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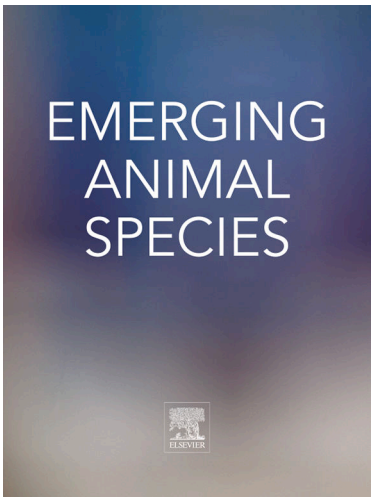

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Hematology and serum chemistry of female South American fur seals (*Arctocephalus australis*) from Lobos Island, Uruguay.

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Abstract

Hematology and serum chemistry are used to diagnose disease in marine mammals in captivity and in the wild. Clinicopathologic variables are the first step in determining treatment and assessing the suitability for rehabilitation and release. The South American fur seal (SAFS; *Arctocephalus australis*) occurs along the Pacific coast from southern Peru to Cape Horn and northward to southern Brazil on the Atlantic coast. The goal of this study was to evaluate hematologic, clinical chemistry, and physical parameters of eight female SAFS from coastal Uruguay. Segmented neutrophils were the major leukocytes followed by lymphocytes. The mean aspartate aminotransferase (AST) was similar to that reported previously for females and pups, except two females that showed higher levels. Alanine aminotransferase (ALT) in SAFS from our study was markedly higher than values reported for Chilean SAFS. AST and ALT values found could be related to liver damage. ALP levels in the females were within the range reported previously. Creatinine was higher compared with females and pups from Chile. The differences found in hematology and

serum chemistry between Chilean and Uruguayan populations may also be related to differences in physiological and immune status. Basophils, myelocytes, metamyelocytes, lymphocyte plasma cells, and immature or pathologic forms were not observed. No unusual values were observed for red blood cells (RBC), white blood cells (WBC), and platelets. Glucose, uricemia, creatine kinase (CK), lactate dehydrogenase (LDH), Na, Cl, Ca, P and K levels are the first report for the species. These are the first results on the hemotology and blood chemistry of otherwise wild SAFS from Isla de Lobos, which can be used to assess the health of wild populations and during rehabilitation.

Abbreviations

RBC	Red blood cell
HGB	Hemoglobin
PCV	Packed cell volume
MCV	Mean corpuscular volume
MCHC	Mean cell hemoglobin concentration
WBC	White blood cell
PC	Platelet count
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
CK	Creatine kinase

SAFS South American fur seal

LDH Lactate dehydrogenase

Key words: blood chemistry, fur seal, hematology, South America.

Introduction

Hematology and serum biochemistry can be important indicators of health and disease in wild animal populations although obtaining these data can be challenging (Schwacke et al., 2009). When a wild animal is considered for rehabilitation center because of disease or poor health, an assessment based on clinicopathologic variables, is the first step in determining treatment and assessing the suitability for rehabilitation and release (Moore et al., 2007).

The South American fur seal (SAFS, *Arctocephalus australis*) is an endemic species, which occurs along the Pacific coast of South America from Mazorca Island in southern Peru to Cape Horn and the South Atlantic coast between Brazil and Argentina, even on Malvinas Islands (Muelbert and Oliveira, 2006; Túnez et al., 2008; Crespo et al., 2015). Three geographic morphotypes of SAFS have been identified based on skull morphology: Peru-North Chile, South Chile, and the Atlantic coast (Oliveira et al., 2008a; Gutiérrez et al., 2021). However, an analysis based on mitochondrial DNA indicates slightly different ranges for the three morphtypes: Peru, South Chile-South Argentine, and Uruguay (Túnez et al., 2006, 2013).

