010). Despite this, there are many areas of overlap and the research methods for animal welfare may solve animal conservation problems and vice versa (Fraser, 2010). For example, conservationists often rescue animals in threatened habitats, breed them in captivity, and

then release them back to the wild, but the success of this has been reported to be very low (Beck et al., 1994; Seddon, 1999). Solutions for this include housing and handling animals in ways that minimise stress and providing environmental enrichment that prepares the animals for the life in the wild (Swaisgood, 2010a). These procedures are well established among animal welfare scientists and can be adapted for use by conservation biologists.

Conservation biologists are interested in maintaining free-ranging wildlife, in their natural habitat. More interest is given to species threatened with extinction. Some wild animals, such as the Arabian oryx, the species on which this thesis is focused are bred in captivity because of the continuous threats they face in the wild. For conservationists they focus on removing or reducing those threats and the reintroduction or restocking of remnant populations of the species using captive-bred individuals.

Free-ranging wildlife faces natural and anthropogenic threats. Natural threats include drought leading to dehydration, flooding, volcanoes and hurricanes, and food shortage leading to starvation. The human-induced threats include hunting, poaching, habitat destruction, urban and infrastructural development, pollution, road and railway constructions, mining and oil explorations. Such activities may directly or indirectly impact on animal welfare and population stability. For the Arabian oryx, the subject of this study, poaching and prolonged drought are the main threats in the wild. Threatened wildlife either faces the risk of extinction or, as for the Arabian oryx, are rescued and bred in captivity, where their welfare becomes an important concern.

The activities practiced by wildlife reintroduction programmes such as housing, feeding, transport, capture, handling, release to novel environment have associated welfare issues and most conservation biologists have so far given little attention to the welfare of animals before and after translocation.

## **7.2 Captive breeding**

Captive breeding programmes are very important for preservation of biodiversity (Rahbek, 1993). Arabian oryx is a famous example of a species that after extinction in the wild in 1972 (Henderson, 1974), was reintroduced from the 'World Herd' established by previous

capture of a small group of wild Arabian oryx before extinction (Grimwood, 1988; Stanley Price, 1989). Keeping captive Arabian oryx in conditions that ensure good welfare will ensure the introduction of healthy individuals to the wild and ultimately a healthy wild population. Good welfare and health are important aspects for conservation of any species (Wasser et al., 2002). Chapter 1 outlines the current conservation status of Arabian oryx and shows that there are many more captive Arabian oryx than wild ones, and at least in the foreseen future, captive breeding will continue. Captive breeding was the solution for the poaching problem that faces Arabian oryx in the wild in Oman (Spalton et al., 1999).

### **7.2.1 Current welfare of captive Arabian oryx**

Captive breeding of wildlife has implications for the welfare of these animals. Animals are held in captivity for short or long –periods, for a variety of reasons, ranging from those with a direct link to conservation to more oblique purposes such as educating and inspiring the public to care about conservation. Many studies highlight serious welfare issues, for example elephants in captivity suffer from a host of issues including high levels of mortality of both infants and adults, morbidity, obesity and abnormal behaviour (Clubb, 2010).

In Oman, there are two major captive breeding programmes of Arabian oryx. First, the Omani Mammals Breeding Center holds more than 150 oryx in a total area of 0.5 km<sup>2</sup> and the numbers are increasing as there is only partial breeding control. As the number increases, within the same area, crowding increases and might potentially have undesirable consequences on the welfare of animals. The second captive breeding programme, at Jaaluni was established in 1998, and numbers of oryx have increased from 38 animals to 250 animals in 2010, held in a total area of 1.898 km<sup>2</sup>. The welfare issues within the captive herd at Jaaluni are related to health such as injuries because of fighting, swayback (posterior paralysis) and exacerbation of ecto-parasitism by ticks (due to high density). In addition, other problems include fighting for dominance between the dominant and subordinate adult males and habituation of captive oryx due to the constant contact with humans (Al-Kharousi, 2003). Habituation threatens the survival of translocated oryx as it increases the risk of capture by poachers after release to the wild because the oryx are familiar with people and vehicles. The poaching threat has been hindered the long-term

survival of oryx released to the wild in Oman, and consequently impeded further releases, thus contributing to a substantial increase in the number of captive oryx. Therefore, a programme of zero-breeding, by separating males from females in different sub-enclosures, was initiated in 2005 to limit further increase in the captive population. This might help avoid the development of poor welfare conditions related to the high density. The programme has kept the number of Arabian oryx almost constant for the last few years. The proposed fencing of the Arabian Oryx Sanctuary will be a relief for the large number of oryx in the enclosure; after release of oryx to the wild, the immediate problem will be solved.

On a global scale, there is no reported breeding control of Arabian oryx in captive breeding programmes and as the species is known to show good reproduction rate in captivity, therefore overall numbers globally are probably increasing. An increase of 19 % in Arabian oryx was reported between 2001 and 2002 assessments of Middle East populations (Ostrowski and Anagariyah, 2003). The current total numbers of Arabian oryx in the Middle East is not known but it is generally increasing. Probably at some stage in the future, the captive breeding programmes will end up resulting in too many Arabian oryx crowded in small areas and the welfare of these animals will be a major issue, if it has not already reached this state.

Captive breeding is very resource demanding process. Because of the increasing number of captive Arabian oryx, there is an increase in the cost of feeding and the need for further housing facilities to meet the extra demand. Captive Arabian oryx in Oman are provided with good feeding and housing needs, which are monitored and maintained at optimum levels, however, there is currently a lack for systematic scientifically-based monitoring of welfare. Arabian oryx are also found globally in many private collections, and in zoos, which have their own captive breeding programmes. The extent to which these animals receive good welfare is largely unknown.

One of the issues with welfare implications for captive-born animals is that they are likely to lack the behavioural skills that are necessary for survival after the release to the wild. For example, captive-bred bank voles (*Clethrionomys glareolus*) were unable to utilise a key food resource and hence were less dominant than wild-bred bank voles (Mathews et al.,

2005). Upon initial releases during the early stages of the Arabian oryx re-introduction in Oman, some specific behaviours needed for survival linked to foraging and social aspects were not well-developed in captive-born oryx (Tear, 1994). After release, learning and experience were found to play important roles in the development of foraging and the operation of social systems; the transition from an inexperienced captive-born state to an animal which is fully adapted to life in the wild occurred over a period of years (Tear, 1994).

Another issue related to captive breeding programmes is that genetically-related disorders may arise through inbreeding and outbreeding suppressions and were simultaneously found in Arabian oryx in Oman (Marshall et al., 1999; Marshall and Spalton, 2000), and have a negative impact on fitness (Marshall, 1999). The manifestation of a very pale colouration of Arabian oryx hair in some herds in the United Arab Emirates, is considered to be a sign of inbreeding (Molnar et al., 2005). In Oman, the appearance of oryx at the Omani Mammals Breeding Center (OMBC) in Muscat differs from that of oryx held at Jaaluni, in the Arabian Oryx Sanctuary (AOS). Those at OMBC have paler hair colour, a shorter tail hair length and a smaller average body size, compared to oryx held in Jaaluni and those free-ranging in the wild. This was repeatedly noticed by AOS staff when oryx were translocated from OMBC to AOS. The reason for these differences is unknown, and further research should be done to validate these observations and investigate whether differences in appearance could be linked to inbreeding. Inbreeding also has consequences for animals translocated from captivity to the wild as breeding between related individuals of a founder herd might lead to a decrease in fitness and longevity of individuals and threaten the viability of the population (Szablewski et al., 2006).

Stress of captive populations may cause suppression of the immune system (Maule and Vanderkooi, 1999; Squires, 2003) and leave the animals vulnerable to diseases. Therefore, poor health and good welfare cannot exist simultaneously.

Many diseases have been reported in Arabian oryx, especially captive ones (Ostrowski and Anagariyah, 2002, 2003). However these surveys of Middle East populations unfortunately have not continued, even though more health problems might be predicted due to the increasing numbers of captive Arabian oryx. The common viral, bacterial and parasitic

diseases related to Arabian oryx was reviewed in the present studies in more details (see Chapter 1, section 1.1.4).

## **7.2.2 Welfare assessment in captivity**

The present studies focussed on the assessment of distress, which can be a good indicator of poor welfare (Broom and Johnson, 1993b), and is widely assessed non-invasively by measuring faecal glucocorticoid metabolites (Möstl and Palme, 2002). In addition, health is an important aspect of welfare assessment and without knowing reference values, the diagnosis of the health abnormalities might not be accurate or reliable (Solberg, 2006). Therefore, studies described in Chapter 3 aimed to establish reference values for haematological, biochemical and some physiological parameters in Arabian oryx, as a baseline for monitoring the health and fitness of oryx for the diagnosis of health related abnormalities.

### *7.2.2.1 Faecal glucocorticoid metabolites*

The use of faecal glucocorticoid metabolites gives information regarding the level of distress and the welfare status of an individual or group of animals, while minimising the effects of the sampling on the animals (Lane, 2006). In Chapter 2, two enzyme immunoassays were validated to measure faecal glucocorticoid metabolites in Arabian oryx. Faecal glucocorticoid metabolites have been used to assess aspects of welfare in many species. For example, Palme (2003) found significantly higher levels of faecal glucocorticoid metabolites in cows housed in standard cubicle housing than herds housed more comfortably on straw yards. Faecal glucocorticoid metabolites have proved useful in evaluating the implications of painful procedures like castration and colic signs in horses (Merl et al., 2000). This non-invasive method of assessing stress and welfare was also found useful in assessment of the effects of management practices such as claw trimming in cows, in which a tilt table method was found to be less disturbing than a walk-in crush method (Pesenhofer et al., 2006; Kofler et al., 2007).

The advantage of faecal glucocorticoid metabolites is that they can be measured non-invasively and do not involve capture of animals (Duncan, 2005). Because the levels of glucocorticoid increase in response to stress challenges, it is often assumed that high levels

of glucocorticoid indicate stress. However, a recent review suggests that glucocorticoids measures do not change consistently in a predictable manner with adverse conditions (Busch and Hayward, 2009). Another recent review also supports the same conclusion of inconsistency of glucocorticoids in predicting relative fitness of individuals (Bonier et al., 2009).

As discussed earlier (Section 1.2, Chapter 1), the concept of allostasis explains that high glucocorticoid levels do not always indicate poor welfare. For example, these hormones could increase in response to normal activities such as mating (Romero, 2002). However, it is widely agreed that a low allostatic load (not very low or zero glucocorticoid levels) is key for good health and good animal welfare (Korte et al., 2007).

After repeated exposure to the same stressor, habituation usually takes place accompanied by a decrease in glucocorticoid production over time, which results in a reduced energy cost to the animal (Korte et al., 2007). However, in some cases animals do not habituate and show either a hypersensitive response (increasing glucocorticoid production over time) or a prolonged response (continuous stable glucocorticoid production) with a severe high cost on the animal's energy budget. In a few cases animals have shown a hyposensitive response (low glucocorticoid production) (Korte et al., 2007).

If a high allostatic overload is uncorrected and glucocorticoids remain high for days or weeks, inducing what is termed "chronic stress", these hormones usually become detrimental to health and fitness (Busch and Hayward, 2009). High glucocorticoids can suppress the immune system, growth and promote severe protein loss, hypertension, disrupt the second messenger system and can cause neuronal cell death (Sapolsky et al., 2000; McEwen and Wingfield, 2003). They can also affect behaviour, for example by inhibiting reproductive behaviour, and decrease performance of memory-related skills or cause depression (Wingfield et al., 1998).

As chronic stress is characterised by prolonged secretion of glucocorticoids, repeated measurements of faecal glucocorticoid metabolites over an extended period of time, and comparison of the levels with established baseline levels, could be a valuable tool for assessment of chronic stress.

In the present study, FGM was the only direct measurement of chronic stress and using additional complementary methods in future investigations for assessment of stress, strengthens the conclusions (see section 7.2.2.3).

