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Hematology, Serum Chemistry, and Early Hematologic Changes in Free-Ranging South American Fur Seals (*Arctocephalus australis*) at Guafo Island, Chilean Patagonia

Mauricio Seguel,[1] Francisco Muñoz,[2] Alessandra Keenan,[1] Diego J. Perez-Venegas,[3] Eugene DeRango,[4,7] Hector Paves,[5] Nicole Gottdenker,[1] and Ananda Müller[6,8] [1]Department of Pathology, College of Veterinary Medicine, University of Georgia, 501 D. W. Brooks, Athens, Georgia 30602, USA; [2]Animal Pathology Department, Facultad de Ciencias Veterinarias, Universidad Austral de Chile, Campus Isla Teja, sin numero, Valdivia, Chile; [3]PhD Program in Conservation Medicine, Facultad de Ecología y Recursos Naturales, Universidad Andres Bello, 252 Republica St., Santiago, Chile; [4]The Marine Mammal Center, Marin Headlands, 2000 Bunker Road, Sausalito, California 94965, USA; [5]Departamento de Ciencias Basicas, Facultad de Ciencias, Universidad Santo Tomas, 753 Los Carrera Av., Osorno, Chile; [6]Clinical Science Department, Facultad de Ciencias Veterinarias, Universidad Austral de Chile, Campus Isla Teja, sin numero, Valdivia, Chile; [7]Current address: Department of Biology, Sonoma State University, 1801 E Cotati Road, Rohnert Park, California 94928, USA; [8]Corresponding author (email: ananda.muller@uach.cl)

ABSTRACT: The establishment of clinical pathology baseline data is critical to evaluate temporal and spatial changes in marine mammal groups. Despite increased availability of studies on hematology and biochemistry of marine mammals, reference ranges are lacking for many populations, especially among fur seal species. During the austral summers of 2014 and 2015, we evaluated basic hematologic and biochemical parameters in clinically healthy, physically restrained South American fur seal (*Arctocephalus australis*) lactating females and 2-mo-old pups. We also assessed the temporal variation of hematology parameters on the pups during their first 2 mo of life. Reference ranges of lactating females were similar to those previously reported in other fur seal species. In the case of pups, reference ranges are similar to values previously reported in sea lion species. As expected, most biochemical and hematologic values differ significantly between adult females and pups. As in other otariids, South American fur seals pups are born with higher values of total red blood cells, hemoglobin, and packed cell volume, and lower numbers of total leukocytes, neutrophils, lymphocytes, and eosinophils. To the best of our knowledge, data on hematology reference values for South American fur seals has not been previously reported and is useful for continued health monitoring of this species, as well as for comparisons with other otariid groups.

Key words: Clinical pathology, Guafo Island, hematology, marine mammal, serum chemistry, South American fur seal.

Marine mammals are considered sentinels of ocean health; therefore, the current increase in factors that can negatively impact marine ecosystems (e.g., climate change) also creates concern for the health of marine mammal populations (Moore and Huntington 2008; Bossart 2011). Basic hematologic parameters are simple and economic tools for evaluating the health and condition of individuals and animal groups, and the development of reference intervals or ranges for otariid colonies allows for the comparison of temporal or interpopulation differences (Lander et al. 2013). However, baseline information on clinical pathology parameters are lacking for many marine mammal populations, especially among fur seal species, likely due to logistic difficulties of working in the rookeries of these pelagic otariids (Norberg et al. 2011; Tryland et al. 2012). We describe clinical pathology reference ranges for healthy free-ranging South American fur seals (SAFS; *Arctocephalus australis*) and identify temporal hematologic changes during the early development of SAFS pups.

Thirty-two lactating SAFS and 103 pups were captured on Guafo Island (43°35′34.9″S, 74°42′48.53″W), southern Chile, during the austral summers of 2014 and 2015. Adult fur seals were captured with a net and physically restrained, and blood (5–10 mL) was drawn from the caudal gluteal vein into ethylenediaminetetraacetic acid (EDTA) and Vacutainer® no additive tubes (Franklin Lakes, New Jersey, USA; total procedure time of less than 5 min). Immediately after this procedure, animals were anesthetized by an isoflurane mask to perform a complete clinical examination, standard

morphometrics, and collect fecal samples. The pups were captured by hand and physically restrained. Blood (up to 4.5 mL) was drawn from the gluteal caudal vein and immediately transferred to EDTA and serum tubes. All blood samples were placed in a cooler with ice packs, transported to the field lab, and processed within 4 h postcollection.