The biology of SAFS has been well described, including 1) ecology and habitat (Thompson et al., 2003; Katz et al., 2013), 2) trophic ecology (Oliveira et al*.,* 2008b; Vallejos, 2010; Franco-Trecu et al., 2012, 2013, 2014), 3) genetics (Oliveira et al*.,* 2008a; Crespo et al., 2015), 4) reproductive behavior and physiology (Franco-Trecu, 2010; Franco-Trecu et al., 2010; Katz, 2009; Katz et al., 2011), 5) pathology and parasitology (Katz et al., 2012) and 6) pollutant exposure (Gerpe et al., 2009; Denuncio et al., 2017). In contrast, there is less information on the hemotology and blood chemistry (Seguel et al., 2016), reproductive hormones (e.g., progesterone; Katz et al., 2013), heavy metal exposure (e.g., cadmium and metallothionein; Polizzi et al., 2017), and serology (e.g., Influenza A and B viruses; Blanc et al., 2009). Of these studies, the first was based on SAFS from Guafo Island (Chile), and

the later three on SAFS from the Isla de Lobos (Uruguay). Here we report on the hemotology and blood chemistry of otherwise wild SAFS from Isla de Lobos, which can be used to assess the health of wild populations and during rehabilitation.

Materials and methods

We obtained blood samples from eight adult female SAFS (body mass: 43 ± 4.2 kg; standard length: 129 ± 3.8 cm; Table 1) on Isla de Lobos (35°01′ 50″ S, 54°53′ 00″ W), Uruguay, in June 2011. Animals were captured by the DINARA (National Direction of Aquatic Research, Uruguay) staff and anesthetized with isoflurane for blood sampling a veterinarian. All females were classified as ASA 1 (normal healthy, American Society Anesthesiologists). Blood samples (5–10 mL) were collected by from an interdigital vein in the flipper using an intravenous catheter (BD Angiocath 20G × 1.16″). A portion of sampled was stored in EDTA-treated tubes of 1 ml (K3 Quisel) and kept on ice until analysis (Fares Taie Laboratory) for clinical hematology. Other portion of the blood samples was placed in serum-tubes with clot activator (Vacuette, 9 ml Greiner Bio-One), centrifuged a 1000xg and the serum fraction was separated and stored in refrigerated boxes until arrival at the laboratory which were placed at -20 °C until analysis (Fares Taie). Blood analysis was carried out in Fares Taie private laboratory in Mar del Plata city, Argentina. The time elapsed between the sampling and its arrival at the laboratory was from 5 to 15 days. It was not possible to obtain the same number (n) of samples for all blood variables because of small blood volumes.

Blood Analysis

Complete blood cell count (CBC) was obtained from blood collected in EDTA-treated

tubes using an automated hematology analyzer (Mindray BC5380) including: white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), hematocrit (PCV), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) and platelet count (PC). WBC differential count and cell morphology were determined manually by a biochemist specialized in hematology using a microscope Carl Zeizz Primo Star objectives 40x and 100x from a blood smear stained with may-grünwald and giemsa.

The serum fraction was used to determined calcium (Ca), phosphorous (P), potassium (K), sodium (Na), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine kinase (CK), lactate dehydrogenase (LDH), chloride, glucemia and uricemia. The analysis were determined using an autoanalyzer AU480 de Beckman Coulter.

Results

Blood count, leukocyte formula and blood chemistry parameters for free-ranging females of *Arctocephalus australis* are presented in table 2 and 3. Basophils, myelocytes, metamyelocytes, lymphocyte plasma cells, and immature or pathologic forms were not observed in any samples of SAFS (Table 2). No unusual values were observed for RBC, WBC, and platelets, which were in the range of values reported for lactating female and pup SAFS from Guafo Island, Chile (Seguel et al, 2016).

Discussion

In all females, segmented neutrophils were the major leukocytes followed by lymphocytes. This is in agreement with the results found in SAFS of Guafo Island and for other pinnipeds (Roletto 1993; Gerlinsky et al., 2018) and porpoises (Nabi et al., 2019).