#### 7.2.2.2 *Reference values*

Health is an important aspect of welfare assessment (Karesh et al., 1997). Evaluation of wildlife health is without doubt important, and the use of reference values for haematological, physiological and clinical parameters is fundamental for reliable diagnosis of health abnormalities (Solberg, 2006). However, there are currently no reliable reference values for haematological, biochemical parameters for Arabian oryx. For example a veterinary laboratory in Salalah (Royal Court Affairs, Oman) that provides regular veterinary services to the Arabian Oryx Project, uses an estimated range of values for haematological and biochemical parameters that is not based on scientific research. This range of values, probably based on occasional collection of blood samples from the Arabian Oryx Sanctuary, and most if not all are from handled oryx captured by immobilisation chemicals such as Immobilin (etorphine and acepromazine in combination) or other chemicals. Therefore, the suitability of using this estimated reference values for diagnosis of health related problems is questionable. All published studies that reported some haematological, physiological and clinical parameters for Arabian oryx obtained the measurements from oryx captured by immobilisation, from mixed sexes or after unspecified method of capture (Chapter 3). Therefore, studies described in Chapter 3 aimed to establish reference values for haematological, biochemical and some physiological parameters in Arabian oryx, as a baseline for monitoring the health and fitness of oryx. In Chapter 3, reference values for 32 parameters were obtained for Arabian oryx that were not captured by immobilisation and the differences between sexes were evaluated. The present studies showed that immobilisation and tranquilisation can induce changes in these parameters. Perphenazine enanthate (a long-acting tranquilliser) was found to cause significant changes in only 3 out of 32 parameters (red blood cells, haemoglobin and haematocrit) (Chapter 4). On the other hand, xylazine (an immobilising agent) was found to cause significant and substantial changes in 20 out of 32 parameters (Chapter 5).

The reference values for haematological, biochemical and physiological parameters that have been established in the present studies (Chapter 3) can aid in the diagnosis process and help to determine when parameters outside the “normal” range of values. The sample size that these values were derived from was small, but currently they fill a gap in knowledge until a reference values can be acquired from a larger sample size of Arabian oryx and additional parameters measured.

### 7.2.2.3 *Other measures of welfare*

In this thesis, welfare assessment has involved measurement of faecal glucocorticoid metabolites and assessment of haematological, biochemical and physiological parameters, but it is acknowledged that the monitoring of welfare of animals needs inclusion of a wider repertoire of approaches (see Chapter 1 section 1.2.1). Although there are many different methods of assessment of welfare, it is universally accepted that there is no single welfare measure (Broom, 1988; Mendl, 2001; Dawkins, 2003). Later the Five Freedoms proposed by the Farm Animal Welfare Council (FAWC, 1993), became a more accepted framework for assessing welfare (Webster, 2001). Recently, Botreau et al. (2007) has developed the Five Freedoms framework into 4 criteria (good feeding, housing, health and behaviour). Generally, the four criteria is widely accepted for assessment of welfare (principally of captive animals) and categorised as either resource-based (feeding and housing) or animal-based (health and behaviour) (Whay, 2007). Under these criteria Botreau et al. (2007) proposed twelve sub-criteria for overall assessment of welfare of captive animals: good feeding assessed as an absence of prolonged hunger or thirst, good housing means provision of comfort for resting, thermal comfort and an ease of movement, good health equals an absence of injuries, diseases or pain and appropriate behaviour means expression of social behaviour, expression of other behaviours, good human-animal relationships and an absence of general fear.

Moreover, Broom (1991) proposed several measures for assessment of animal welfare in captivity. These include impaired biological fitness (indicated by decreased lifetime reproductive success, lengthened intervals between successive breeding and early death), body damage, disease level, adrenal hormones (glucocorticoids), heart rate, changes in

behaviour, reduced life expectancy, prolonged inactivity and the occurrence of behavioural stereotypies.

Most of the criteria for assessment of welfare have been developed for assessing of the welfare of captive animals. However, wildlife has special requirements (as discussed later) and need to be prepared for life in the wild after release which might contradict with some of the above mentioned good welfare criteria.

### **7.2.3 Future welfare of captive Arabian oryx**

As Arabian oryx face several issues that adversely affect their welfare in captivity, some guidelines to improve their welfare are outlined.

#### *7.2.3.1 Genetic management*

To prevent inbreeding and outbreeding depressions that affect the fitness of animals (Lynch, 1991), captive herds should be genetically managed and studbooks employed for all captive breeding programmes, as for example applied for bonobos (*Pan paniscus*) (Van Coillie et al., 2008). Genetic diversity is correlated to fitness (Reed and Frankham, 2003). Inbreeding or mating between closely-related individuals can result in increase of homozygosity and therefore decrease fecundity, increase mortality and reduce growth (Van Coillie et al., 2008). The use of genetic methods like amplification of microsatellites for the analysis of genetic structure and to monitor and manage inbreeding has proved useful. For example, Armstrong et al. (2010) utilised such technique to analyse the genetic structure of a highly inbred captive population of the African antelope (*Addax nasomaculatus*).

#### *7.2.3.2 Control of overcrowding*

Overcrowding among Arabian oryx in captivity needs to be avoided so as to minimise the close contact between individuals and allow expression of normal behaviour, allow ease of movement, reduce fighting and injuries, and avoid transmission of diseases.

For Arabian oryx in Oman, to solve the problem of fighting for dominance between adult males, surplus adult males are separated from the herd and until recently were released to the wild. No females are released to the wild at the current time because of the poaching

problem and once the current fencing of the reserve is completed, both male and female oryx will be released to the wild in a balanced sex ratio. This practice of releasing only males to the wild could be questioned ethically, as they could not breed and were faced with the risk of poaching. It is no longer applied because it has been observed recently that keeping males in a separate enclosure without females, solves the problem and very little fighting occurs between adult males.

### 7.2.3.3 *Health screening and control of diseases in captivity*

Health screening involves check-ups for viral, bacterial, fungal, parasitic and other pathological diseases. Health monitoring also involves the frequent examination of physical health by qualified veterinarians and cases that show signs of illness should be subjected to further clinical examination and treated. Other illnesses that can be monitored and treated include serious injuries, abscesses and swayback or posterior paralysis which has been observed in some Arabian oryx at Jaaluni. It is believed that swayback is attributed to severe copper deficiency. In Oman, goats and a few sheep were found to suffer from swayback in spite of sufficient copper content in food (Ivan et al., 1990). It was concluded that the copper deficiency was due to copper-sulphur and copper-iron interactions in the rumen, enhanced by feeding of fresh roughage containing rumen degradable proteins.

In captivity, it is important not to mix any other species with healthy Arabian oryx herds or other conservation-bred wildlife. The current captive breeding programmes that have Arabian oryx mixed with other species, should first screen oryx for diseases, vaccinate them and separate the oryx from the other species. For example, the presence of foxes near or inside captive collections such as at Jaaluni, in Oman, might expose oryx to infection with rabies, since rabies-positive samples were collected from some red foxes at Jaaluni (J. A. Spalton, personal communication; Al Ismaily et al. (2002)). Most mortalities of Arabian oryx are from unknown cause, and rabies could be one of them. Therefore, introduction of electric wiring above the fence that repels foxes, is a good practice.

As many herds of Arabian oryx suffer from endo- and ecto-parasites, frequent deworming is also a good practice (Flach, 1997). For deworming and treatment of endo- and ecto-parasites, injectable ivermectin, doramectin, thiabendazole or fenbendazole are usually used (Ostrowski and Anagariyah, 2002).

#### 7.2.3.4 *Management of stress levels*

Provision of good housing and feeding and the freedom to express normal behaviour should reduce the level of stress in captive oryx (see section 7.2.2). Animals caught from the wild are prone to problems that include poor health, repetitive stereotypic behaviour and breeding difficulties, particularly when housed in areas that are a fraction of the home-range size in the wild (Clubb and Mason, 2003). Therefore, wildlife kept in captivity are prone to stress and frustration. Assessment of stress levels in herds of Arabian oryx, could be assessed by measurement of faecal glucocorticoid metabolites by comparison to baseline levels (Chapter 2) if considered with care (see section 7.2.2.1) and alongside other complementary measures, such as body condition and behaviour. Faecal glucocorticoid metabolites could be used in the future to assess the effect of different numbers of oryx within similar-sized enclosures on faecal glucocorticoids metabolites, in order to optimise housing conditions and assess how crowding affects their welfare.

#### 7.2.3.5 *Assessment of behaviour*

In the present studies, the behaviour of oryx was not used in assessment of welfare, although such approaches could be useful in future studies for further understanding of the welfare of Arabian oryx (see section 7.2.2.3). There are some behavioural observations of Arabian oryx, mainly in the wild, that were described by Stanley Price (1989) and Tear (Tear, 1992, 1994). Previous behavioural descriptions could be useful in future studies to compare to the behaviour of Arabian oryx in captivity and investigate deviations of “wild-type” behaviour that might indicate poor welfare (Dawkins, 2003).

#### 7.2.3.6 *Welfare standards*

The Coordination Committee for the Conservation of Arabian Oryx (CCCAO), based in Abu Dhabi, UAE, was established in 2001 to coordinate the efforts for conservation of Arabian oryx, within its natural range. Under this Committee, regulations and guidelines should be established to promote welfare of captive and free-ranging Arabian oryx throughout the natural range. Governmental and private collections should adopt these standards and embrace conservation goals and plans for translocating oryx to the wild. Recognised standards and criteria for oryx that can be released to the wild for conservation,

such as freedom from diseases, vaccinated, trained for life in the wild and preferably from a known genetic line should be part of future plans.

#### **7.2.4 Improvement of welfare in captivity**

After assessment of welfare, its improvement is the next vital step (Whay, 2007), although intervention to improve the welfare of wildlife species housed in captivity might differ from that for farm animals. Enrichment of captive conditions aids welfare (Young, 2003). Enrichment could include, for example, hiding food to entice animals into more natural searching behaviours. The purpose of enrichment is to stimulate the psychological and physiological wellbeing of animals (Shepherdson et al., 1998). Environmental enrichment has been reported to play a role in alleviating the stress in wildlife kept in captivity (Carlstead and Shepherdson, 2000). Reducing the density of individuals in captivity by controlling breeding or releasing some animals to the wild, might also help improve the welfare of the captive animals. Improvements should also include provision of extra space by enlarging the size of enclosures, to avoid problems such as poor health, repetitive stereotypic behaviour and breeding difficulties (Clubb and Mason, 2003). In addition, periodical health checkups and treatment of diseases are without doubt beneficial for health and well-being of animals.

The improvement of wildlife welfare should take into consideration that these animals should eventually be released to the wild. This requires that application of welfare standards such as the proposed “Five Freedoms” or the criteria proposed by Botreau et al. (2007) should not lead to compromising the conservation goal of establishing a self-sustaining population. Therefore, captive-bred animals should be prepared for the life in the wild, even at the expense of their good welfare in captivity (Swaisgood, 2010a). For instance, animals housed in predation-free captive environment do not have knowledge about predators and the skills to avoid them (Blumstein et al., 2001). Such animals are more likely to be killed or harmed by predators after release. For example, houbara bustards were given an anti-predator training by introducing a hand-reared red fox, muzzled and controlled on a long lead. The training with a live predator caused stress, shock and some birds were touched and wounded. However, this training improved post-release survival of houbara in comparison to groups trained with a model predator or those that had no training

(Van Heezik et al., 1999). This anti-predator training with a live red fox likely evoked a stress response in captive houbara i.e. there is a short-term welfare cost, but with a long-term conservation value which is the improved post-release survival. Therefore, it is important to be aware that compromising individual short-term animal welfare may be for the benefit of the long-term conservation goals (Swaisgood, 2010b).

### **7.3 Welfare of animals during release to the wild**

Translocation of Arabian oryx involves several practices such as capture, special preparation in captivity, temporary confinement prior to transport, transport and finally release to the wild, or a new enclosure. As mentioned earlier, poaching has prevented the release of Arabian oryx to the wild and currently the numbers of Arabian oryx have risen in captivity. As the Arabian Oryx Sanctuary is being fenced, there will need to be release of oryx from captivity after completion of fencing of the Arabian Oryx Sanctuary.

#### **7.3.1 Preparation for release**

##### *7.3.1.1 Welfare*

Good health is part of good welfare and part of the preparation for release of wildlife is veterinary investigations of the release site for risk assessment. This includes determination of diseases of wildlife and domestic animals prevalent in the area of release. This helps in planning for vaccination programmes against the common infectious diseases in the area (Kock et al., 2010). Each country generally vaccinates its translocated wildlife according to the most easily spread infectious diseases present in the release area.

