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Chapter 5

REFERENCE VALUES FOR HAEMATOLOGICAL, BIOCHEMICAL AND PHYSIOLOGICAL PARAMETERS IN ARABIAN ORYX

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ABSTRACT

The vulnerable 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 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. 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. 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

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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 Arabian oryx. 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.

Keywords: Biochemistry, Biotechnology, Arabian oryx, Oryx leucoryx

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 (Karesh & Cook, 1995; Kirkwood, 1993). 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, it is important 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 (Deem et al., 2001; Karesh et al., 1997). These reference values aid in the diagnosis of certain pathologies (Deem et al., 2001; Kaneko et al., 1997). 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; Junqueria & 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 confusion (Solberg, 2006; Walton, 2001). 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 (Dimauro et al., 2008; Harris & Boyd, 1995; Horn & Pesce, 2003; Lumsden, 1998; Solberg, 2006; Walton, 2001). 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), Spanish ibex (*Capra pyrenaica*) (Perez et al., 2003), black-faced impala (*Aepyceros melampus petersi*) (Karesh et al., 1997), 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 of their health and well-being. The availability of accepted reference values for haematology, biochemistry, ions and hormones and clinical parameters will help in this monitoring and 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 & Lavin, 1999) and koalas (*Phascolarctos cinereus*) (Hajduk et al., 1992). Some haematological and biochemical parameters, and data for body temperature, heart and respiratory rates were reported for neonates of Arabian oryx (Bounous-Dalton & 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 & 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 cannot 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) (Ancrenaz et al., 1996; Greth et al., 1993; Vassart & Greth, 1991) 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 & 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 data points 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, this study 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 (Kocan et al., 1981; Presidente et al., 1973; Seal et al., 1972; Wesson III et al., 1979). In this study, we 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 are examined. Thirty six adult Arabian oryx (24 males and 12 females) were used for the establishment of reference values. All reference values presented in this study were obtained from oryx that were captured physically without using chemical immobilisation or tranquillisation. 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 represented about 8 % of Oman's total population of captive and wild oryx (430 individuals, at the end of 2008). Arabian oryx at the Omani Mammals Breeding Centre are held in two enclosures of areas 37,331 m^2 and 12,674 m^2. 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. The inclusion criteria included adults of age between 1 and 5 years, apparently healthy and from both sexes. Exclusion criteria included calves, old oryx, dominant males, pregnant and lactating females, and apparently sick animals. 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. The oryx were blind-folded immediately after capture and blood samples were collected by a qualified veterinarian. 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 18G (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. 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.

BLOOD SAMPLES ANALYSES

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. The inter- and intra-variations of data for haematological parameters were measured for 8 males using duplicate samples (Table 1) as recommended by (Murray et al., 1993).

Table 1. Inter- and intra-variations (%) of haematological parameters calculated for 8 male Arabian oryx after measurement by Cell-Dyn 4000

Parameters	Intra-variation	Inter-variation
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

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. 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), at the Department of Biochemisty, Sultan Qaboos University Hospital 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 where analysed by immuno-assays (Access® immuno-assay system, Beckman Coulter Inc.). The studied parameters were cortisol, free thyroxine (T_4), free triiodothyronine (T_3), and insulin. The instruments that measured blood ions, biochemistry and hormones (Cobas Integra 800 and Access, respectively) were calibrated as a routine practice. 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 to saline to give: 0 % 25 % 50 % 75 % and 100 % serum. The dilution of serum with saline resulted in the expected decline in the concentration of selected representative parameters. Interassay and intraassay variability in the concentrations of ions, biochemical parameters and hormones calculated from 4 duplicated samples are shown in Table 2.

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).

Table 2. Inter- and intra-variations (%) of serum ions, biochemistry and hormones calculated from 4 duplicated samples

Parameters	Intra-variation	Inter-variation
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_4	7.73	9.02
Free T_3	21.79	12.86
Insulin	30.30	56.84

The statistical differences between sexes (male versus female) were compared by one way ANOVAs followed by multiple comparison tests (Holm-Sidak method). Where data could not be tested by one 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 study, the data from the present studies are presented as mean and standard error of mean, unless stated otherwise.