The mean AST was similar to that reported for Chilean lactating females and pups (Table 3; Seguel et al., 2016). However, two females (7 and 8) showed levels higher than those reported for the range found in SAFS from Chile. On the other hand, the mean for ALTs in SAFS from our study was markedly higher than values reported for Chilean lactating females and pups. Biomarkers of liver damage based on serological enzymes are grouped into those that indicate hepatocellular leakage due to tissue damage (ALT, SDH, AST, and LDH), and those that reflect increased enzyme production caused by impaired bile flow or drug induction (ALP and GGT) (Fauquier et al., 2008). As a result, the elevated AST and ALT values found in this study could be related to liver damage because of toxin, illness, toxic compounds, among others.

ALP levels in the females were within the range reported for lactating females in Chile (Seguel et al., 2016). Chilean pups presented considerably higher values than lactating females, which may result from physiological changes during rapid maturation and body growth, as in other mammals (Thrall et al., 2012).

In contrast, the mean creatinine was higher in our females compared with females and pups from Chile. Gerlinsky et al. (2018) reported that their levels increased with age in Steller sea lion females (*Eumetopias jubatus*). On the other hand, in pinnipeds, it was reported that creatinine levels can also increase with infections and parasites (Roletto, 1993).

The concentrations of Na, Cl, Ca, P and K are the first report for this species. Because there is no other information on these electrolytes for this species. Compared with values from South American sea lion (*Otaria flavescens*), also from Isla de Lobos, there were no differences (Polizzi et al., 2016).

With regards to CK, LDH, glucose, uricemia, they are also the first information for the species. Because females in our study and those at Guafo Island belong to different populations, the differences found in hematology and serum chemistry may also be related to differences in physiological and immune status as has been observed in other pinniped species (Bowen et al., 2006; Kennedy et al., 2021).

Hematology and serum chemistry variables are used to diagnose disease in marine mammals in the wild and undergoing rehabilitation and may indicate recovery or well-being. When wild populations are assessed, it is difficult to study individuals over time, so values are often compared with reference levels from healthy captive animals. For many marine mammal populations, these reference data are often unavailable or difficult to obtain because of logistical challenges in animal capture and blood sampling (Champagne et al., 2015). Hence, our values contribute to the available data for SAFS adult females in the western coast of the South Atlantic .

Conclusion

We present some of the first hematological and blood chemistry values for wild SAFS females from Uruguay. Our results will contribute to the limited data on the blood biomarkers for this species, which will be useful for veterinary clinical analysis and conservation of the species.

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Female	Weight (BM, kg)	Standard length (SL, cm)
1	47	136

2	49	126
3	40	124
4	37	125
5	41	128
6	49	133
7	40	128
8	40	130
Mean	*43*	*129*
s.d.	*4.2*	*3.8*

Table 1: Morphometrics for eight female *Arctocephalus australis.*

	Females Lobos Island Uruguay							Lactating females Guafo Island Chile[1]		Pups Guafo Island Chile[1]	
Blood count	1	3	4	5	6	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
RBC (x10^6/µl)	4.3	4.7	4.3	4.5	4.1	4.4±0.2	4.1-4.7	5.4±0.9	3.7-7.7	4.2±0.7	3.0-5.9
HGB (g.dl^{-1})	13,7	12,2	13,1	13,5	13,7	13.2± 0.6	12.2-13.7	16.5±2.5	10.1-19.8	12.2±1.4	9.9-16.7
PCV (%)	40	38	39	41	39	39.4±1.1	38-41	52.1±4.7	36-51	38.5±4.2	30-48
MCV (um3)	93	81	91	91	95	90.1±5.4	81-95	99.5±18.3	61.9-147.5	95±16.8	58.5-135.3
MCHC (mg Hb/100mL)	340	320	340	330	350	336±11	320-350	31800±4200**	22400-36900**	31800±3500**	23700-39400**
WBC (x10^3/µl)	6.1	13.2	14.1	6.6	6.6	8.6±3.9	6.1-13.2	16.6±3.9	10.8-24.8	16.7±3.6	10.6-26.6
PC (x10^4/mm^{-3})	26.8	28.4	24.8	47.6	13.5	26.1±12.3	13.5-47.6	--	--	--	--