Based on information from the Ministry of Agriculture (Oman), Directorate of Animal Health, the most common viral and bacterial diseases in livestock and wildlife (e.g. foxes) in the Central area of Oman, the area where the Arabian Oryx Sanctuary is located, are peste des petits ruminants (PPR), rabies, foot and mouth disease (FMD), enterotoxaemia, and brucella (Dr Sultan AlIsmaili, epizootiologist, Personal Communication, 2010). Even when the reserve is fenced some livestock might still exist inside and therefore the chance of transmitting diseases to Arabian oryx and Arabian gazelles still exists. Ostrowski and Anagariyah (2003) recommended vaccination of oryx against rinderpest, PPR (one

injection provides a life-long protection) and FMD (booster injections are needed). There is an urgent need for regularly updated information about diseases of Arabian oryx in the Middle East. Accordingly, vaccination of Arabian oryx against infectious disease common in livestock or wildlife in a country should be considered for the wild animals present in that country to prevent catastrophic outbreak of such diseases, which can result in huge losses of free-ranging herds of wildlife.

Screening for endo and ecto-parasites is recommended for animals to be translocated (Woodford, 2000). Arabian oryx bred alongside other wildlife in zoos are prone to parasite infections, as found from faecal egg counts (Goossens et al., 2005). The area where Arabian oryx were reintroduced in Oman contains camels, goats, sheep and feral donkeys. Although this area is very large, there might be some interaction of the Arabian oryx with these animals, leading to transmission of parasites as well. To prepare Arabian oryx for release to the wild, animals should be screened and treated for endo- and ecto-parasites using the common injectable ivermectin or oral suspensions like albendazole.

Temporary confinement before release to the wild is a useful strategy for various reasons. First, it acts as an extended quarantine during which clinical signs of infectious or contagious diseases might be detected (Kock et al., 2010). Second, it enables formation of socially cohesive groups (see next section).

#### 7.3.1.2 Conservation

The IUCN, the International Union for Conservation of Nature, produced guidelines for reintroduction of endangered captive animals to the wild (Kleiman, 1989; IUCN, 1998) and these guidelines were updated in 1995 (Soorae, 2008). The guidelines recommend that reintroduction candidates (captive-bred) should be given the opportunity to acquire the necessary information to enable survival in the wild, through training in their captive environment. It is very useful for re-introduction programmes to adopt extensive modifications and enrichment of captive environments so as to prepare captive-bred animals for survival after release (Kleiman, 1989, 1996). Captivity has a number of effects on behavioural development and some of them are detrimental to reintroduction and survival in the wild (Carlstead, 1996).

From the conservation point of view, preparing Arabian oryx for release should consider their behaviour. To select candidates for release, it is very useful to have a mixture of wild-born if available (e.g. some rescued wild-born oryx still live at Jaaluni in Oman), and captive-bred individuals. The wild-born animals have the knowledge and skills of survival in the wild and are likely to pass them on to the inexperienced captive-born animals after release to the wild. Therefore, mixing wild-born with captive-born candidates for release should be a good strategy to pass on foraging skills to the captive-born animals that lack wild experience (Tear, 1994; Tear et al., 1997). For example, Arabian oryx released into an established population developed efficient strategies for using the habitat range, more quickly than oryx released into unoccupied habitat, and this was likely to reflect the benefits of learning from the established free-ranging population (Tear, 1992). Indeed, in previous studies, wild born-oryx were found more capable of adaptation to life in the wild and have better survival success, compared to captive-born oryx (Bedin and Ostrowski, 2003).

The preparation for releasing animals to the wild involves consideration of the social aspects of animals as Arabian oryx are known to have a social hierarchy (Stanley Price, 1989). Formation of a socially cohesive herd of Arabian oryx before release is a prerequisite and may take from few months to more than a year to achieve (Stanley Price, 1989; Tear, 1992). Otherwise, the animals might scatter and disperse after release as learned from the present studies after releasing groups of male oryx (Section 6.4.2). Social cohesion of the reintroduced group is very important in keeping the released herd together and increases the rate of reintroduction success especially in social animals like Arabian oryx (Stanley Price, 1989; Whitehead, 2010). In carnivores, recent studies with black-tailed prairie dogs (*Cynomys ludovicianus*) showed that animals translocated in family groups were less likely to be killed by predators, had a five-fold higher rate of survival, and higher reproductive rates than animals released in groups of unfamiliar individuals (Shier, 2006).

One of the major problems that captive-bred Arabian oryx have is familiarity with the presence of humans and vehicles. As poaching is the main threat to Arabian oryx in the wild in Oman, the release of tame animals puts them at high risk of being poached. Solutions for this should be sought to decrease the level of familiarity to human and vehicles or, prior to release to the wild, to train them to run away from vehicles. One

suggestion, to solve this problem is to shoot oryx just before or after release, with rubber bullets that do not cause harm to the animal but are painful enough to make them escape from vehicles and humans. Such ideas should be tried or other creative solutions should be found.

In Arabian oryx, release of family groups might contradict with the goal of restricting inbreeding (see section 7.2.3.1), and could lead to a decrease in the fitness of the individuals and threaten the viability of the population in the long-term. However, a group of unrelated individuals housed together for a long time might lead to establishment of a socially cohesive herd to be released as one group. If this can be achieved, although it could be seen as having some welfare cost (a long period of confinement), it would be likely to have a long-term benefit for the population. Further research is needed to investigate whether unrelated individuals of Arabian oryx can form a socially cohesive herd or whether family groups are more likely to be more successful in this regard.

### **7.3.2 The process of releasing animals to the wild**

#### *7.3.2.1 Capture*

Capture of animals causes acute stress and affects their welfare. For example, capture of vicuña (*Vicugna vicugna*) for wool harvest resulted in injuries, death, separation from mothers, muscle damage, body trauma and crushing, and stress of pregnant females predisposing to abortion (Bonacic et al., 2006). The restraint of vicuña resulted in significant increases in creatine kinase, haematocrit and rectal temperature (Bonacic et al., 2006).

Capture of Arabian oryx can be achieved chemically, with immobilising agents, or physically without using chemicals. Immobilisation has advantages and disadvantages and sometimes it is inevitable. Xylazine, a widely used immobilising chemical, was evaluated in Arabian oryx (Chapter 5) by comparison to the reference values established in Chapter 3. Although xylazine gave a satisfactory immobilising status, it caused significant changes in 20 out of 32 parameters. However, chemical immobilisation was reported to reduce stress in some species, such as the free-ranging hog deer (*Axis porcinus*) captured in drive nets (Arnemo et al., 2005). On the other hand, Kock et al.(1987) evaluated four methods of

capture and found that physical capture methods (drop-net, net-gun and drive-net) resulted in less alteration to biological parameters, and less capture stress and mortality incidents than use of chemical immobilisation. Therefore, wildlife managers need to consider which method is most suitable for the species in question, and has fewest and the lowest level of negative impacts on the animals. Physical capture may be better in terms of causing less physiological changes that accompany chemical immobilisation, which might jeopardise the health or life of animals. However, physical capture has to be done in a way that is safe for both handlers and animals to avoid causing injuries to the animals or adverse affects such as capture myopathy (Montan, et al., 2002). Capture myopathy, as a result of restraint, has been reported in Arabian oryx (Greth and Vassart, 1989; Vassart et al., 1992).

Recently, for physical restraint of antelopes without immobilisation, a restraint device (TAMER) has been used in the UAE (MacNamara and Blue, 2007). Using this device, seventy oryx could be handled in one day without injuries, losses or capture myopathy (Cardas et al., 2010). The device enables safer and easier provision of medication, such as vaccinations, health examination, weighing, ear tagging, hoof trimming and even minor surgery (MacNamara and Blue, 2007).

#### 7.3.2.2 *Temporary confinement*

Temporary confinement of animals in a holding pen before transport to a release site is sometimes used for group transport until the required number of individuals is captured. This temporary confinement is useful as a preparative step as capturing animals that have not been handled before and then transporting them immediately after capture is very stressful (Ancrenaz et al., 1995). Temporary confinement and familiarisation, sometimes referred to as “boma-training”, helps animals get accustomed to handling and the presence of humans (Ancrenaz et al., 1995; Ostrowski and Bedin, 2001; Chassot et al., 2005). In the present studies, two groups (8 males and 8 females) were housed for 5 and 3 months in individual pens, respectively. These two groups of Arabian oryx showed a significant decline over time in serum cortisol, as a result of familiarisation (See section 3.4.2 and Figure 3.1). Habituation occurs when animals are repeatedly exposed to a stressful stimulus (e.g. unfamiliar confinement) and results in a decline in the response to the stimulus, for

example by showing a lower serum cortisol or reduced faecal content of glucocorticoids and their metabolites (Broom and Johnson, 1993a; Korte et al., 2007).

Another advantage of temporary confinement (see also 7.3.1.1) is to acclimatise animals to the release area by moving animals to pre-release enclosures close to the release site. During this period, they get adapted to grazing natural plants and to the weather while closely monitored and provided with supplementary feeding and water if necessary.

### 7.3.2.3 *Transport*

Transport is one of the inevitable management practices in translocation except in 'soft release'. For animals that are 'hard-released' to the wild, they are captured and loaded into a crate or straight into a vehicle and translocated to the release site. Transport has various deleterious effects on the welfare of animals such as stress, possible injuries and sometimes even death. The main causes of mortality during transport are stress, extreme temperatures and injuries (Openshaw, 1993). Transport stress resulted in a significant increase in faecal glucocorticoid metabolites in cattle (Palme et al., 2000). Laws et al. (2007) also observed an increase in faecal glucocorticoid metabolites after relocation of African elephants and an increase in stereotypic behaviour 24 h after transport. In the present studies, faecal glucocorticoid metabolites were not measured one day after transport but at day 2, the metabolites were high in some individuals (Figure 6.6) and returned to baseline in the following days. Some stereotypic behaviour was also observed in Arabian oryx after transport, like pacing back and forth along the fence of the holding pen.

### **Assessment of welfare during transport**

For assessment of short-term stress and discomfort, the use of both behavioural and physiological measurements are suggested (Grandin, 1997). The assessment of welfare during and after transport using behavioural, physiological and pathological indicators was reviewed by Broom (2003, 2005). The behavioural indicators of poor welfare during transport include attempting to escape, vocalisation, kicking and struggling. Behavioural measures may also include aversion tests which is the time required to induce an animal to re-enter a chute where it was previously handled (Rushen, 1996).

The use of physiological indicators of welfare during transport have been recommended to evaluate the effects of food deprivation (free fatty acids,  $\beta$ -hydroxybutyrate, glucose and urea), dehydration (osmolality, total protein, albumin and haematocrit), physical excretion (creatinine kinase and lactate), fear and arousal (cortisol, heart rate and respiratory rate) (Warriss et al., 1994; Knowles and Warriss, 2000). Measures of welfare during and after transport also include measures of immune system function, injury, clinical disease, mortality and measures of later reproduction and growth (Broom, 2005).

The studies described in Chapter 6 examined the effects of translocation of Arabian oryx on faecal glucocorticoids metabolites. Transport and exposure to novel habitat resulted in an increase in faecal glucocorticoid metabolites on day 2 after transport and then levels returned to baseline (Figure 6.6 and Figure 6.7). This agrees with the more comprehensive studies by Viljoen et al. (2008) who found a significantly higher faecal content of glucocorticoid metabolites after translocation of African savannah elephants in comparison to the levels before and after recovery from transport to the wild.