The haematological data for male and female Arabian oryx are presented in (Table 3). All reported haematological parameters other than white blood cells and the percentages of neutrophils, lymphocytes and basophils, did not differ between males and females (Table 3). 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).

The values for biochemical and clinical parameters, ions, osmolality and hormones for male and female oryx are shown in Table 4. Most parameters did not differ significantly between males and females. The only parameters that differed significantly between males and females were sodium ($P = 0.027$), urea ($P < 0.001$), glucose ($P < 0.001$), cortisol ($P < 0.001$) and free T_3 ($P = 0.016$). Males have significantly higher values of sodium, urea, glucose and free T_3 and significantly lower cortisol than females (Tables 4).

The main aim of this study 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 animals. 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 AlWusta Wildlife Reserve, known previously as 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.

Table 3. 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	Sex	Unit	n	Mean	SD	SEM	2.5 %	97.5 %
WBC	Males	$x10^9$/L	24	8.03	1.60	0.33	5.11	11.20
	Females	$x10^9$/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	$x10^{12}$/L	24	12.22	0.75	0.15	10.70	13.70
	Females	$x10^{12}$/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	$x10^9$/L	24	215.64	109.51	22.35	44.90	429.00
	Females	$x10^9$/L	12	157.97	177.78	51.32	38.30	663.00

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; Solberg, 2006). However, sample size should take account of the total number of available animals, particularly for endangered species. The total number of Arabian oryx in Oman is (2010, time of study) 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 & 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' relating to welfare that encourage reduction of the number of animals used to obtain information (Russell & Burch, 1959).

Table 4. 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_3, triiodothyronine, T_4, thyroxine; bpm, breaths per min (for respiratory rate) or beats per min (for heart rate)

	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	mosmol/Kg	16	309.53	10.53	2.63	286.50	325.00
		Females	mosmol/Kg	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_4	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_3	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

The samples in the present study were exclusively from Arabian oryx housed at the Omani Mammals Breeding Centre, 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 centre at the time of the study, which represents a relatively high proportion, about a quarter (24 %) of the herd at the centre. 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 the previous 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 & Lavin, 1999) and therefore, the values reported by those studies might differ from the reference values reported in present studies.

Immobilisation has significant effects on many haematological and biochemical parameters, as found in Arabian oryx using xylazine (AlJahdhami 2010). This agrees with the effects of chemical capture by immobilisation that 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 report haematological and biochemical parameters from immobilised oryx are not compared to those obtained in the present study. Future researchers who measure blood parameters of Arabian oryx should consider the method of capture (i.e. with or without immobilisation) before comparing their results with the reference values obtained in the present studies.

The reference values obtained in the present study are compared with those reported in the few previous 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”.

HAEMATOLOGY STUDIES

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 also been measured by Bounous-Dalton & Hood (1980) and Ferrell et al., (2001). Comparing their haematological values for neonatal Arabian oryx, the values of red blood cell counts, haematocrit and haemoglobin concentrations were clearly lower in neonates than in the combined values of male and female adults included in the present study (Table 5 and Table 6).

The mean leukocyte counts in the present study are comparable to those of neonates reported by Bounous-Dalton and Hood (1980) (Table 5). However, Ferrell et al. (2001) reported a lower median WBC count than that obtained in the present study (Table 6). The values of MCV, MCH and MCHC are comparable between neonates and adult Arabian oryx (Bounous-Dalton & Hood, 1980) (Table 5). 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.