Pups' age was estimated based on the peak parturition date for the Guafo Island rookery (15 December of each year; Paves and Schlatter 2008). The animals selected for this study were considered clinically healthy (assessed by detailed physical examination) and were negative for hookworm infection on flotation fecal analysis.

White blood cell (WBC) and red blood cell (RBC) counts were performed manually in the field laboratory by using a hemocytometer and following hematology techniques and calculations, as described by Thrall et al. (2012). Blood smears were made in duplicate, air dried, fixed, and stained by using Diff-Quick fixative stain method (Dade Behring Inc., Newark, Delaware, USA). Blood smears were examined, and differential WBCs were performed at the mainland laboratory 1–3 mo after collection. Hemoglobin (HGB) concentration was determined from a drop of blood placed on a disposable cartridge reader of a portable point of care human HGB photometer (STAT-Site®, Stanbio Company, Boerne, Texas, USA) previously validated with dog blood in the Veterinary Clinical Pathology Laboratory of Universidad Austral de Chile (UACh), Valdivia, Chile, by using an automated hematology analyzer (KX-21N Sysmex, Kobe, Japan). Mean cell volume and mean cell HGB concentration were calculated by using routine veterinary diagnostic hematology formulas (Thrall et al. 2012).

For serum chemistry, whole blood was centrifuged within 4 h postcollection at 1,200 × G for 10 min. Serum without evidence of lipemia or hemolysis was carefully collected and frozen at –20 C until transport to the mainland laboratory, where serum was then placed in a –80 C freezer until processed within 2–12 mo postcollection. Selected serum chemistry parameters (Table 1) were analyzed using a Metrolab® GL 20300 Chemistry Analyzer (Metrolab, Buenos Aires, Argentina) at the Veterinary Clinical Pathology Laboratory of UACh.

To examine temporal variation on hematologic values, we captured and marked with hair dye eight, 1- to 2-d-old pups and recaptured them every 15 d until they were 2 mo old. In this group, age was determined by observation of parturition or of large pieces of fresh placenta attached to the umbilicus. On each capture, we collected blood and performed hematologic analyses, as previously described.

Normality of data was assessed by the Anderson-Darling test. The packed cell volume (PCV), alanine aminotransferase, aspartate aminotransferase, triglyceride, and eosinophil values were not normally distributed and were logarithmically transformed to fit a normal distribution and assessed by parametric methods (linear regression) or were assessed by nonparametric methods (Mann-Whitney) when two groups were compared. Outliers were identified with Grubb's test and excluded from further analyses. All outliers were considered typing errors (e.g., 3% PCV). The mean, SE, and range for each clinical pathology value were calculated for lactating females and pups. The temporal changes of hematologic parameters for eight pups followed for 2 mo were assessed by using a nonparametric Friedman test. Linear regression models were fitted by using each hematologic and biochemical parameter value as a response variable and sex, year, and body condition (calculated as body length/weight quotient) or length and weight as explanatory variables.

Using regression models, there was not a significant association between sex, year, or total length, and any of the biochemical and hematologic values of pups. Body condition was positively associated with lymphocyte counts (P=0.001).

The reference ranges for serum chemistry values of SAFS adult females and pups and published ranges for adult female Antarctic fur seals (*Arctocephalus gazella*) are shown in Table 1. As expected, most biochemical values, with the exception of albumin and

TABLE 1. Values for selected serum chemistry analytes for South American fur seal (*Arctocephalus australis*) lactating females and pups at Guafo Island, Chile, and published ranges for adult female Antarctic fur seals (*Arctocephalus gazella*).

