Ultrasonographic Assessment of the Corpus Luteum at the end of Embryonic Diapause in Free-ranging Northern Fur Seals (*Callorhinus ursinus*)

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Abstract

A field study was done to determine if the corpus luteum (CL) could be detected by ultrasonography and to examine the concept of structural and functional resurgence of the CL at the end of embryonic diapause in free-ranging Northern fur seals. Earlier studies done post-mortem suggest an increase in CL diameter and progesterone in association with gross observation of an embryonic vesicle (EV) at the end of diapause. Data were collected from 96 females in 2007 (11-16 Nov) and 65 in 2008 (18-24 Nov) on St. Paul Island, Alaska. Transrectal ultrasonography was done using physical restraint with or without sedation to detect and measure the EV (diameter, ≥2 mm) and CL. A blood sample was collected from an interdigital vein of a hind flipper for radioimmunoassay of serum progesterone. In 2007 and 2008, one or two CL were detected in 91% and 95% of the seals and an EV in 65% and 85%, respectively. For animals with a single CL (75 in 2007 and 62 in 2008), there was no effect of year on diameter and no CL-by-year interaction, but mean (±SEM) diameter was larger (P=0.006) in seals with a detectable EV (15.8±0.3 mm) than those with no EV (13.8±0.6 mm). Similarly, mean progesterone concentrations tended to be higher (P=0.13) in seals with a detectable EV (11.1±0.6 ng/ml) than those with no EV (8.2±0.7 ng/ml). There was no EV-by-year interaction on progesterone but higher concentrations were measured in 2008 than 2007 (P=0.0001), which may have been a result of a later (1 week) sample collection period in 2008. In conclusion, the use of ultrasonography provided a noninvasive approach to detect the CL and study the concept of structural and functional resurgence of the luteal gland in association with early embryonic development following diapause in Northern fur seals.

Overview

- Northern fur seals (*Callorhinus ursinus*) of Pribilof Islands declared depleted under Marine Mammal Protection Act (NMFS, 2007)
- Reason for population decline not known but may involve factors that have direct or indirect effect on reproduction
- Production of progesterone by CL is essential for development & maintenance of early pregnancy in mammals
- Early necropsy studies of Northern fur seals suggest increase in CL diameter & systemic progesterone concentrations by end of embryonic diapause (Craig, 1964; Daniel, 1971 & 1975), but recent blood sampling study during peri-implantation did not support concept of CL resurgence (Browne et al., 2006)
- Lethal methods for collecting data from wild fur seals present ethical challenges & strictly regulated
- Ultrasonographic imaging is a noninvasive method to effectively evaluate ovaries & diagnose pregnancy in free-ranging Northern fur seals (Adams et. al., 2007)

Objective

Relate EV detection and November calendar dates to changes in CL size and progesterone concentrations during early implantation at end of diapause in free-ranging Northern fur seals.

Hypotheses

1) Size of CL and concentrations of progesterone are greater in fur seals with a detectable EV than those with no detectable EV.

2) Size of CL and concentrations of progesterone are greater in fur seals later in November than earlier.

Materials & Methods

Location:
- St. Paul Island, Alaska
  - Polovina Cliffs rookery
  - Tolstoi rookery

Calendar dates & seals:
- November 11 - 16, 2007; n = 96 mature females
- November 18 - 24, 2008; n = 65 mature females

Ultrasound machine for field operation:
- Portable Aloka SSD 500 with 7.5 MHz human prostate probe
- Protected by aluminum or canvas carrying case
- Powered by 12 v/15 amp battery through an inverter
- Ambient light blocked with cardboard shroud

Seal restraint for ultrasound imaging & blood sampling:
- Flat board & headstock, with or without gas anesthesia
- Vest to secure fore flippers
- Technician to secure hind flippers & pelvis for transrectal scanning

Ultrasound imaging to detect EV & measure CL:
- EV detected & delineated as mostly spherical anechoic structure ≥2 mm within uterine lumen
- Ovaries, often adjacent to kidneys, examined for presence of CL
- CL detected & delineated as mostly spherical echoic structure distinct from ovarian stroma
- CL cross-sectional diameter estimated & recorded