The use of short- and long-term tranquillisers such as perphenazine enanthate, zuclopenthixol, haloperidol and acepromazine, have been reported to be useful in reducing the disturbance during transport (Ebedes, 1993b; Portas, 2004; Redrobe, 2004; Lopez-Olvera et al., 2006b). The use of a combination of boma training (temporary confinement, section 7.3.2.2) prior to transport and long-acting tranquillisers during transport have been reported to alleviate stress during transport (Openshaw, 1993). These methods proved useful for translocation of Arabian oryx and resulted in no injuries or deaths (Ancrenaz et al., 1995; Ostrowski and Bedin, 2001; Strauss and Anagariyah, 2007), and were successfully employed in the present studies (Chapter 4 and 6)

#### 7.3.2.4 *Translocating animals to the wild*

The last stage of the translocation process is the release of animals to the wild. The stage of freeing animals either transported ('hard-release') or let out of an enclosure ('soft-release') has several welfare implications. At this stage, animals face a novel environment and novelty is one of the causes of psychological stress on animals (Grandin, 1997). In arid environments such as Arabian Peninsula, the release site should contain enough food to sustainably hold the released herd and also enough trees for shade (Mesochina et al.,

2003b). Another issue related to welfare of released animals is that animals tend to escape or run away usually after hard-release by transport. Usually the release site is selected very carefully, based on the availability of good food and shade trees within a large hyper-arid desert area. Animals running away might end up in areas with poor vegetation and suffer from dehydration, stress and exhaustion, which could compromise their survival. The use of long-acting tranquillisers such as perphenazine enanthate (Chapter 4) or zuclopenthixol acetate or acepromazine might be useful in minimising the migration from the release site to help retain them in the selected site at least for several days. Perphenazine enanthate was shown to have only limited effects on haematological and physiological parameters (Chapter 4), and therefore could be utilised for release of wildlife.

Another issue is related to the dependency of captive-bred Arabian oryx on the food and water supply provided in captivity, which will not be readily available in the wild. Pre-release training for prolonged thirst, by deprivation of water for extended periods, might help to decrease the level of dependence. Post-release monitoring should account for the possible need to supply food and/or water to the released cohort to prevent any adverse welfare consequences or mortalities due to hunger or thirst.

Arabian oryx in the wild can live for several months without drinking water (Stanley Price, 1989; Ostrowski et al., 2003). However, if they have access to free water they tend to drink (Tear, 1992; van Heezik et al., 2003). The availability of shade, whether artificial or natural, is very important for their survival (Seddon and Ismail, 2002; van Heezik et al., 2003) since Arabian oryx need shade for thermal regulation and to limit the need for evaporative water loss, thus conserving water (Ostrowski et al., 2002b; 2003; 2006). To conserve water in their bodies, Arabian oryx employ heterothermy by increasing its body temperature up to an amplitude of 5°C during hot months (Hetem et al., 2010). A lack of shade will adversely affect their capability to conserve water and hence their survival will be at risk.

#### **7.4 Welfare of translocated animals in the wild**

Wildlife that are found naturally in the wild, might suffer from various welfare issues, however, many more welfare issues probably apply to translocated animals. The

consideration of welfare for free-ranging wildlife is conspicuously missing, mainly because environmental discomforts affecting wild animals are recognised by people as natural processes or stressors (Paquet and Darimont, 2010). Therefore, more concern is given to welfare issues associated with humans and their activities that involve wildlife, such as captive breeding. However, there are obvious welfare concerns for animals reintroduced to the wild to meet the conservation aims. For this reason, Swaisgood (2010b) stated that two rights (good animal welfare in captivity and the reintroduction of wildlife to their natural habitat), should not make a wrong (compromising the welfare or life of reintroduced animals).

#### **7.4.1 Welfare issues in the wild**

First of all, there are reports that captive-bred animals face much greater post-release challenges than animals translocated from one place in the wild to another, mainly because the former have less knowledge of how to cope in the wild (Swaisgood, 2010a). The novelty of being free may stress a freed animal until it adapts. Chronic stress often results in immunosuppression which may make it vulnerable to diseases and it may succumb as a result (Dickens et al., 2010). In chapter 6, one oryx (10 B), which found its way back to Jaaluni after hard release provided a good example of stress-experienced in the wild and later recovery after re-housing when a sharp reduction in the immunoreactive faecal glucocorticoid metabolites occurred (Figure 6.12).

The level of success of captive-bred animals released back into the wild is generally low and hand-reared animals have even lower level of success (Kelly et al., 2008). For Arabian oryx, there are few cases of hand rearing in Oman, when mothers abandoned their calves or died (Stanley Price, 1989). Hand-reared oryx should not be translocated to the wild unless under exceptional circumstances they have been successfully integrated within the herd and close human care was completely stopped (Stanley Price, 1989).

A recent review (Shobrak, 2007) evaluated the Arabian oryx reintroduction programmes in seven countries that face the similar problems: (1) poaching, (2) natural (drought), (3) selection of inappropriate release sites, (4) lack of socio-economic studies, (5) lack of law enforcement, (6) diseases, (7) poor public awareness, (8) low financial support in some

cases, and (9) mining and oil exploration activities. Some of these challenges can directly or indirectly affect the welfare of Arabian oryx in the wild.

#### 7.4.1.1 *Poaching*

Poaching of Arabian oryx can occur in Oman because the Arabian Oryx Sanctuary is not fenced, in contrast to the fenced Mahazat as-Sayd reserve in Saudi Arabia where poaching never reported as a problem. Probably the major attraction of poaching is the high price of selling Arabian oryx on the black market, rather than poaching for food. Uruq Bani Ma'arid (Saudi Arabia) is a non-fenced protected area that also faces poaching of Arabian oryx (Bedin and Ostrowski, 2003; Chassot et al., 2005). Poaching has inclined Oman to consider fencing, as a last resort. Fencing for conservation has advantages and disadvantages. Fencing can mitigate major threatening processes to wildlife that were identified by the IUCN such as mining, oil drilling, hunting, roads, human disturbance and pollution (Hayward and Kerley, 2009).

Poaching of live Arabian oryx and other wildlife involves chasing, capture, restraint and without doubt these cause immense stress and a poor welfare state on the poached animal which might culminate in its death. As mentioned earlier, the lack of anti-poaching behaviour, such as escape responses to an approaching vehicle is due to familiarity with humans and the vehicles of rangers and the feeding staff while captive. The oryx cannot distinguish between "enemies and friends", i.e. the vehicles of poachers and rangers, respectively. This is one the serious problems that has led to the setback of the reintroduction of Arabian oryx in Oman, and possibly in other countries. Seeking a solution for this is very important, and hopefully fencing of the reserve in Oman will largely solve the poaching problem, but the familiarity problem remains. Hopefully the wild-born generation of to be released oryx will be not familiar to humans and vehicles.

Fencing can create a problem of accumulating wind-blown rubbish, and in the Mahazat as-Sayd protected area in Saudi Arabia, post mortem of seven dead Arabian oryx revealed that the rumen contained between 2-5 kilograms of fragments of plastic bags (Anagariyah et al., 2008).

#### 7.4.1.2 Drought

One of the major problems that wildlife face in arid and hyper-arid areas such as the Arabian Peninsula is the prolonged drought periods. In a country like Oman, some parts of the country for example, the Jiddat AlHarasis where the Arabian Oryx Sanctuary is located, sometimes don't receive rain for many years. Therefore, some wildlife, especially large mammals such as the Arabian oryx, Arabian gazelles and Nubian ibex may suffer from starvation and thirst. The problem of drought might be exacerbated by fencing of the Arabian Oryx Sanctuary that was originally planned to solve the problem of poaching.

Drought is one of the main physiological challenges that face Arabian oryx in the wild and may result in thirst, loss of body condition and even death (Spalton, 1999). A two year drought (1999-2000), in Uruq Bani Ma'arid in Saudi Arabia (located at the western edge of the Empty Quarter, one of the most arid places in the world), resulted in the mortality of about half of the population of Arabian oryx (75 out of 149 oryx) (Bedin and Ostrowski, 2003). The other causes of death were maladaptation to the wild and interspecific fights (Bedin and Ostrowski, 1998, 2003). The water content of plants favoured by Arabian oryx, for example, *Panicum turgidum*, *Lasiurus scindicus*, and *Stipagrostis sp.* varies seasonally and ranges from 5 to 56 % (Williams et al., 2001). As oryx mainly depend on preformed water in plants and metabolic water from consumed food, they may face severe thirst during droughts (Spalton, 1999; Ostrowski et al., 2002b), although in the drought of 1992 immigrant oryx did not return to Jaaluni for water. Thirst caused some Arabian oryx to abandon their newly-born calves (Ostrowski and Anagariyah, 2002). As yet we have no information on the faecal glucocorticoid metabolites of free-ranging oryx during prolonged drought conditions, but stress seems inevitable and increases would be expected as seen in studies of other mammals. For example, African elephants showed significantly elevated faecal glucocorticoid metabolites during the dry season compared to the wet season (Foley et al., 2001).

Drought was seen to lead to mass mortalities of reintroduced Arabian oryx, sand gazelles and introduced red-necked ostriches in the fenced Mahazat as-Sayd protected area in Saudi Arabia. Since the reintroduction project began in 1990 until 2008, deaths of 560 oryx and 2815 gazelle were recorded (Islam et al., 2010a). Most carcasses were found close to the

fence suggesting that starved and thirst animals tried to move outside the reserve, but the fence prevented them from doing so (Islam et al., 2010a). So, fencing may reinforced the effects of a drought by preventing natural movements of animals to other areas. To reduce the catastrophic effects of mass mortalities in the Mahazat as-Sayd protected area of Saudi Arabia, the wildlife conservation authorities developed a strategy and action plan (Islam and Knutson, 2008; Islam et al., 2010b) focused on determination of the carrying capacity of the reserve, and monitoring and management of oryx and sand gazelle population dynamics to keep animal numbers within 60-70 % of the carrying capacity.

The Arabian Oryx Sanctuary in Oman was not fenced since it was established in 1980 and it held a large number of Arabian oryx (more than 400 oryx in mid 1990s). Nevertheless, many 'natural' mortalities occurred, with highest rates among the youngest and oldest animals, which were reported to be due to malnutrition as a direct results of drought (Spalton, 1995), despite the fact that the oryx were able to move long distances searching for food in the unfenced reserve. The range of oryx exceeded 35,000 km<sup>2</sup> at that time. As the current plan is to fence a much smaller area (2824 km<sup>2</sup>), the problem of drought will be much worse after fencing and may be the major threat to the viability of the oryx population in Oman rather than poaching which used to be the major threat before fencing. The Arabian oryx project in Oman could learn a lesson from the story of mass mortalities in Saudi Arabia and adopt and develop similar management strategies and action plans, in particular in relation to determining the carrying capacity under drought conditions.

Conservation biologists have to play a role, in avoiding release of animals into conditions that adversely affect their welfare or threaten their survival. For example, during prolonged drought when the body condition of animals starts to deteriorate, supplementary food and water could be provided. Provision of food such as hay and/or lucerne can rescue the animals from starvation. However, the use of vehicles might not be practical because of the difficulty of spotting oryx in undulated terrains such as the escarpment of the Arabian Oryx Sanctuary in Oman, over a relatively large area. Therefore, provision of food by aircraft might be a better option. Linked to these plans, the monitoring of faecal glucocorticoid metabolites using the techniques outlined in Chapter 2 will provide a very useful tool for evaluating stress in the newly-released oryx and for remnant oryx that will face challenging environmental conditions.

Dickens et al. (2010) reviewed the role of chronic stress on the success of translocation. They illustrated that several acute stressors apply to translocation activities such as capture, handling, temporary confinement, transport, release procedures and the novelty of the new site, and collectively will inevitably result in a chronic stress state. Low food and water availability (drought) will exacerbate this status. As a result, chronically-stressed translocated animals will be vulnerable to several factors such as disease, a decrease in reproduction rate, predation, starvation and movement from the release site. These factors might potentially decrease the likelihood of translocation success. However, these factors should not preclude translocation as a conservation tool, because there are suggested ways to reduce the vulnerability of translocated animals to stressors related to translocation which are also reviewed by Dickens et al. (2010).