		Lactating South American fur seals				South American fur seal pups				Adult female Antarctic fur seals[c]	
Analyte[a]	P[b]	n	Mean±SD	SE	Range	n	Mean±SD	SE	Range	n	Range
AST (U/L)	0.01*	30	31.7±17.7	3.5	10–71	42	43.9±19.1	2.95	17–88	46	13–290
ALT (U/L)	0.01*	32	8.2±11.5	2.1	2–38	45	9.1±8.22	1.20	2–38	46	0–14
ALP (U/L)	<0.01	30	90.3±66.2	12.5	49–307	41	479.5±177.8	27.8	84–854	46	64–806
GGT (U/L)	0.02	32	75.9±25.9	4.6	17–122	41	92.10±33.6	5.25	19–172	46	NR[d]
Protein, total (g/L)	0.03	30	70.2±8.0	1.5	51–83	40	64.40±8.4	1.32	44–79	46	50–79
Albumin (g/L)	0.34	30	42.1±8.6	1.6	24–54	41	43.85±5.1	0.80	44–53	46	24–39
Globulin (g/L)	<0.01	29	28.2±7.3	1.4	17–46	40	19.48±8.1	1.21	7–37	46	NR[d]
Urea (mmol/L)	0.03	32	11.1±2.6	0.4	7.3–15.9	43	10.55±3.0	0.45	5.8–16.3	46	5.3–21.3
Creatinine (μmol/L)	<0.01	32	94.8±15.1	4.2	70–114	40	68.32±21.3	3.36	17.4–110	46	53–122
Cholesterol (mmol/L)	<0.01	30	5.4±1.1	0.2	3.9–7.7	45	4.63±1.2	0.16	2.3–6.9	46	2.3–9.2
Triglycerides (mmol/L)	0.16*	17	0.9±0.4	0.1	0.2–1.7	42	1.20±0.7	0.14	0.40–3.0	46	0.4–1.0
Phosphate, inorganic (mmol/L)	<0.01	31	2.0±0.4	0.4	1.2–2.6	40	2.67±0.8	1.12	1.8–3.9	46	1.3–4.0

[a] AST = aspartate aminotransferase; ALT = alanine aminotransferase; ALP = alkaline phosphatase; GGT = gamma-glutamyl transferase.

[b] P value for comparison between South American fur seal lactating females and pups. All two-sample Student's t-tests, except those marked with an asterisk, were compared using Mann-Whitney U-tests.

[c] Tryland et al. 2012.

[d] NR = not reported.

triglycerides, were significantly different between pups and adult SAFS.

Reference ranges for hematology parameters of SAFS adult females and pups and published ranges for adult female northern fur seals (*Callorhinus ursinus*) are shown in Table 2. The RBC, HGB, PCV, segmented neutrophils, monocytes, and eosinophil numbers are significantly different between SAFS adult females and pups.

South American fur seal pups are born with higher values of absolute RBC, PCV, HGB, and lower numbers of total WBC, neutrophils, lymphocytes, and eosinophils than adult and 2-mo-old SAFS (Friedman test S=15.09–17.26, df=4, P=0.002–0.005). The newborn pups' erythrocyte values decreased over the first month of life and then stayed low until the end of the study, while leukocytes increased rapidly within the first 2 wk of life (Fig. 1).

The lack of significant effects of year of capture and body measurements on hematologic and serum chemistry values is probably related to the selection of healthy pups, which have little variation in body condition and other morphometrics among seasons (M.S. unpubl. data). Despite the homogeneity in this study population, body condition had a positive effect on lymphocyte count in pups, which confirms what has been described in other otariids: cellular immune response is more developed in well-nourished pups (Vera-Massieu et al. 2015).

Considering differences among species, populations, sampling, and analytic methods, reference ranges of hematologic and serum chemistry values in SAFS adult females are comparable to those reported in lactating northern fur seals (Norberg et al. 2011) and Antarctic fur seal adult females (Tryland et al. 2012). There are fewer studies on fur seal pup hematology and serum chemistry; however, the reference ranges reported here can be compared and are similar to those reported for healthy Steller sea lion pups (*Eumetopias jubatus*; Lander et al. 2013) and Australian sea lions (*Neophoca cinerea*) at postpatent hookworm infection (Marcus et

