Progesterone in Luteal Bodies of Bobcats

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Abstract: Historically, corpora luteal counts have been used to index reproductive output; however, there has been skepticism as to their usefulness in bobcats because bobcats may retain their corpora lutea from one season to the next. We conducted this study to determine if bobcats retain corpora lutea and if they are functional. Luteal bodies were monitored throughout multiple breeding seasons. The functionality of luteal bodies of previous cycles (LBPCs) in bobcats was explored using radioimmunoassay, and compared to that of corpora lutea (CL). LBCPs continued to produce progesterone, although CL tissue had a greater progesterone concentration than LBPC tissue. This study offers evidence against using luteal body counts to determine reproductive output. Further, we suggest that retention of luteal bodies may be a unique reproductive strategy by bobcats to maintain pregnancy and, as such, may affect restoration efforts and should be considered in establishment of harvest quotas.

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Historically, corpora lutea (CL) counts were used to determine reproductive output for various species (Cheatum 1949, Provost 1962, Simkin 1965, Wright and Coulter 1967, James and Seabloom 1969, Oleyar and McGinnes 1974). Controversy behind the accuracy of this technique when applied to the bobcat *(Lynx rufus)* resulted in its discontinuance for that species (Duke 1949, Crowe 1975, Beeler 1985). One reason

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for abandonment was the suspected retention of CL in the bobcat, first suggested by Duke (1949) who noted that luteal bodies were present outside the breeding season and in greater numbers than could be reasonably attributed to 1 breeding cycle or season. Crowe (1975) observed an increase in luteal bodies with bobcat age and concluded these retained CLs were luteal bodies of previous cycles (LBPCs) because they differed from classic corpora albicantia in age and appearance. He also asserted the unlikelihood of CL retention without function in bobcats and speculated that LBPCs may secrete progestins in conjunction with CLs of a current pregnancy. Perry (1972), however, warned against the inclination to equate presence of a morphological entity with existence of a functional, steroid-producing body. Our study was undertaken to determine whether bobcat LBPCs retain any functional capacity.

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Methods

We conducted periodic laparoscopies on 8 bobcats 2–9 years of age to document progressive changes in their ovaries. All cats were housed at the Blackjack Research Site of the Forest and Wildlife Research Center and the Mississippi Agricultural and Forestry and Experiment Station. Each pen measured approximately 6 × 6 × 3 m and was constructed of rigid net wire fencing with cement floors and roofed with chicken wire. Pens were equipped with horizontal tunnels, log scratching posts, elevated runs, and a plywood den measuring 0.6 × 1 × 1 m. Pens were cleaned daily. Cats were fed a commercially prepared beef feline diet or chicken parts, supplemented with vitamins and minerals Monday through Friday, whole carcass chickens on Saturday, and fasted on Sunday. Fresh drinking water was available *ad libitum*, and a litter pan containing water was provided as excretion habitat.

Females were captured and sedated with ketamine HCI (20 to 30 mg/kg intramuscularly (IM)), transported to the Mississippi State University (MSU) College of Veterinary Medicine, anesthetized by mask induction with oxygen and isoflurane, intubated and then maintained on the anesthetic gas/O_2 mixture. Vital signs including heart rate, body temperature, pulse rate, and electrocardiogram (ECG) were monitored. A 10 × 25-cm area of the ventral abdomen was clipped, scrubbed with surgical prep solution, draped, insufflation probes placed, and the abdomen insufflated to aid in viewing. A 1-cm ventral midline incision was made for insertion of the laparoscope through which the ovaries were visualized.

Ovaries were photographed during each surgery with an Olympus CLE-F system and Olympus OM-1 camera (Olympus Corp., Woodbury, N.Y.). Observations were made on all luteal bodies regarding their location on the ovary, diameter, amount of protrusion from the ovarian surface, coloration, vascularity, and apparent

degree of connective tissue. This information was used to map the luteal body locations on each ovary for identification and aging at subsequent surgeries.

The surgical incision was sutured in a 2-layer closure. The linea alba was closed together with peritoneum using 2-0 polydioxanone suture (PDS) (Ethicon, Inc., Johnson and Johnson Co., Somerville, N.J.) in a simple interrupted pattern. The subcutis was apposed using 3-0 PDS in a simple continuous pattern. Finally, the wound was "sealed" with colloidion to waterproof the wound temporarily. Insufflation wounds were not sutured or sealed. Animals were returned to their pens and confined to dens until ambulatory.