A study by Kilgallon et al. (2008) is the only investigation of haematological parameters of adult Arabian oryx without using any immobilising or tranquilising chemicals. Comparison of the values reported by Kilgallon et al. (2008) and combined values of males and females in the present study is presented in Table 7. 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 the study of Kilgallon et al. (2008) 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 than 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 (Burton et al., 1995; Iseki et al., 1991; 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 & Johnson, 1993). Cortisol and catecholamine-mediated distortions of haematology and blood biochemistry have been reported in captured impala and red deer (Marco & Lavin, 1999).

The mean haematocrit reported by Kilgallon et al. (2008) (41.79 %, Table 7) and ISIS (2002) (44.00 %, Table 8) 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 1) which are below the satisfactory accepted percentage of variation (10 %) suggested by Murray et al. (1993). Arabian oryx in the holding pens were provided with water twice a day (morning and evening) in a water container, but could have been dehydrated by the time of handling, as water was provided after handling. Serum osmolality is another indicator of hydration status but urine osmolality is a better 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 & McKenzie, 2002), and consequently causing an increase in haematocrit. Probably the stress of capture caused an increase in haematocrit in Arabian oryx in the present study. However, 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.

Table 5. Comparison of haematological values for neonates as reported by Bounous-Dalton and Hood (1980) and for combined values of male and female adults as reported in the present study. n, number of animals; SD, standard deviation of mean; SEM, standard error of mean; Abrreviations as in Table 3

Parameter	Bounous-Dalton and Hood, 1980					The present study			
	Unit	n	Means	SD	SEM	n	Mean	SD	SEM
WBC	$x10^9$/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	$x101^2$/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 6. 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

Parameter	Ferrell et al., (2001)				The present study			
	Median	Min	Max	n	Median	Min	Max	n
WBC ($x10^9$/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 7. Comparison between some haematological parameters reported by Kilgallon et al., (2008) and the combined values for males and females in the present study. SD, standard deviation of mean, SEM, standard error of mean, n, number of animals and WBC, white blood cells

Parameter	Kilgallon et al., (2008)				The present study			
	n	Mean	SD	SEM	n	Mean	SD	SEM
WBC ($x10^9$/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 8. Comparison between some haematological parameters reported by the database of ISIS (2002) and the combined values for males and females in 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

		ISIS, 2002				The present study		
Parameter	Unit	Mean	SEM	Samples	Animals	Mean	SEM	N
WBC	$x10^9$/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	$x10^{12}$/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	$x10^9$/L	330.00	30.00	35	26	196.42	22.72	36

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).

In the present study significant differences between male and female Arabian oryx occurred 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.

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 between male and female Arabian oryx.

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 ± 0.19 mmol/L and 1.16 ± 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 ± 1.24 mmol/L and 2.29 ± 0.04 mmol/L respectively) and values did not differ significantly in males and females (Table 4).

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 9).

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), 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.

In the present study, there was a significant difference between the serum glucose concentrations of male and female oryx (6.08 ± 1.94 mmol/L in males; 5.14 ± 2.21 mmol/L in females). A number of studies have reported the concentration of glucose in Arabian oryx, but for mixed sex groups. Ostrowski et al. (2006) reported a lower glucose concentration of 3.70 ± 0.30 mmol/L (n = 7), while Kilgallon et al. (2008) and Vassart and Greth (1991) reported higher glucose concentrations of 10.24 ± 2.27 mmol/L (n = 19), and 10.82 ± 0.83 mmol/L (n = 73, immobilised) respectively. Finally, the ISIS database gives a value of 7.99 ± 3.11 mmol/L (n = 163 samples from 98 animals). The concentration of glucose for merged sexes in the present study was 5.76 ± 2.05 mmol/L.

Table 9. Comparison between the concentration of chloride, magnesium and body temperature reported by ISIS, 2002 data base and the present study

		ISIS, 2002				The present study		
Parameter	Units	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

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, other studies (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. 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 & Hoffmann, 1997). This study presents a broad range of valuable parameters that are useful for diagnosis of abnormalities in Arabian oryx and aid the conservation programmes to keep the oryx populations healthy. Using no chemical immobilisation makes the presented reference values closer to normality. 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.

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