#### **7.4.2 Monitoring the welfare of reintroduced animals**

Once animals have been released to the wild, they should be controlled as a population to ensure the integrity and viability of the population. Monitoring of Arabian oryx populations after release to the wild is very important for the success of reintroduction programmes (Kleiman, 1989; Stanley Price, 1989; Seddon, 1999; Mesochina et al., 2003b). Post-release monitoring of the population has been found to be even more important than the number of released animals (Mesochina et al., 2003b) as shown by computer modelling that probability of extinction was high when no management was applied to the population of Arabian oryx (Treydte et al., 2001). Estimation of the population size is part of post-release monitoring and several estimators have been recommended (Mesochina et al., 2003b). Seddon et al. (2003) recommended the mark-resighting method as the most precise one. However, total count was found to be more precise (Chassot et al., 2005). Estimating population size (Chassot et al., 2005) should be coupled with assessment of the carrying capacity of the area (Treydte et al., 2001) and such methods need to be applied after fencing the Arabian Oryx Sanctuary in Oman. Other population monitoring tasks include satellite tracking for home range determination, tagging for identification, recording of births and deaths, recording individual details (sex and age status), habitat quality and meteorological data. The dominant social status of individuals of the herd can be evaluated by observing the behaviour of animals and utilising non-invasive methods that measure sex steroid hormones might be useful in this regard. Monitoring the reintroduction of wildlife using

such practices is common and well established, however monitoring the welfare of free-ranging wildlife is not as common. Obviously the approaches used in considering the welfare for captive animals such as the “Five Freedoms” cannot be applied to free-ranging wildlife, because most of the freedom criteria move outside the control of humans once an animal is free to roam.

There are various methods for assessment and monitoring of welfare of free-ranging wildlife. Kirkwood et al. (1994) suggested that assessment of the scale and severity of harm on welfare requires consideration of several factors which are: 1) the number of animals affected, 2) the cause and nature of harm, 3) the duration of the harm and 4) the capacity of the animal to suffer. The practicality of using such factors for assessment of welfare is not straightforward.

As mentioned earlier, Dickens et al. (2010) stated that translocated wildlife are most likely exposed to chronic stress, that adversely affects their welfare for some time. The assessment of stress of free-ranging wildlife especially large mammals like Arabian oryx that are difficult to capture, with methods that involve blood sampling, for example for measurement of blood cortisol or ‘leukocyte coping capacity’ (McLaren et al., 2003; Gelling et al., 2009), or measurement of body weight (McLaren et al., 2004), heart rate, or any haematological or blood biochemical parameters are not practical.

The establishment of baseline data for haematological, biochemical and physiological parameters against which to compare results of health surveys remain important mainly for captive animals or for small animals in the wild that can be easily captured, like water voles in the UK, where haematological parameters offer a useful indicator for survival (Mathews et al., 2006).

Therefore, only methods that do not involve repeat captures are useful for long term monitoring of welfare in free-ranging large mammals like the Arabian oryx. One of the increasingly used methods is measurement of faecal glucocorticoid metabolites, as an indicator of adrenocorticosteroid activity (Lane, 2006; Swaisgood, 2010a). The use of this method along with other complementary methods such as faecal thyroxine ( $T_4$ ) and triiodothyronine ( $T_3$ ) (Keech, 2008; Keech et al., 2010), body condition index and behaviour, would provide a good picture about animal welfare. The monitoring of

reproductive status of animals in the wild can be achieved by measuring faecal progesterone metabolites as was established for Arabian oryx (Ostrowski et al., 2005). Breeding of the released herd is a good indicator of reintroduction success (Seddon, 1999).

Evaluation of the body condition of animals after release to the wild, is an indicator of the availability of food or stress-induced depletion of body resources (Moberg, 2000). The physical condition index relies on a 5 point-scale, with 1 indicating a very low condition and 5 an excellent condition: (1) well-defined skeletal details and concave rump, (2) ribs protrude, rump flat, (3) ribs well defined, rump well rounded, (4) ribs show slightly, rump well rounded and (5) fully muscled with no ribs show (Hamilton et al., 1977; Gerhart et al., 1996). This index was adopted by the Arabian Oryx Project in Oman in 1990 (Spalton, 1995). In the present studies, the application of the method of body condition index on released oryx (Chapter 6) was not practical because of the short time scale of investigation after release (11 days) and difficulties in locating oryx in the wild. Utilisation of tracking techniques in future studies should help in locating animals after release. Emaciation is very common during drought (Gregory, 2004) and body condition index should be used to monitor body condition of released animals.

Another important aspect of monitoring animal welfare is the surveillance of diseases in the free-ranging wildlife. It is intrinsically more difficult to monitor diseases in wildlife than in domestic animals. Mass mortality of wildlife and animals showing obvious morbidity signs should be investigated by capture if feasible, and examined and treated if possible (Mörner et al., 2002). Translocation of wildlife without adequate risk assessment might result in introduction of destructive pathogens into naïve resident wildlife populations (Kock et al., 2010). In addition, the reverse process might also occur, as the released animals might carry pathogens that could infect the remnant population or other species existing in the area. Thus, the released animal should not be viewed as a single animal but as a package of potentially dangerous viruses, bacteria, protozoa, helminths and arthropods (Woodford, 2000). The newly-released animals might experience stress due to the novelty of the new location, and this might have applied in the studies reported in Chapter 6. In these studies, after transport and release to the wild there was an increase in faecal glucocorticoid metabolites (Figure 6.10 and Figure 6.11). Such stress might induce suppression of the immune system (Maule and Vanderkooi, 1999) of the newly-released animals, which

would probably make them less tolerant of infectious diseases. Prophylactic vaccination of the released animals against common diseases in the area should help in this regard. It is advisable to employ an inactivated vaccine, if available, because live vaccine might be pathogenic to other species (Kock et al., 2010).

#### 7.4.2.1 Intervention

There is a debate about intervention to capture and treat translocated or wild animals showing signs of disease. Some conservation biologists prefer no intervention and to “let nature take its course” (Deem et al., 2001). However, veterinary medicine recommends intervention, involving diagnosis, vaccination and treatment of diseases of wildlife (Woodford and Kock, 1991; Kirkwood, 1993; Woodford, 1993; Karesh and Cook, 1995). Infectious diseases in free-ranging wildlife are a threat that conservationists are often ill-equipped to manage. Small populations could be driven to extinction by virulent pathogens. Any poorly planned and funded intervention to vaccinate or treat infected animals has the potential to do more harm than good. The best approach for conservation biologists who lack veterinary knowledge and skills, probably is managing population size, structure and contact between host species as an alternative to veterinary intervention (Woodroffe, 1999).

Veterinarians can assist conservation biologists by playing a major role in re-introductions and their primary role is to ensure the health and welfare of animals both those selected for re-introduction and sympatric species in the recipient area. The suggested veterinary intervention role involves selection and application of appropriate health screening in both the source and recipient area, disease prophylaxis, use of chemical restraint technique, physiological monitoring during restraint and treatment of injuries (Kock et al., 2007).

Apparently, veterinary intervention with free-ranging animals in the wild is challenging as animals are usually difficult to capture for treatment. This in turn limits the role of veterinarians to be involved in maintaining health of free-ranging wildlife.

There are reported reintroduction failures due to disease for example, ten white rhinoceros (*Ceratotherium simum*) re-introduced from South Africa to Kenya, carried trypanosomes identified by veterinary checks. Despite advise to avoid moving them, translocation went ahead and all the re-introduced animals died (Kock et al., 2007). Management of diseases

by veterinarians on the other hand made some re-introductions successful such as mountain and sand gazelles in Saudi Arabian (Dunham, 1997; Abu Elzein et al., 1998; Dunham, 2001). At an outbreak of tuberculosis, the gazelles tested negative for the disease, were isolated in separate enclosures and then reintroduced successfully to the wild.

### **7.4.3 Assessment of reintroduction success**

Many reintroduction programmes (75-90 %) fail to establish a viable population (Beck et al., 1994; Wolf et al., 1998). Based on IUCN guidelines, reintroduction was defined as “an attempt to establish a species in area which was once part of its historical range” (IUCN, 1998). This definition implies that any reintroduction project that establishes a species in its historical range is a success. Several definitions have been proposed as to what constitutes a successful reintroduction (Seddon, 1999) and these include (i) breeding by the first wild-born generation (Sarrazin and Barbault, 1996), (ii) a three-year breeding population with recruitment exceeding adult death rate (Sarrazin and Barbault, 1996), (iii) an unsupported wild population of at least 500 individuals (Beck et al., 1994) and (iv) the establishment of a self-sustaining population (Griffith et al., 1989). Regardless of definitions, the problem of describing reintroduction projects as successful or unsuccessful (failed) is that the assessments are influenced by the point in time at which a population is assessed as self-sustaining, or not, and hence the success or failure of the reintroduction does not take account of long-term persistence (Seddon, 1999). For example, the reintroduction of Arabian oryx in Oman was considered one of few successful reintroductions at the time of assessment, but now there is no viable population in the wild. Therefore Seddon (1999) proposed a sequence of three objectives to determine success which are (i) the survival of the release generation, (ii) breeding by the release generation and their offspring, and (iii) persistence of the re-established population assessed through extinction probability modelling.

## **7.5 Future directions for conservation of Arabian oryx and their welfare**

### **7.5.1 Captive oryx**

There are now more than 10 ‘official’ captive breeding programmes for Arabian oryx in the Middle East. There are also many more oryx held in private collections (Ostrowski and Anagariyah, 2002). A recent estimate put the total number of Arabian oryx in the region of 8000 animals, and the majority of which are in captivity (Strauss, 2008). Of these, it is estimated that at most 20 % of all the oryx in the Arabian peninsula are currently of conservation value, because of the general lack of genetic management in many of the captive populations (Strauss, 2008). Therefore, the captive breeding programmes need to manage their herds of Arabian oryx by keeping a studbook of all individuals and managing the breeding in such a way as to ensure maximisation of genetic diversity of the herd. However, cross-breeding between individuals from populations that are bred separately for many generations, would also participate in maximisation of genetic diversity. This should keep the herd in good fitness and achieve higher survival rates after release to the wild.

Most Arabian oryx currently live in captivity, but there is a lack of information as to the extent the captive breeding programmes have plans for translocation of animals and to what extent they truly participate in the conservation of this endangered species. Therefore, awareness among those involved in captive breeding programmes needs to be raised in order to encourage contribution towards conserving this endangered species by reintroduction of healthy individuals into their natural habitat.

Currently there is a lack of information about the welfare state of Arabian oryx in the captive-breeding programmes and lack of welfare regulations related to their well-being and health. So, there is an urgent need to investigate the welfare status of Arabian oryx in the captive-breeding programmes. Moreover, it is equally important to advocate regulations to ensure that Arabian oryx and other endangered wildlife are housed and cared for in captive conditions that maintain good welfare. The Coordination Committee for Conservation of Arabian Oryx (CCCAO), based on Abu Dhabi, should play an important role in this regard. Official inspections from the committee should assess animal welfare standards and participation in conservation. For example, official inspection of UK zoos for

participation in biodiversity conservation and animal welfare judged most to be non-compliant with one or more animal welfare standard, only 24 % to have implemented all of the optional conservation measures in the legislation, and 11 % to participate in any conservation measures at all (Draper, 2010). Future research should focus on establishing welfare standards for holding Arabian oryx in captivity and the best methods of assessment and conservation-oriented improvement.

The majority of captive breeding programmes mix Arabian oryx with other species such as gazelles, sheep, ibex and deer (Ostrowski and Anagariyah, 2002) and consequently there is the risk of disease transmission to individual Arabian oryx rendering them of dubious conservation value. The regulations should discourage mixing Arabian oryx with other species in captivity to limit disease transmission. It is highly recommended that current captive populations are screened for infectious diseases, particularly those populations mixed with other species. Infected animals should be treated, vaccinated and isolated in enclosures without any other species. In the case of importing or exporting oryx from one country to another or when there is movement between captive groups within a country, screening and vaccination against diseases is necessary.

Surveillance of diseases present in captive and re-introduced populations of Arabian oryx should be carried out in all captive populations and the results published and circulated to inform all countries that hold Arabian oryx.

The acceptable density of captive populations of breeding animals needs to be considered with care and an agreed maximum derived to avoid overcrowding and the risks of poor welfare and distress. The bodies responsible for captive collections should adapt strategies to avoid overcrowding by providing adequate space, and control breeding or release surplus individuals to augment remnant populations.

The reference values established in the present studies (Chapter 3) should be utilised to aid in the clinical diagnosis of health and welfare of animals by wildlife veterinarians, with consideration of the effects of the method of capture and chemicals used for immobilisation (Chapter 5).

The method of measuring faecal glucocorticoid metabolites established in the present studies (Chapter 2) should be utilised for monitoring the welfare of animals in captivity and further studies of Arabian oryx in the wild (begun in the present studies: Chapter 6). This method should help in optimising the housing and feeding conditions, further assessing transportation procedures (Chapter 6) and assessing the relative effects of 'soft' and 'hard' release of captive animals, and therefore improve the welfare of captive Arabian oryx.