Blood sampling:
- Approximately 10 ml from interdigital vein of hind flipper using tourniquet at base of flipper
- Serum divided into aliquots & frozen

Progesterone radioimmunoassay (RIA):
- Diagnostic Systems Laboratories (Kit no. DSL-3400)
- Validated for fur seal serum
  - Serial dilutions of ether-extracted seal serum parallel to kit standard curve in human serum
  - Gel fraction of ether-extracted seal serum pool immunoreactively similar to progesterone reference
- Within- & between-assay coefficients of variation 5% & 8%
- Assay sensitivity 0.05 ng/ml

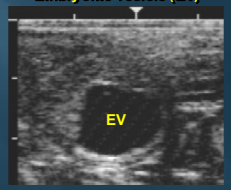
Statistical analysis:
- CL & progesterone data checked for outliers & ranked
- Non-parametric 2 x 2 ANOVA using SAS PROC MIXED
  - Main effects, no detectable EV vs detectable EV & early vs late November calendar dates
  - Interaction, EV-by-date
- Fisher's exact test for proportional data
- Statistical significance, P≤0.05

Ultrasonographic Imaging

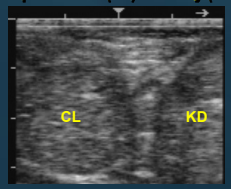
Ultrasound machine & probe

Ultrasound scanning

Embryonic vesicle (EV)



Corpus luteum (CL) & kidney (KD)



Results

Table 1. Proportion of seals with a detectable EV & CL[1].

	EV	CL
Nov 11 – 16	62/96 (65%)[a]	75/96 (78%)[x]
Nov 18 – 24	55/65 (85%)[b]	62/65 (95%)[y]

[1]Seals with no CL detected & more than 1 CL excluded from analyses
Difference between dates for [a,b]EV (P=0.007) & [x,y]CL (P=0.003)

Table 2. Mean (± SEM) changes in CL diameter (mm) in relation to detectable EV & November calendar dates.

	No EV	EV	Combined
Nov 11 – 16	13.9 ± 0.6 (n=20)	15.6 ± 0.5 (n=53)	[x]15.1 ± 0.4 (n=73)
Nov 18 – 24	13.5 ± 1.2 (n=8)	16.1 ± 0.3 (n=53)	[y]15.8 ± 0.3 (n=61)
Combined	[a]13.8 ± 0.6 (n=28)	[b]15.8 ± 0.3 (n=106)	

Main effects of [a,b]EV (P=0.006), [x,y]date (P=0.77) & interaction (P=0.47)

Table 3. Mean (± SEM) changes in progesterone concentrations (ng/ml) in relation to detectable EV & November calendar dates.

	No EV	EV	Combined
Nov 11 – 16	6.6 ± 0.4 (n=21)	7.9 ± 0.4 (n=39)	[x]7.4 ± 0.3 (n=60)
Nov 18 – 24	12.5 ± 1.3 (n=8)	13.9 ± 0.8 (n=45)	[y]13.7 ± 0.7 (n=53)
Combined	[a]8.2 ± 0.7 (n=29)	[b]11.1 ± 0.6 (n=84)	

Main effects of [a,b]EV (P=0.13), [x,y]date (P=0.0001) & interaction (P=0.5)

Discussion

- In seals with EV detected vs no EV detected:
  - larger CL (Table 2)
  - higher progesterone (Table 3)

First hypothesis supported

- In seals later vs earlier in November:
  - more EV & single CL detected (Table 1)
  - higher progesterone (Table 3)

Second hypothesis supported for progesterone but not CL size

Conclusions

- Results support concept of CL resurgence at end of embryonic diapause in Northern fur seals
- Ultrasonography & RIA are effective noninvasive approaches for basic & applied research in reproduction in threatened seal species

Future Research

- Examine fur seal placentae for chorionic gonadotropin (CG) as detected in other seal species (Hobson & Boyd, 1984)
- Investigate luteotrophic role of CG in CL resurgence during early pregnancy as discovered in humans & equids (Bergfelt et al., 1989)

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