Tissue Collection

In 1987, captive breeding females underwent laparoscopies subsequent to the last observed mating. Two females (Nos. 15 and 8) were unilaterally ovariectomized 3 weeks into gestation. A bilateral ovariectomy was performed on a third, anestrous female (No. 12) following the 1987 breeding season. Additionally, 3 wild females obtained from trappers during spring 1987 were euthanized by lethal injection and immediately ovariectomized. Upon removal, ovaries were submerged in 0.1 M HEPES buffered salt solution supplemented with 120 mMol NaC1, 5 mMol KC1, 1 mMol $CaC1_2$, 5 mMol glucose, and 1.5% bovine serum albumin at pH 7.4 and placed on ice for transport to the laboratory. All luteal bodies were dissected from the ovaries, and a biopsy of each was frozen. Tissue biopsies were also taken from CLs of 4 pregnant and 2 non-pregnant females during the luteal phase of the estrous cycle, including the 3 females that were later ovariectomized. Three LBPCs from cat No. 12 that were 3 breeding seasons old (as documented through previous laparotomies), were treated as a single LBPC because their individual integrity had been destroyed by previous biopsy and electrocautery. All luteal biopsies were frozen in phosphate-buffered saline solution with 0.01% thiomersal and 0.1% gelatin (PBS-MG) and later homogenized in a 30-ml tissue grinder (Wheaton, Millville, N.J.). Progesterone levels of the homogenates were determined by RIA (Stevenson et al. 1981).

Average recovery of 3H-progesterone from Dulbecco's Modified Eagle Medium (DMEM) or serum was 90% ($N = 6$). Crossreactivity (%) for the RIA procedure established for the laboratory were: progesterone, 100.00; corticosterone, 4.22; dihydrotestosterone, 0.174; estrone, 0.44; estradiol, 17-α, 0.20; androsterone, <0.01; 5-androstan 3, 17-α diol, <0.01; androstenedione, <0.01; estradiol 17-α, <0.01; and estriol, < 0.01 (Matamoros 1986). Sensitivity for progesterone assays averaged 0.026 ng/dl, which reduced binding of radiolabelled progesterone to antibody to 89.8%. The within-assay mean CV was 7.48 ($N = 442$). The interassay CV for 1987 assays was 7.88 ($N = 2$). Interassay CVs for 2 serum pools run in 1988 assays were 13.54 ($N = 7$) and 27.29 ($N = 6$). Progesterone levels were analyzed using a RIA SAS program developed by the Animal and Dairy Science Department at MSU.

Cell Incubations

Luteal tissue from 4 females (Nos. 10, 12, 08, and 15) was treated to promote dispersion of cells that were incubated subsequently with hCG (Lyphomed, Inc.,

Rosemont, Ill.) following the procedure of Kineman et al. (1987). Tissue was placed in supplemented 0.1 Mol HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffered salt solution on ice for transport to the laboratory, where the medium was decanted. Luteal tissue from the cats was pooled into 1 of 2 groups: LBPC or CL tissue (classification based on previous laparoscopic examinations). After weighing and adding fresh medium, the tissue was subjected to 2 15-minute serial enzymatic digestions (Kineman et al. 1987) and filtered. A 200-μl aliqout of each resultant cell suspension was stained with trypan blue, and viable luteal cells were counted. During cell staining and counting, the remaining cell suspensions were exposed to 95% 0_2:5% CO_2, capped, and incubated at 37 C for 1 hour. The suspensions were centrifuged for 5 minutes at 120 × g, and the supernatants were discarded. Pellets were resuspended in a volume of DMEM that would yield a known concentration of cells. We placed 100 μl of the cell suspensions in each of 4 tubes with 0, 100, 1000, or 10,000 ng of hGC in 0.9 saline and brought to 1 ml with DMEM. The tubes were exposed to 95% 0_2:5%CO_2, capped, and incubated for 2 hours at 37 C. Tubes were then centrifuged at 1,800 × g for 10 minutes at 4 C. The supernatants were decanted, frozen, and later analyzed by RIA for progesterone.