In the present studies, the behaviour of oryx was not used in assessment of welfare, although such approaches would be useful in future for a more integrated understanding of the welfare of Arabian oryx.

Methods should be developed for improving the welfare of Arabian oryx in captivity such that life in captivity provides a better preparation for life in the wild.

Ultimately, keeping captive Arabian oryx in conditions that ensure good welfare will ensure introduction of healthy individuals to the wild and a healthy wild population.

### **7.5.2 Translocation of Arabian oryx**

There is a need for establishment of guidelines for selecting the suitable release site, assessing the need for provision of food and water for the released herd until animals become physiologically adjusted to life in the wild.

Mixing wild-born with captive-born oryx after 'hard' release, might be a good strategy as the wild-born individuals could pass on foraging skills to the captive born animals that lack wild experience.

The preparation for releasing animals to the wild involves the consideration of the social aspects of animals as Arabian oryx are known to have a social hierarchy.

Creative solutions should be found to solve the problem of oryx familiarity with the presence of humans and vehicles that puts them at risk of poaching after release.

The use of long- and short-acting tranquillisers during translocation in combination with temporary confinement should increase the chance of successful translocation and reduce the associated stress of transportation (as revealed by the studies in Chapters 6).

### **7.5.3 Free-ranging oryx in the wild**

For any reintroduction programme to succeed, it has to consider the local people who live on the area. Tear and Forester (1992) suggested the use of social theory that was presented by Firey (1960) as a suitable conceptual framework to help in planning reintroductions and improve success rates. The reintroduction programme for Arabian oryx in Oman started in 1980 and was considered one of the most successful reintroduction programmes of a large animal in the world (Kleiman, 1989). Unfortunately, the project started to collapse in the mid 1990s, because of poaching. The poaching problem might be mainly a social problem, expressed as a reaction of local people (tribes) that were not involved in the reintroduction project and recruitment of rangers from only one tribe (Spalton, 2003). This stresses the importance of involving the local community for the success of wildlife reintroduction and conservation (Hackel, 1999; Berkes, 2004).

Surveillance of diseases present in re-introduced populations of Arabian oryx (see Chapter 1, section 1.1.4) should be carried out. Intervention policies to prevent or treat diseases in free-ranging oryx should be developed and implemented. Further research is needed to help decreasing the accumulated stress associated with the re-introduction process as a result of capture, confinement, transport, and then exposure to a novel area without feeding.

Post release monitoring is important for evaluating the success of the re-introduction and to ensure that individuals in the population are in a good welfare state.

Although fencing might solve the poaching problem, it could create a bigger problem of suffering by animals during prolonged drought periods as normally oryx would move vast distances in search of suitable food and maintaining their water balance (see Chapter 7 section 7.4.1.2). Hence post-release monitoring of the welfare of individuals is indispensable.

## Appendices

### Appendix I

List of all Arabian oryx used in different experiments of this thesis and the dates when different experiment started.

No.	Animal	sex	First capture	ACTH (Ch 2)	Dexamethasone (Ch 2)	Storage (Ch 2)	Reference values (Ch 3)	Perphenazine enanthate (Ch 4)	saline (Ch 4)	xylazine (Ch 5)	Transport (Ch 6)	Release (Ch 6)
1	998	female	30/08/2006	No	No	No	30/08/2006	No	No	No	No	No
2	13A	male	NR*	No	No	No	18/09/2006	No	No	No	No	No
3	995	female	10/07/2006	No	No	No	25/12/2006	No	No	No	25/12/2006	No
4	996	female	18/07/2006	No	No	No	25/12/2006	No	No	No	26/12/2006	No
5	997	female	30/07/2006	No	No	No	25/12/2006	No	No	No	27/12/2006	No
6	10A	male	Sep-2006	No	No	No	25/12/2006	No	No	No	28/12/2006	22/02/2007
7	1A	male	Sep-2006	No	No	No	25/12/2006	No	No	No	29/12/2006	22/02/2007
8	5A	male	Sep-2006	No	No	No	25/12/2006	No	No	No	30/12/2006	22/02/2007
9	10B	male	Jan-2007	No	No	No	20/02/2007	No	No	No	20/02/2007	19/04/2007
10	16B	male	Jan-2007	No	No	No	20/02/2007	No	No	No	20/02/2007	19/04/2007
11	17B	male	Jan-2007	No	No	No	20/02/2007	No	No	No	20/02/2007	19/04/2007
12	19B	male	Jan-2007	No	No	No	20/02/2007	No	No	No	20/02/2007	19/04/2007
13	1B	male	Jan-2007	No	No	No	20/02/2007	No	No	No	20/02/2007	19/04/2007
14	20B	male	Jan-2007	No	No	No	20/02/2007	No	No	No	20/02/2007	19/04/2007
15	10C	male	Mar-2007	No	No	No	16/04/2007	No	No	No	16/04/2007	16/05/2007
16	16C	male	Mar-2007	No	No	No	16/04/2007	No	No	No	16/04/2007	16/05/2007
17	17C	male	Mar-2007	No	No	No	16/04/2007	No	No	No	16/04/2007	16/05/2007

No.	Animal	sex	First capture	ACTH (Ch 2)	Dexamethasone (Ch 2)	Storage (Ch 2)	Reference values (Ch 3)	Perphenazine enanthate (Ch 4)	saline (Ch 4)	xylazine (Ch 5)	Transport (Ch 6)	Release (Ch 6)
18	19C	male	Mar-2007	No	No	No	16/04/2007	No	No	No	16/04/2007	16/05/2007
19	1C	male	Mar-2007	No	No	No	16/04/2007	No	No	No	16/04/2007	16/05/2007
20	20C	male	Mar-2007	No	No	No	16/04/2007	No	No	No	16/04/2007	16/05/2007
21	F15	female	Sep-2007	04/05/2008	11/11/2007	05/05/2008	20/01/2008	18/11/2007	20/01/2008	06/02/2008	No	No
22	M1	male	Jul-2007	04/05/2008	16/09/2007	05/05/2008	03/02/2008	24/09/2007	12/01/2008	03/02/2008	No	No
23	M3	male	Jul-2007		16/09/2007		03/02/2008	24/09/2007	12/01/2008	03/02/2008	No	No
24	M5	male	Jul-2007	04/05/2008	16/09/2007	05/05/2008	03/02/2008	24/09/2007	12/01/2008	03/02/2008	No	No
25	M9	male	Jul-2007		16/09/2007		03/02/2008	24/09/2007	12/01/2008	03/02/2008	No	No
26	M10	male	Jul-2007		16/09/2007		04/02/2008	24/09/2007	12/01/2008	04/02/2008	No	No
27	M14	male	Sep-2007		11/11/2007		04/02/2008	18/11/2007	12/01/2008	04/02/2008	No	No
28	M12	male	Sep-2007		11/11/2007		04/02/2008	18/11/2007	12/01/2008	04/02/2008	No	No
29	M10B	male	Sep-2007		11/11/2007		04/02/2008	18/11/2007	12/01/2008	04/02/2008	No	No
30	F12	female	Sep-2007		11/11/2007		05/02/2008	18/11/2007	20/01/2008	05/02/2008	No	No
31	F14	female	Sep-2007		11/11/2007		05/02/2008	18/11/2007	20/01/2008	05/02/2008	No	No
32	F16	female	Sep-2007		11/11/2007		05/02/2008	18/11/2007	20/01/2008	05/02/2008	No	No
33	F18	female	Sep-2007		11/11/2007		05/02/2008	18/11/2007	20/01/2008	05/02/2008	No	No
34	F17	female	Jul-2007	04/05/2008	16/09/2007	05/05/2008	06/02/2008	24/09/2007	20/01/2008	06/02/2008	No	No
35	F19	female	Sep-2007	04/05/2008	11/11/2007	05/05/2008	06/02/2008	18/11/2007	20/01/2008	06/02/2008	No	No
36	F20	female	Sep-2007		11/11/2007		06/02/2008	18/11/2007	20/01/2008	06/02/2008	No	No

\* NR, not recorded.

**Appendix II**

Morphology of blood cells of Arabian oryx compared to human blood cells

**Methods****1. Examination of blood cells by light microscope**

Blood smear slides were prepared from blood samples within 2 h of collection in glass tubes containing the anticoagulant EDTA. The slides were stained with Giemsa-Wright stain (Hematek stain pack, 4405, Bayer health care). The slides were examined under a light microscope (Leica DMLB) attached to a digital camera (Leica DFC300 FX), and images of blood cell types were captured using the camera software (Leica AF6000E).

**2. Examination of blood cells by transmission electron microscopy**

Blood samples were collected in vacutainer tubes containing an anticoagulant (EDTA). The blood was centrifuged and the buffy coat (the white layer between plasma and erythrocytes) was removed, fragmented into small pieces (about 2 mm<sup>3</sup>) using a razor blade under a stereo light microscope and placed in Karnovsky fixative (2.5 % gluteraldehyde, 4 % paraformaldehyde in 1M cacodylate buffer, pH 7.2) for 2 h at 4 °C. After fixation, the buffy coat fragments were washed in 1M sodium phosphate buffer (3 x 10 min). Secondary fixation was carried out in 1 % osmium tetroxide in distilled water for 60 minutes. The buffy coat was washed again in 1M sodium phosphate buffer (3 x 10 min). For dehydration, the specimens were treated with a graded series of acetones (25 %, 50 %, 75 %, and 90 %) and four changes of 100 % acetone at room temperature for 15 min each. The specimens were infiltrated with araldite epoxy resin with three different ratios of acetone:araldite, 3:1 for 1 h, 1:1 for 1 h, 1:3 for 30 min and then with 3 changes of 100 % araldite at 37°C for 1 h, 30 min and 30 min, respectively. The specimens were then embedded in beam capsules and placed in the oven at 60 °C overnight to polymerize. Once the specimens were polymerized in resin, they were ready for sectioning (ultramicrotomy). Glass knives of 45° angle were produced using a glass knife maker (Leica). Semi-thin sections of 1 µm thickness were produced using an ultra-microtome (Reichert-Jung, Leica, Ultra-cut 701701), placed on a slide and stained with toluidine blue. From the area of interest, ultra-thin sections of 60-90 nm thickness were cut at 1 mm/sec. Sections were picked up with copper grids. The sections were stained first with supersaturated uranyl acetate for 30 min at room temperature in the dark, rinsed thoroughly with distilled water and stained secondly

with lead citrate for 10 min at room temperature. The sections were examined using a transmission electron microscope (JEOL JEM -1230, Japan) equipped with a digital camera (Gatan 792-CCD) operated at 100kV.

## Results

Typical morphology of white blood cells in humans is shown in Figure 8.1 (Junquera and Carnerio, 2005) for comparison to Arabian oryx blood cells. Neutrophils of Arabian oryx have multi-lobes linked by fine thread of chromatin and contain granules (Figure 8.2 and Figure 8.3). The Arabian oryx neutrophils are 5.0 – 10.3  $\mu\text{m}$  in diameter ( $n = 8$  EM images). The basophils of Arabian oryx contain basophilic granules (Figure 8.4), which stain pink with Wright-Giemsa stain (Figure 8.5). The diameter of basophils of Arabian oryx ranges from 5.9 – 6.7  $\mu\text{m}$  ( $n = 4$ ). Eosinophils are characterised by granules that contain disk-shaped electron dense crystalline core (Figure 8.6) and a characteristic bi-lobed nucleus with filament thread between the two lobes; the granules are not stained with Wright-Giemsa stain (Figure 8.7). Eosinophils range from 5.9 to 10.0  $\mu\text{m}$  in diameter ( $n = 5$ ). Monocytes of Arabian oryx lack specific granules (agranulocyte) and are characterised by an irregular horse-shoe like nucleus (Figure 8.8 and Figure 8.9). Monocytes range in size from 5.8 to 6.8  $\mu\text{m}$  ( $n = 3$ ). Lymphocytes of Arabian oryx contain a distinct spherical or round nucleus, which occupies most of the cytoplasm (Figure 8.10 and Figure 8.11). Only two lymphocytes were measured and had diameters of 5.6 and 6.8  $\mu\text{m}$ .