Leukocyte formula %									
Eosinophils	1	0	1	1	2	1.18±0.5	0.1-2.0	18.08*	9.25*
Lymphocytes	26	25	19	24	25	23.6±2.7	19.0-26.0	25.31*	27.74*
Monocyte	5	5	4	4	3	4.1±0.80	3.0-5.0	3.6*	1.73*
Segmented neutrophils	68	70	76	68	70	70.0±3.2	68.0-76.0	48.82*	58.95*
Band neutrophils	0	0	0	3	0		0-3.0	4.16*	2.3*

Table 2: Blood count and leukocyte formula for free-ranging females of *Arctocephalus australis* that inhabit Lobos Island (Uruguay), and lactating females and pups from Guafo Island (Chile). RBC: red blood cell count; HGB: hemoglobin; PCV: packed cell volume; MCV: mean cell volume; MCHC: mean cell hemoglobin concentration; WBC: white blood cell count; PC: plateled count; [1]: Seguel et al., 2016; *the data were transformed to be able to compare.

	Females Lobos Island Uruguay										Lactating females Guafo Island Chile[1]		Pups Guafo Island Chile[1]	
Parameter	1	2	3	4	5	6	7	8	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
AST ($U \cdot L^{-1}$)	77	10	25	16	40	24	93	188	38.8±59.9	10-188	31.7±17.7	10-71	43.9±19.1	17-88
ALT ($U \cdot L^{-1}$)	42	17	18	35	69	26	81	36	35.2±23.2	18-81	8.2±11.5	2-38	9.1±8.2	2-38
ALP ($U \cdot L^{-1}$)	72	81	62	98	80	nd	57	104	77.5±17.4	57-104	90.3±66.2	49-307	479.5±177.8	84-854
Urea ($mmol \cdot L^{-1}$)	16.6	13.9	18.5	17.5	15.3	nd	11.1	16.5	15.5±2.5	11.1-18.5	11.1±2.6	73-15.9	10.5±3.0	5.8-16.3
Creatinine ($\mu mol \cdot L^{-1}$)	114.9	141.4	132.6	150.3	159.1	141.4	123.7	132.6	141.4±8.8	123.7-159.1	94.8±15.1	70-114	68.3±21.3	17.4-110
CK ($U \cdot L^{-1}$)	2447	nd	403	389	689	nd	2194	104	626.2±1013.9	104-2447	--	--	--	--
LDH ($U \cdot L^{-1}$)	311	107	255	294	311	241	846	508	307.7±225.7	107-846	--	--	--	--

Glucose ($mg \cdot dl^{-1}$)	62	122	71	123	145	167	nd	45	95.4±45.9	45-167	--	--	--	--
Uricemia ($mg \cdot dl^{-1}$)	1	0.8	0.8	0.4	0.8	0.7	0.9	1.7	0.8± 0.4	0.4-1.7	--	--	--	--
Ca ($mg \cdot .dl^{-1}$)	8.7	8.6	8.2	8.8	9.3	nd	8.9	8.6	8.7±0.3	8.2-9.3	--	--	--	--
P ($mg \cdot dl^{-1}$)	7.4	9.7	8.4	11.6	7.7	nd	9.1	7.8	8.7±1.5	7.4-11.6	--	--	--	--
Na ($mEq \cdot L^{-1}$)	148	146	148	150	146	165	144	149	149.4±4.3	144-165	--	--	--	--
K ($mEq \cdot L^{-1}$)	4.4	4.5	4.1	5.1	3.8	4.4	4.1	3.8	6.5±0.4	3.8-5.1	--	--	--	--
Cl ($mEq \cdot L^{-1}$)	109	107	111	109	106	120	114	108	110.4±4.6	106-120	--	--	--	--

Table 3: Blood chemistry parameters for free-ranging females of *Arctocephalus australis* that inhabit in Lobos Island (Uruguay), and lactating females and pups from Guafo Island (Chile). AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; CK: creatine kinase; LDH: lactate dehydrogenase; nd: not determined; SD: standard deviation. [1]: Seguel et al., 2016