Approximately 1×10^6 cells/100μl were used in incubations of LBPC and CL cells from cats Nos. 15 and 08. However, dispersals of CL cells from cats Nos. 10 and 12 yielded fewer cells, and incubations used different levels of hCG. Consequently, data from the incubations of cells from cats Nos. 10 and 12 were analyzed separately. Least-squares means of progesterone production by incubated cells over all hCG levels within cat (cats Nos. 15 and 08) were compared with a level of $P < 0.05$ considered significant (SAS 1985).

Results

Tissue Progesterone

Peak tissue progesterone levels occurred around day 22 of gestation. With a single exception, active CLs biopsied up to approximately 1 month post-ovulation (in either pregnant or non-pregnant cats) had tissue progesterone concentrations exceeding 10,000 ng/g ($x = 20183$ ng/g, SD = 6803, $N = 15$), whereas tissue progesterone of LBPCs biopsied during the first month post-ovulation were below 10,000 ng/g ($x = 2384$ ng/g, SD = 1304, $N = 8$), regardless of pregnancy status.

Tissue progesterone in individual CLs of 6 captive females ranged from 8,645 ng/g on day 14 of gestation to 29,537 ng/g on day 22 (Fig. 1). Tissue progesterone in individual CLs of non-pregnant captive cats in the luteal phase of their cycle was 10,727 and 21,233 ng/g 22 days post ovulation (cat No. 04) and 21,458 ng/g 29 days post ovulation (cat No. 08). Tissue progesterone in LBPCs of 2 pregnant cats and 1 non-pregnant cat in the luteal phase of her cycle are given in Table 1. Progesterone content in LBPCs of No. 12 decreased with increasing age of the structure (Fig. 2).

Mean tissue progesterone was 602 ± 443 ($N = 3$), 3,181 ± 1,492 ($N = 3$), and 987 ± 453 ($N = 14$) ng/g in luteal bodies from 3 wild bobcats. One cat was post par-

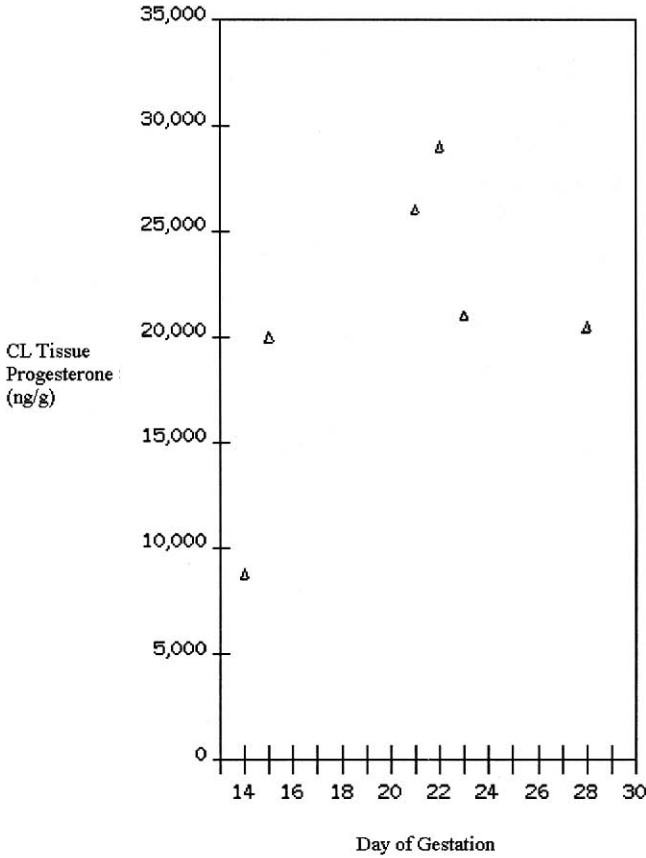

Figure 1. Progesterone levels from corpora lutea (CL) sampled at or around weeks 2, 3, and 4 of gestation in 6 captive bobcats.

Table 1. Tissue progesterone levels (P4) of luteal bodies of previous cycles (LBPCs) in 2 pregnant captive bobcats at 3 weeks gestation and 1 non-pregnant captive bobcat in the luteal phase of her estrous cycle.