Erythrocytes of Arabian oryx are biconcave in shape (Figure 8.12) with a diameter of 3.7 – 6.7  $\mu\text{m}$  ( $n = 3$ ). Platelets of Arabian oryx are non-nucleated and contain an open canalicular system (Figure 8.13). Under the light microscope, they are seen to be smaller than erythrocytes and sometimes cluster in groups (Figure 8.14).

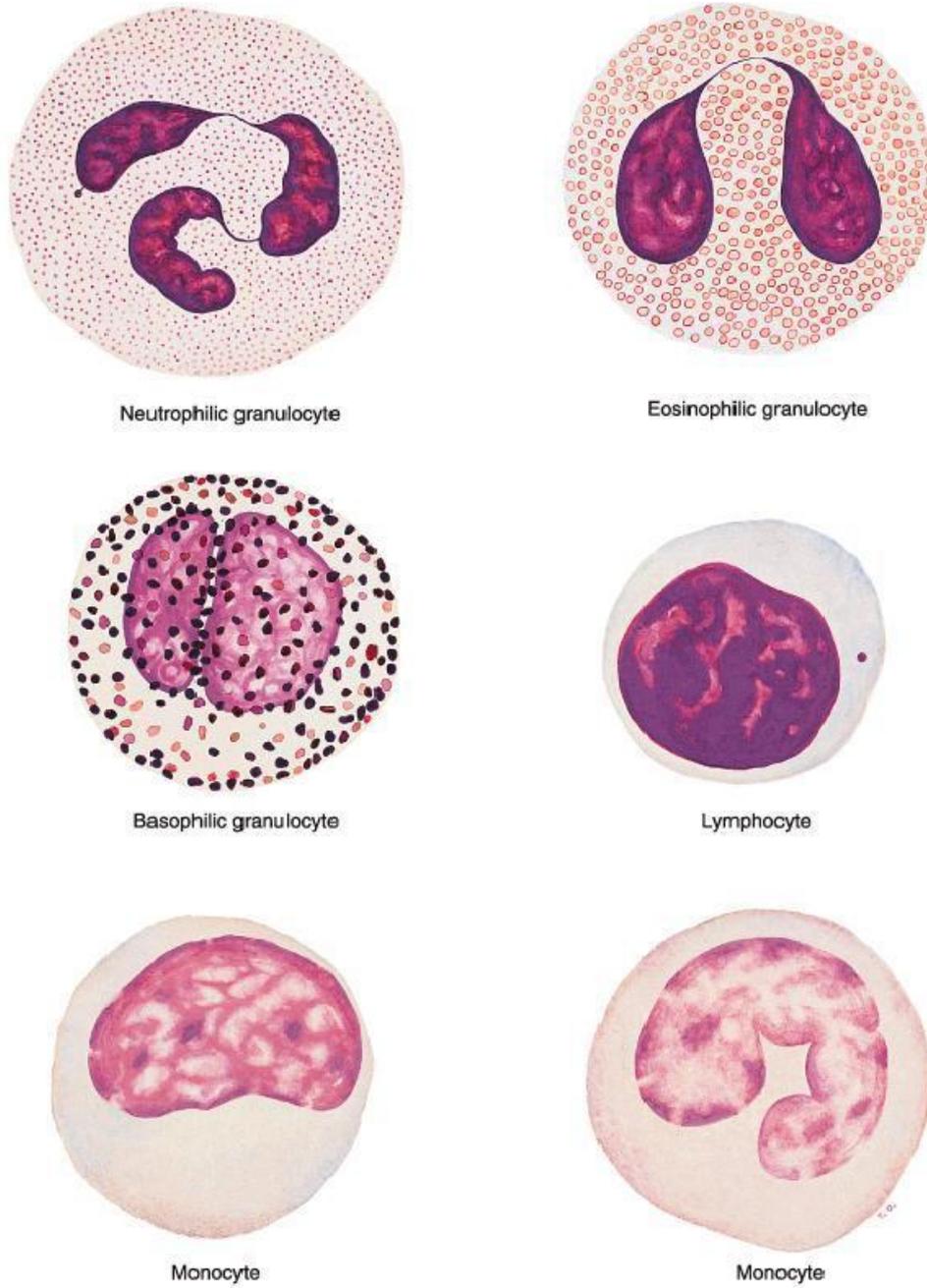


Figure 8.1 Typical illustration of morphology of white blood cell types (Junquera and Carnerio, 2005).

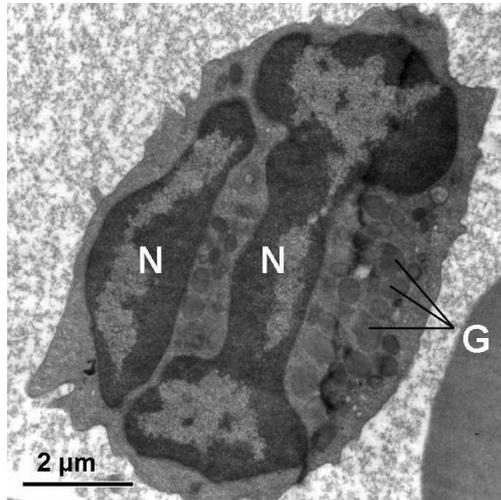
**White blood cells****Neutrophils**

Figure 8.2 An electron micrograph of Arabian oryx neutrophil (x20000). N, nucleus and G, granules.

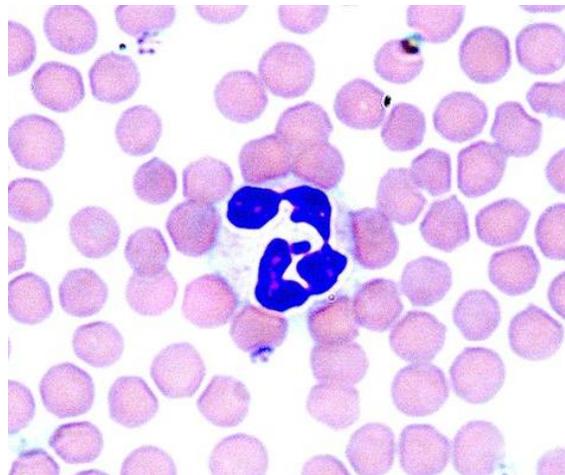


Figure 8.3 A Micrograph of Arabian Oryx neutrophil. Light microscope, high magnification, Wright-Giemsa stain, (x2000).

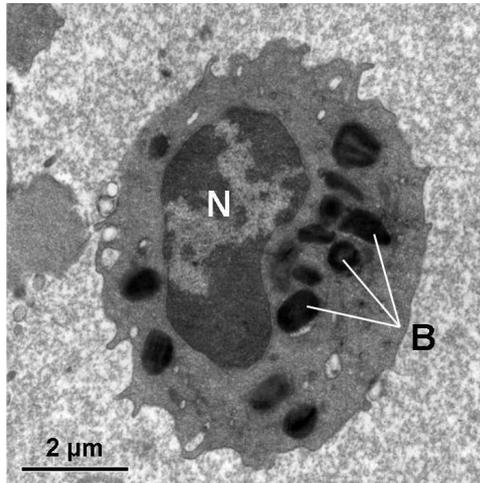
**Basophils**

Figure 8.4 An electron micrograph of an Arabian oryx basophil (x20000). N, nucleus and B, basophilic granules.

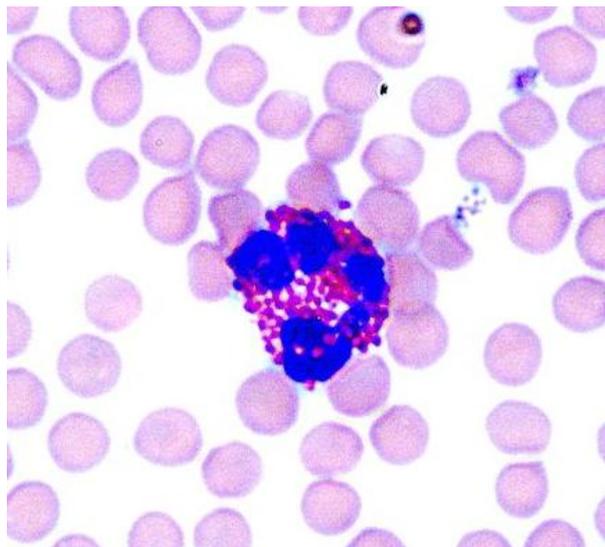


Figure 8.5 A micrograph of Arabian oryx basophil. Light microscope, high magnification, Wright-Giemsa stain, (x2000).

## Eosinophils

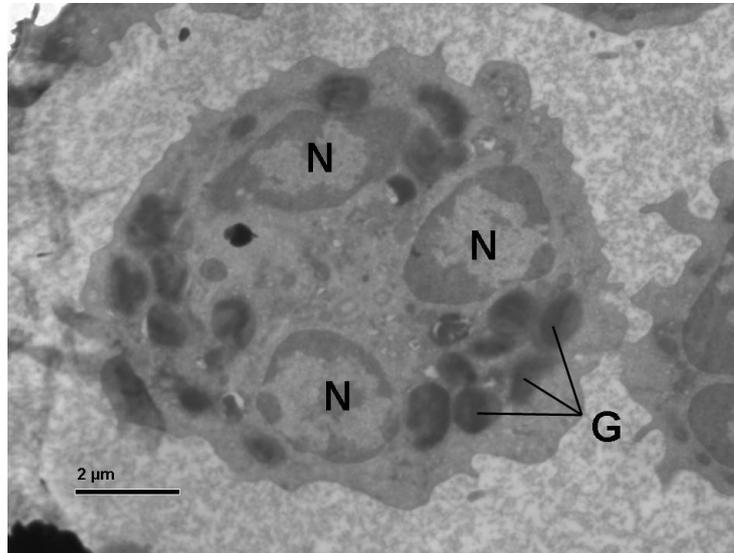


Figure 8.6 An electron micrograph of an Arabian oryx eosinophil (X20000). N, nucleus and G, granules with disk-like electron dense crystalline core.

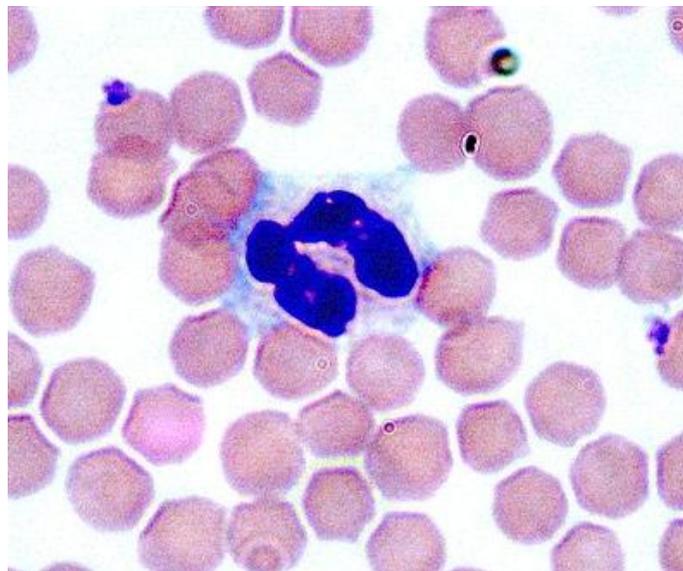


Figure 8.7 A micrograph of Arabian oryx eosinophil. Light microscope, high magnification, Wright-Giemsa stain, (x2000).

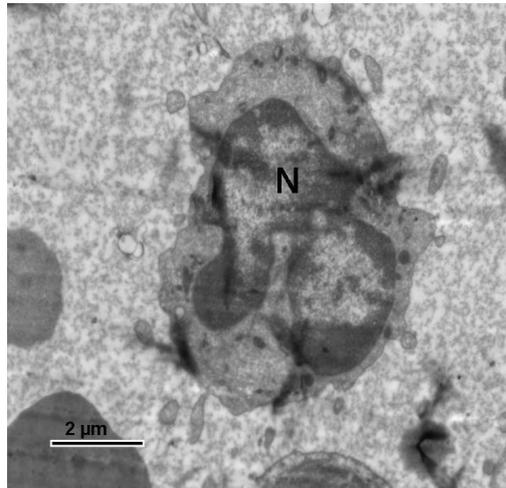
**Monocytes**

Figure 8.8 An electron micrograph of an Arabian oryx monocyte (x25,000). N, nucleus.