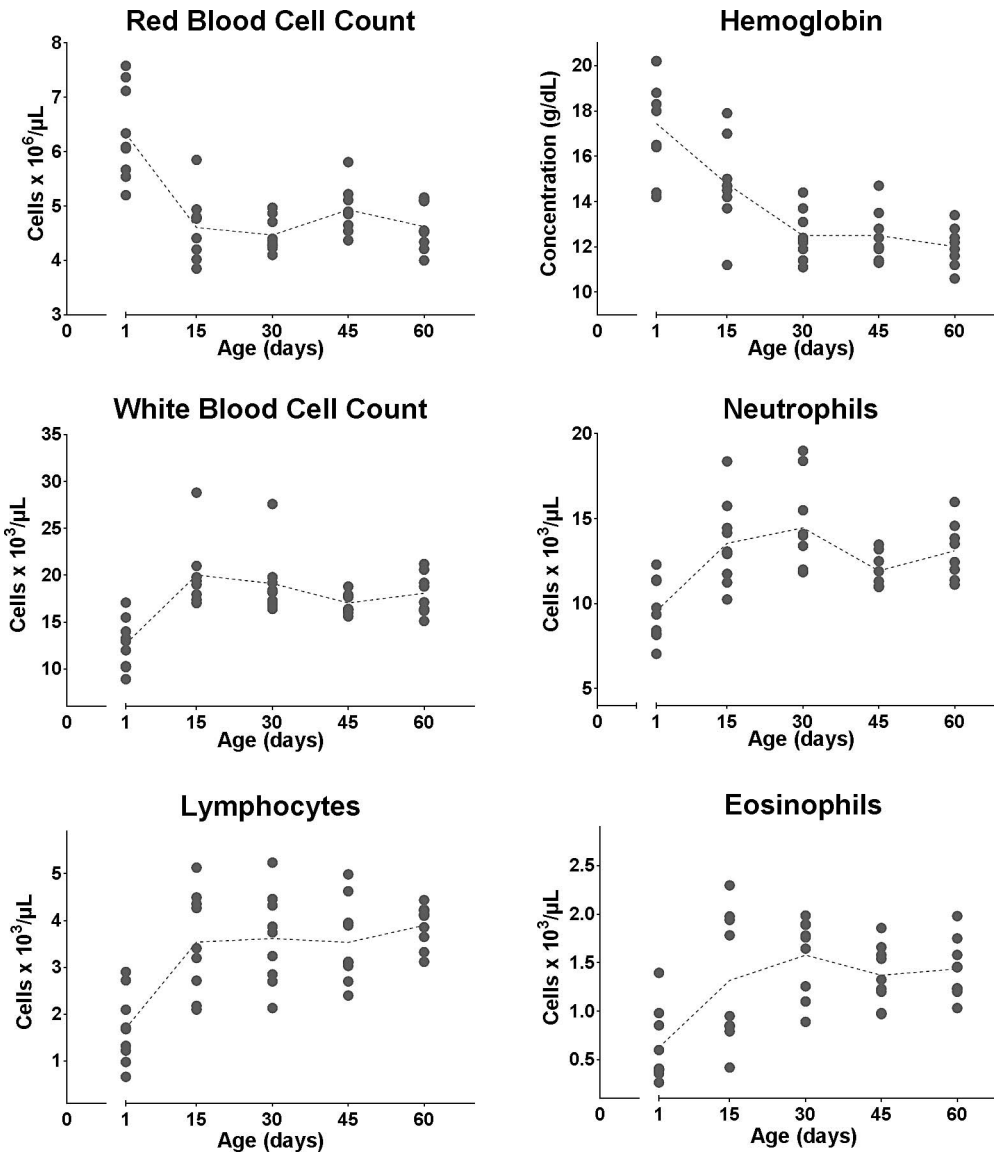

FIGURE 1. Early changes on hematologic parameters in South American fur seal (*Arctocephalus australis*) pups. Fur seal pups experienced a decline in the number of red blood cells, packed cell volume (not shown), and hemoglobin during the first 2 wk of life. In contrast, most leukocytes increased significantly over the first 2 wk of age. All models were statistically significant (Friedman test S=15.09–17.26, df=4, P=0.002–0.005).

al. 2015). Hookworm disease is endemic in this SAFS population (Seguel et al. 2013), and although in this study, the fecal hookworm-negative status of pups does not completely rule out hookworm infection, pups were in good health, and their hematologic parameters differed from those observed in hookworm-infected SAFS and Australian sea lions (Marcus et al. 2015; M.S. unpubl. data).