Cat	*N* Breeding seasons old	Day of cycle(C) or gestation(G)	P4 (ng/g)
No. 15	0[a]	G = 22	1,638
	0		3,985
	0		1,667
No. 08	3	G = 21	1,792
	3		4,745
No. 04	1[b]	C = 22	906

a. A zero denotes a luteal body from a previous cycle in the current breeding season.
b. Estimate, based on appearance at laparoscopy.

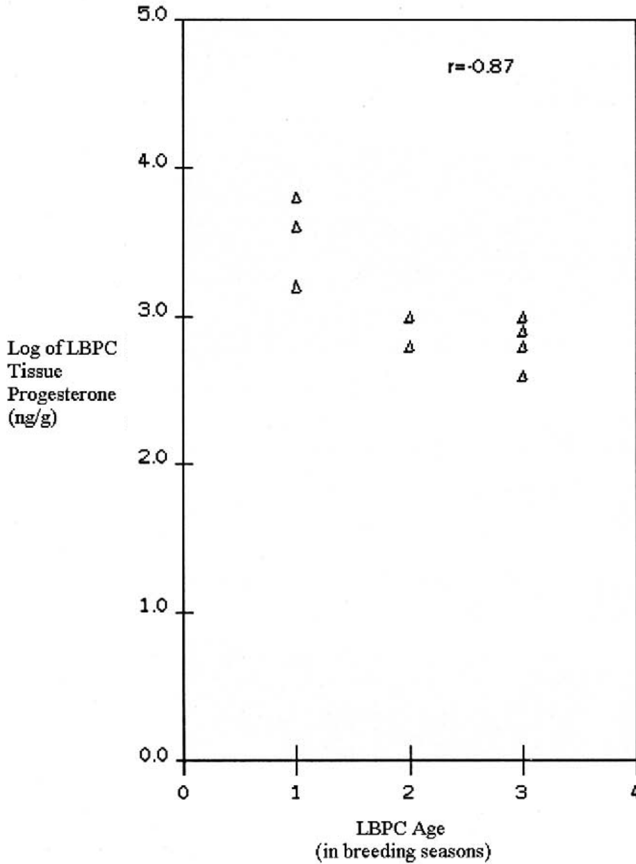

Figure 2. Tissue progesterone levels from multiple-aged luteal bodies of previous cycles (LBPCs) in the ovaries of an anestrous bobcat (cat No. 12) following ovariectomy at the end of the breeding season.

tum (mean progesterone = 987 ng/g) and another was pregnant (mean progesterone = 3,181 ng/g) with 2 feti estimated at about 30 days of gestation based on comparisons with prior observations in captive animals.

Cell Incubations

Data from the incubations of cells from cats Nos. 10 and 12 were analyzed separately because lower cell dispersal yields necessitated that different levels of hCG be used in the incubations (Table 2). Progesterone production differed ($P < 0.05$) between all hCG treatments in CL cells from cat No. 08, and between incubations with 1,000 ng/100µl hCG and those with 10,000 ng/100µl hCG for LBPC cells from cat No. 08. Among cell incubations from cat No. 15, a significant difference in progesterone production was detected between the 1,000 ng/100µl hCG and the 10,000 ng/100µl hCG levels of hCG stimulation (Table 3) for both LBPC and CL cells.

Table 2. Mean progesterone production of bobcat corpora luteal cells after incubation with human chorionic gonadotropin (hCG).

hCG Level (ng/100 ul media)	Progesterone (ng/ml media)	*N*	SD
Cat: No. 10[a]			
0.0	0.1712	7	0.0673
0.1	0.4704	7	0.1161
1.0	0.4203	6	0.1101
10.0	0.8886	5	0.1491
100.0	1.0875	6	0.1169
Cat: No. 12[b]			
0.0	1.2907	5	0.2098
0.1	1.2953	7	0.1718
1.0	1.3752	8	0.3850
10.0	1.2504	8	0.2863
100.0	1.2256	6	0.2863

a. Estimated number of corpora luteal cells used in incubations: 21,000/100 ul media.

b. Estimated number of corpora luteal cells used in incubations: 144,000 cells/100 ul media.