Figure 8.9 A micrograph of Arabian oryx monocyte. Note the horse-shoe like nucleus. Light microscope, high magnification, Wright-Giemsa stain, (x2000).

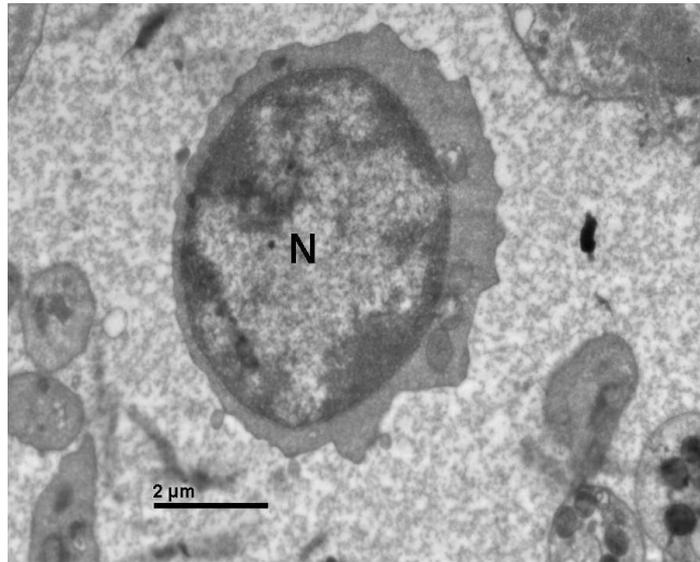
**Lymphocytes**

Figure 8.10 An electron micrograph of an Arabian oryx lymphocyte (x30,000). N, nucleus.

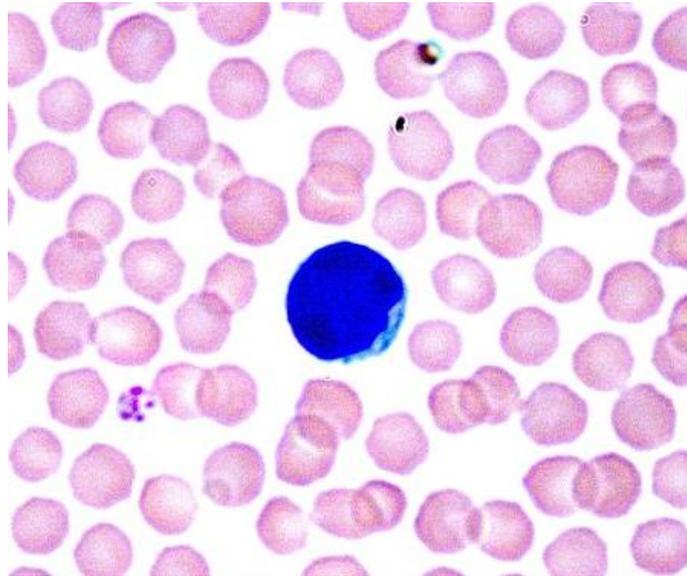


Figure 8.11 A micrograph of Arabian oryx lymphocyte. Light microscope, high magnification, Wright-Giemsa stain, (x2000).

Erythrocytes

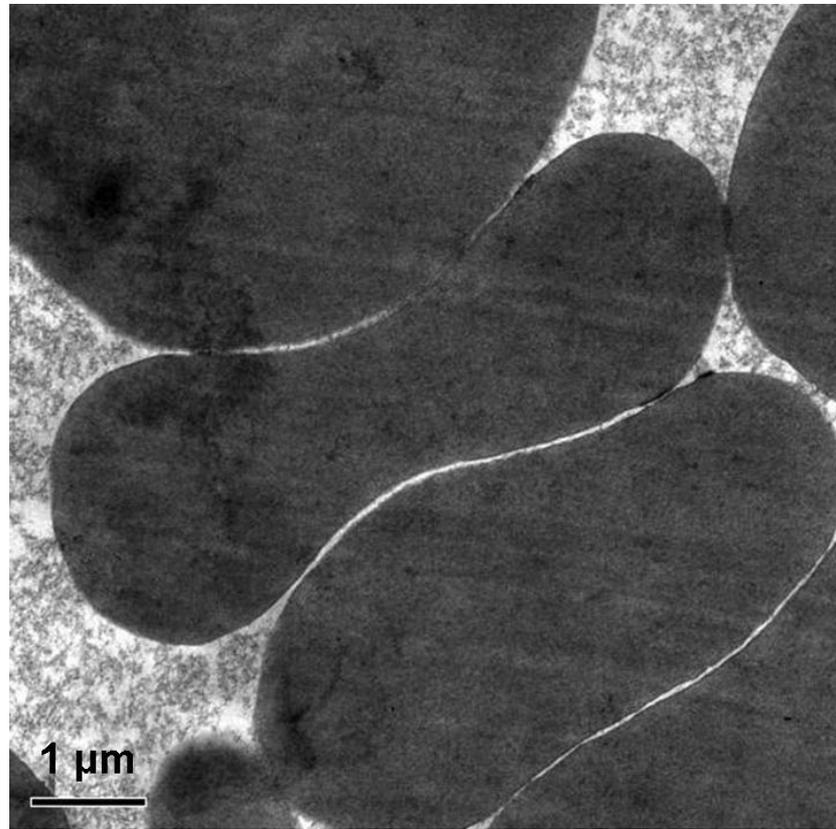


Figure 8.12 An electron micrograph of Arabian oryx erythrocytes (x25,000).

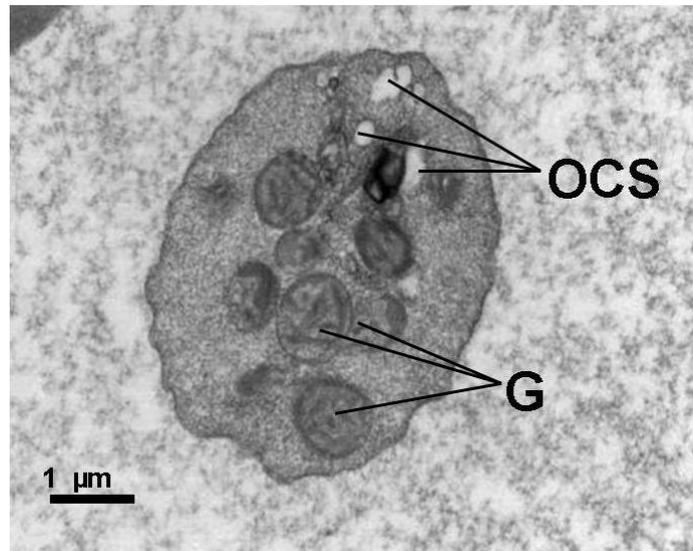


Figure 8.13 An electron micrograph of an Arabian oryx platelet (x30,000). OCS, open canalicular system and G, granules.

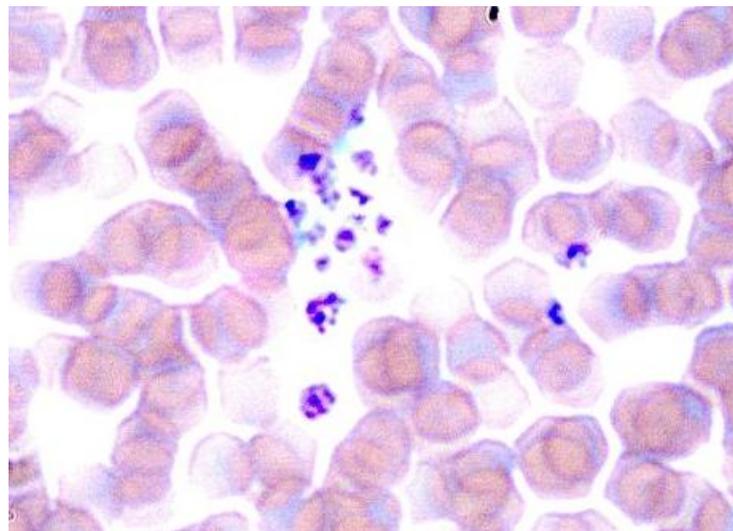


Figure 8.14 A group of platelets from Arabian oryx with red blood cells. Light microscope, high magnification, Wright-Giemsa stain, (x2000).

### Discussoin

The neutrophils of Arabian oryx have a multi-lobed nucleus that is similar to that of humans. The basophils of Arabian oryx have stained granules that appear pinkish with Wright-Giemsa stain, under light microscope, like the basophils of humans. The nucleus of Arabian oryx eosinophils is bi-lobed and connected by a thin filament, which again is a similar feature in humans. The horse-shoe like monocytes of Arabian oryx also resemble those of humans. The spherical nucleus of lymphocytes is an unmistakable feature in those of Arabian oryx and humans. Despite the similarity in morphology of most blood cell types, there were some differences from humans. For example, the volume of erythrocytes is slightly smaller in Arabian oryx than humans. The corpuscular volume of Arabian oryx erythrocyte ranges from 40 to 50 fl (Table 3.7), while that of humans ranges from 80 to 100 fl (Davidson and Hamilton, 1978; Tonnesen et al., 1986), but comparable to 49 fL reported for scimitar horned oryx (*Oryx dammah*) (Hawkey, 1975). In Arabian oryx and humans, erythrocytes are biconcave in shape (Figure 8.12). From Table 8.1, it can be seen that most blood cells of Arabian oryx and humans have similar sizes and similar nuclear lobularity, but erythrocytes are slightly smaller in size in Arabian oryx.

Cell size is one factor beside others, like cytoplasmic granularity and nuclear lobularity, that the instrument uses to identify blood cell types. For lymphocytes and monocytes that were slightly smaller in size in Arabian oryx than in humans (Table 8.1), the instrument identifies them mainly by cytoplasmic granularity and nuclear lobularity. Therefore, calibration of the instrument for these parameters is not necessary and measurements should be reliable.

In the present study, the Cell-DYN 4000 successfully identified and counted red blood cells of Arabian oryx by measurement of optical scatter and electrical impedance. Red blood cells of oryx were smaller than that of humans (Table 8.1), and therefore in future studies of Arabian oryx blood samples, calibration of the analytical instrument would be desirable.

The variability of measurements for duplicated haematological samples was between 0.23 and 3.36 % for most parameters (leukocytes, neutrophils, lymphocytes, erythrocytes, haemoglobin, haematocrit, MCV, MCH and MCHC), which indicates high level of precision of the analytical method. A coefficient of variation of 10 % or less is considered

satisfactory (Murray et al., 1993). However, counts for monocytes, eosinophils and basophils gave coefficients of variation of 126.2 %, 20.6 % and 21.8 % respectively. This high variability between samples might be due to the fact that these leukocytes represent only a small percentage (3-6 %) of the total leukocyte count, so variability can be expected. The platelets count also showed high variability (36.3 %), which indicates low precision in platelet counts.

Based on similarity between Arabian oryx blood cells and those of humans and the low coefficient of variations for most haematological parameters, it can be concluded that the Cell-Dyn 4000 is consistent and can be used for analysing haematological parameters of Arabian oryx.

Blood Cell	Diameter		Nucleus features	
	Human	Arabian oryx	Human	Arabian oryx
Erythrocyte	7.5 µm	3.7 - 6.7 µm	No nucleus, biconcave	No nucleus, biconcave
Neutrophil	8 - 9 µm	5.0 - 10.3 µm	3 to 4 lobes	3 to 4 lobes
Eosinophil	9 - 11 µm	5.9 - 10.0 µm	2 lobes (sausage shaped)	2 lobes (sausage shaped)
Basophil	7 - 8 µm	5.9 - 8.7 µm	S-shaped	S-shaped
Lymphocyte	7 - 8 µm	5.6 - 6.8 µm	Round	Round
Monocyte	10 - 12 µm	5.8 - 6.8 µm	Horse-shoe shaped	Horse-shoe shaped
Platelet	2 - 4 µm	2.0 - 3.3 µm	No nucleus	No nucleus

Table 8.1 Comparison between the size and the nuclear features of blood cell types of Arabian oryx and humans. Information about the size and the features of nucleus for humans were obtained from Junquera and Carnerio (2005).

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