We documented a transient decline in hematocrit and other hematology parameters consistent with that observed in otariids with life histories comparable to SAFS (Beckmen et al. 2003; Richmond et al. 2005; Trillmich et al. 2008). However, we did not sample animals

TABLE 2. Mean, SD, SE, and range (minimum-maximum) of hematology values for free-ranging South American fur seals (*Arctocephalus australis*) at Guafo Island, Chile, and published values for free-ranging lactating Northern fur seals (*Callorhinus ursinus*).

Parameter[a]	P[b]	Lactating South American fur seals				South American fur seal pups				Lactating Northern fur seals[c]	
		n	Mean±SD	SE	Range	n	Mean±SD	SE	Range	n	Range
RBC ($\times 10^6$/μL)	<0.01	30	5.4±0.95	0.17	3.7–7.7	103	4.2±0.7	0.07	3–5.9	24	3.5–5.8
HGB (g/dL)	<0.01	29	16.5±2.5	0.47	10.1–19.8	96	12.2±1.4	0.15	9.9–16.7	24	10–17.3
PCV (%)	<0.01*	29	52.1±4.7	0.88	36–51	100	38.5±4.2	0.42	30–48	24	35–55
MCV (fL)	0.21	28	99.5±18.3	3.46	61.9–147.5	99	95±16.8	1.7	58.5–135.3	24	72–139
MCHC (g/dL)	1.00	27	31.8±4.2	8.09	22.4–36.9	93	31.8±3.5	0.37	23.7–39.4	24	28–39
WBC ($\times 10^3$/μL)	0.76	28	16.6±3.9	0.74	10.8–24.8	99	16.7±3.6	0.36	10.6–26.6	24	4.2–14.8
Segmented neutrophils ($\times 10^3$/μL)	<0.01	28	8.1±2.7	0.51	2.8–13.5	52	10.2±2.3	0.32	5.1–16	24	3.35–11.1
Band neutrophils ($\times 10^3$/μL)	0.05	28	0.69±0.7	0.13	0.1–2.7	52	0.4±0.4	0.05	0.1–2.0	24	NR[d]
Lymphocytes ($\times 10^3$/μL)	0.21	28	4.2±1.5	0.28	2.3–7.4	52	4.8±2.5	0.34	1.1–12.5	24	0.53–2.15
Monocytes ($\times 10^3$/μL)	<0.01	28	0.6±0.5	0.11	0.2–2.0	52	0.3±0.2	0.02	0.1–0.8	24	0–0.77
Eosinophils ($\times 10^3$/μL)	<0.01*	28	3±1.5	0.28	1–8.2	48	1.6±1.1	0.13	0.1–4.3	24	0.11–0.31

[a] 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.

[b] P value between South American fur seal lactating females and pups. All tests are two-sample Student's t-tests, except those marked with an asterisk, were compared by Mann-Whitney U-tests.

[c] Norberg et al. 2011.

[d] NR = not reported.

at the time point (1 yr old) when hematologic parameters may be expected to approach adult values (Trillmich et al. 2008). This pattern of hematocrit shifts, along with elevated alkaline phosphatase and gamma-glutamyl transferase are commonly observed in young domestic mammals and probably related to physiologic changes during the period of fast body growth (Thrall et al. 2012).

South American fur seal pups are born with low numbers of leukocytes, neutrophils, eosinophils, and lymphocytes, and similar changes have been described in other otariids. Steller sea lions undergo a decrease in the number of total leukocytes, neutrophils, and T-lymphocyte proliferation over the first 40 d after birth; however, the number of lymphocytes, eosinophils, and monocytes does not change (Keogh et al. 2010). In Galapagos sea lions (*Zalophus wollebaeki*), total leukocytes, eosinophils, and neutrophils decrease the first 3 mo of life and then increase until they are 6 mo old (Brock et al. 2013). The functional consequences of this neonatal leukopenia in pinnipeds are unknown, but experimental studies in California sea lions (*Zalophus californianus*) have shown that otariid neonates have a milder cellular inflammatory response than older age classes (Vera-Massieu et al. 2015).

To the best of our knowledge, these are the first hematology and serum chemistry reference values reported on SAFS adult females and pups. The data presented here will serve as a point of comparison for health assessments during upcoming years in this population and allow for comparison between other fur seal species or reproductive groups.

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