Table 3. Least squares means of progesterone for bobcat luteal cells collected from either corpora lutea (CL) or luteal bodies of previous cycles (LBPCs) and incubated with human chorionic gonadotropin (hCG). Approximately 1×10^6 cells were incubated for each level of hCG.

Cat	Luteal body (0[a] = *N* of breeding seasons old)	hCG Level (ng/100ul media)	Means of progesterone (ng/ml media)[b]
08	CL(0)	0	2.51A
		100	3.05B
		1,000	3.40C
		10,000	4.26D
	LBPC(3)	0	0.70A
		100	0.71A
		1,000	0.86A
		10,000	1.12B
15	CL(0)	0	1.43A
		100	1.23A
		1,000	1.01A
		10,000	1.69B
	LBPC(0)	0	0.63A
		100	0.85A
		1,000	0.79A
		10,000	1.54B

a. A zero denotes an LBPC in the current breeding season.

b. Means within tissue type with uppercase letters are significantly different at $P < 0.5$

Discussion

Tissue progesterone from bobcats demonstrated that CLs of the luteal phase of the estrous cycle had similar progesterone concentrations to those of pregnancy. Our data suggested that tissue progesterone rises steadily over the first trimester of an approximately 9-week gestation, coinciding with a rise in serum progesterone (Woshner 1988). However, a wild-caught bobcat estimated to be about 30 days pregnant (with 2 feti) had tissue progesterone levels below 5,000 ng/g in all 3 luteal bodies present, suggesting that tissue progestrone levels of CLS only exceed 10,000 ng/g from approximately the second to the fourth week of gestation and then decline.

Progesterone concentration in LBPC tissue from an anestrous cat (cat No. 12) decreased with increasing LBPC age. This decline was most dramatic between LBPCs that were 1 breeding season old and those that were 2 breeding seasons of age. Cat No. 08's LBPC cells were harvested from luteal bodies that were 3 breeding seasons old, whereas No. 15's LBPC cells were from structures that arose in a prior cycle, but in the same breeding season, as the CLs. The increase in progesterone synthesis with increasing amounts off the stimulatory hCG in cell incubations suggests that both CL cells and LBPC cells were capable of synthesizing progesterone. Comparisons between LBPC and CL cells receiving the same level of stimulus within cats showed a significant difference between LBPCs and CLs for all levels of hCG in No. 08 and for the 0 ng/100µl and the 100 ng/100µl levels of hCG in No. 15. These differences show that CL cells produced significantly more progesterone than LBPC cells as expected, although when stimulated with 10,000 ng/100µl hCG, LBPC cells from No. 15 synthesized amounts of progesterone equal to those produced by unstimulated CL cells.

Cell incubations indicated that LBPC cells were able to synthesize progesterone, but at a much slower rate than CLs. Thus, LBPCs may contribute to the maintenance of early pregnancy by reinitiating progesterone secretion, or they might aid in the regulation of gonadotropin secretion via feedback along the hypothalamo-hypophyseal axis. Perhaps for the bobcat, even, CLs of a non-pregnant cycle might be useful in a subsequent pregnancy. Retention of luteal bodies has not been documented in other felids yet the concept of accessory luteal bodies is not a new one. In fact, accessory CLs are involved in the maintenance of pregnancy in horses (Short 1961). The accessory CLs in horses are those CLs that develop later in pregnancy (Short 1961); however, in horses, these accessory structures are not retained. A final comment is that the functional role for LBPCs may relate to the finding that bobcats ovulate spontaneously (Woshner 1988), whereas other felids (including domestic cats) studied to date appear to be induced ovulators (Greulich 1934, Wildt et al. 1979, Bonney et al. 1981); although the significance of such a relationship is unclear.

This study offers evidence against using CL counts to determine reproductive output. Further, it suggests that the bobcat may have a unique reproductive strategy to maintain pregnancy through the functional use of LBPCs. If so, then reproductive performance of populations harvested extensively, with subsequent loss of older-aged animals, may be reduced. Additionally restoration efforts of bobcats into former range, as currently being conducted in the northeastern United States, should ex-

pect reduced fecundity of released animals until sufficient luteal bodies of previous pregnancies accumulate. Alternatively, animals released should be older and have demonstrated prior reproductive activities for increased and earlier success of translocations.

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