PW819 - Southern elephant seal (Mirounga leonina): reproductive phisiology contributions

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The aim of this work was to study postpartum vaginal cytology, E2 and P4 concentrations in postpartum southern elephant seals (Mirounga leonina) in 25 de Mayo/King George Island, South Shetland Island, Antarctica. Vaginal cytology samples and blood sample were taken in nine females from the reproductive colony between 17 and 23 days postpartum (dpp). Three additional females were selected and samples were collected at three different dpp (7, 14 and 21). Vaginal cytology smears were scored based upon the epithelial cell and blood cell type (epithelial cell score [ECS], 0=predominant parabasal cells, 1=predominant intermediate cells, 2=predominant superficial cells; polymorph nuclear neutrophil score [PMNNS], (1=high, 2=moderate) and presence of spermatozoa to evidence mating. All blood samples were centrifuged and serum was stored at -20°C until P4 and E2 concentrations were measured by chemiluminescence immunoassay (Elecsys®, Progesterone II and Estradiol; Roche, Mannheim, Germany). At 7 dpp, all vaginal smears clearly showed postpartum cytology (high presence of leukocytes, erythrocytes, intermediate cells and a few parabasal and superficial cells, (ECS=1; PMNNS=1). Moreover, at 14 and 21 dpp, PMNNS decreased (2, $P<0.05$) but ECS did not change ($P>0.05$). It is noteworthy to point out that one animal sample obtained at 21 dpp showed predominance of superficial cells (ECS=2) and presence of sperm suggesting estrous and mating. The P4 concentrations were basal during the study period (3.4±0.5 ng/mL; $P<0.92$), suggesting that ovulation most likely took place later. Conversely, E2 concentrations increased during the study period (d7=5.0, d14=11.0, d21=32.0±2.4; $P<0.001$) in samples obtained between 17 and 21 dpp; suggesting follicular development. This increase in E2 concentrations was not followed by a change in the vaginal cytology score (an increase of superficial cells). In conclusion, by 21 dpp, most cows had follicular development and high E2 concentrations but no indication of ovulation because P4 concentrations remained at basal levels. This finding would indicate that first ovulation most likely takes place after 21 dpp or even after they leave land.

PW820 - Improved sperm freezing in the endangered African wild dog (Lycaon pictus) using a two-step dilution TRIS-egg yolk extender containing Equex STM.

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Development of assisted breeding techniques can aid conservation and management of the endangered African wild dog (Lycaon pictus). Previous attempts to freeze sperm from this species has proven unsuccessful with sperm motility dropping to nearly 0% within 2 h of thawing. The aim of this study was to improve the freezing success of African wild dog sperm by testing two routinely used canine cryopreservation protocols.

Sperm was frozen from n=3 captive African wild dog males housed at Albuquerque BioPark (Albuquerque, NM, USA) and Binder Park Zoo (Battle Creek, MI, USA) during the breeding season (Aug-Sep 2014). Freshly collected semen samples were evaluated for volume, colour, pH, motility, viability, morphology, sperm number, acrosome status and DNA integrity. Each sample was split and frozen using two different protocols. Protocol 1: semen was diluted with a Tris-egg yolk extender containing 8% glycerol and 20% egg yolk, and slowly

cooled from 37 C to 4 C over 2.5 h. The sample was then loaded into 0.25 mL straws, suspended 4 cm over liquid nitrogen vapour for 10 min, then plunged in liquid nitrogen. Protocol 2: semen was first diluted with a Tris-egg yolk extender containing only 3% glycerol and 20% egg yolk, followed by a second extender (same composition) now containing 7% glycerol and 1% Equex STM, added after the 2.5 h refrigeration period. The freezing procedure was the same as Protocol 1. Straws from both protocols were thawed in a 37 C water bath, but Protocol 2 straws were further diluted with a thawing solution that consisted of the initial extender solution without glycerol and egg yolk. Sperm were incubated at 37 C and motility evaluated at 5 min and every 2 h for 8 h after thawing. Viability, morphology and acrosome integrity was evaluated over 6 h and DNA integrity was evaluated immediately post-thaw.

Sperm motility declined significantly for both protocols immediately after thawing (fresh 78.9 ± 2.6%; Protocol 1 24.4 ± 5.0%; Protocol 2 36.7 ± 4.2%; $P < 0.05$). Motility was significantly higher for Protocol 2 from 2 h after thawing (Protocol 1 1.0 ± 0.8%; Protocol 2 30.8 ± 1.9%; $P < 0.05$) and sperm remained motile for up to 8 h. Sperm frozen with Protocol 2 also had significantly higher viability (Protocol 1 37.0 ± 5.7%; Protocol 2 65.3 ± 9.9%; $P < 0.05$) and acrosome integrity (Protocol 1 22.8 ± 8.2%; Protocol 2 69.3 ± 8.8%; $P < 0.05$) immediately after thawing. There was no difference in the proportion of normal morphology or DNA fragmentation between both protocols.

Our results demonstrate that using a two-step dilution with TRIS-egg yolk extender containing Equex STM yields greatly improved post-thaw quality and longevity in African wild dog sperm; making it suitable for use in artificial insemination.

PW821 - Serum progesterone levels in pregnant wild Amazon river dolphin (Inia geoffrensis)

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The Amazon river dolphin (Inia geoffrensis) is a threated cetacean species endemic of the South America. Information about the reproductive endocrinology of this species is scarce; therefore, the aim of this study was to evaluate the serum progesterone levels in pregnant wild Amazon river dolphins and to compare the hormonal levels between the two halves of the pregnancy. Single serum samples were collected from 60 females captured in annual capture-recapture campaigns of a research program. The pregnancy was diagnosed by ultrasound. The gestational age was determined by fetal size and some of them were confirmed by observation of the newborn calf. The serum progesterone was measured by enzyme immunoassay. The progesterone levels of the pregnancy halves were compared by Mann Whitney test. The average of serum progesterone levels observed was 11.74±10.08 g/ml (range 0.47 – 68.60 ng/ml). No differences were observed between the first and second halves of pregnancy (11.81±11.58 vs 11.12±7.65; P=0.7101). This is the first report on serum progesterone in pregnant Amazon river dolphin. These results suggest the progesterone levels remain elevated throughout gestation in I. geoffrensis, without wide variations. These findings corroborate with hormonal pattern already reported for pregnant bottlenose dolphins. However, the origin of the progesterone (corpus luteum or placenta), and the interaction with other hormones should be investigated to better understand the pregnancy physiology in this species.

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