BIOLOGY OF REPRODUCTION **53**, 1022–1029 (1995)

Relationship of Circulating Steroid Hormones, Luteal Luteinizing Hormone Receptor and Progesterone Concentration, and Embryonic Mortality during Early Embryogenesis in the Domestic Cat[1]

William F. Swanson,[2] Terri L. Roth, Janine L. Brown, and David E. Wildt

Conservation and Research Center, National Zoological Park, Smithsonian Institution, Front Royal, Virginia

ABSTRACT

Serum hormones, corpus luteum (CL) progesterone, and CL LH receptors were characterized and interrelated to ovarian follicle and CL number and preimplantation embryo quality/survival in the cat. Blood samples were collected from queens ovariohysterectomized at 64 (n = 7), 76 (n = 11), 100 (n = 8), 124 (n = 7), 148 (n = 6), or 480 (n = 8) h after first copulation (3-times-daily matings on Days 2 and 3 of estrus). Ovarian CL were enucleated, weighed, and bisected; one hemi-CL was assayed for progesterone and the other for LH receptors. Serum was assessed for estradiol-17β and progesterone. Serum estradiol-17β concentrations did not return ($p > 0.05$) to baseline (~20 pg/ml) until 124 h after first copulation, whereas serum progesterone began to increase (> 1 ng/ml) by 76 h, was elevated ($p < 0.05$) by 124 h, and continued to rise thereafter. Serum progesterone was highly correlated with CL mass and LH receptor and progesterone concentration (range, $r = 0.69–0.82$; $p < 0.01$). CL progesterone and LH receptor concentrations were similar ($p > 0.05$) at 64 and 76 h, and both increased ($p < 0.05$) at subsequent time intervals and were correlated closely ($r = 0.65$; $p < 0.01$). Although ovarian CL were distinct and well organized by 64 h, pronounced elevations in serum progesterone and CL LH receptors and progesterone did not occur until at least 36 h later. The rapid increase in serum progesterone concentrations between 100 and 148 h was related to accelerated LH receptor synthesis and CL progesterone production and not entirely to enhanced CL growth. There were few discernible differences in hormonal and luteal traits between queens with viable embryos or high implantation rates and females with degenerate embryos, unfertilized oocytes, or poor implantation, with one consistent exception. Queens with poor fertility in the 64-, 76-, and 100-h groups had higher ($p < 0.05$) CL progesterone concentrations than cats with viable embryos, suggesting that altered follicular dynamics (perhaps premature luteinization) adversely impacted oocyte/embryo quality. In summary, there is a direct and significant relationship between circulating progesterone, CL mass, and CL progesterone/LH receptors during preimplantation embryogenesis in the domestic cat. The temporal kinetics of these events are remarkably similar among mated females and, with the possible exception of CL progesterone, appear unrelated to embryonic viability.

INTRODUCTION

Information on developmental events during early pregnancy in the domestic cat is extremely limited. We recently provided a chronology of events associated with in vivo embryogenesis in the cat by characterizing embryonic developmental rates, the incidence of in utero migration, and embryonic mortality associated with early natural pregnancy [1]. Although ~90% of naturally mated, ovulating queens conceive, a high percentage (~30%) of ovulated oocytes experience either fertilization failure, embryonic degeneration, or an inability to implant. One possible cause is an abnormal or suboptimal endocrine milieu in queens with poor fertility compared to females of higher fertility.

Serum hormone profiles after natural mating and during pseudopregnancy and pregnancy are well documented in the domestic cat [2–9], but hormonal interrelationships to luteal characteristics and embryogenesis have never been investigated. This information is needed to define further the natural reproductive physiology of the species while determining if poor endocrine or luteal function is related to the high incidence of embryonic mortality occurring after natural mating [1] or embryo transfer [10, 11]. We also are interested in these data as comparative information for our investigation of the 36 nondomestic species in the Felidae family, most of which are endangered by extinction.

This study is the fourth and final in a series characterizing developmental [1, 12], histological [13], and endocrine changes associated with the periovulatory, conceptive, and preimplantation/early implantation interval in the cat. The specific objectives were to 1) characterize the dynamics of circulating estradiol-17β and progesterone, corpus luteum (CL) mass, and luteal progesterone and LH receptor concentration and content at defined stages during early pregnancy; 2) correlate luteal changes with serum hormonal kinetics; and 3) relate all of these findings to embryonic development and mortality.

MATERIALS AND METHODS

Natural Mating, Ovariohysterectomy, and Embryo Recovery

Adult female and male domestic cats (Liberty Research Inc., Waverly, NY) were housed in stainless steel cages or gender-specific communal pens under controlled artificial

Accepted June 3, 1995.
Received March 1, 1995.
[1]This research was supported, in part, by grant HD 23853 from the National Institute of Child Health and Human Development, the Philip Reed Foundation, Friends of the National Zoo (FONZ), and the Ralston Purina Company.
[2]Correspondence: Dr. William Swanson, Department of Reproductive Physiology/NOAHS Center, Conservation and Research Center, 1500 Remount Road, Front Royal, VA 22630. FAX: (703) 635-6571.

lighting and provided with a commercial cat food diet (Purina Cat Chow; Ralston-Purina Co., St. Louis, MO) as previously described [1]. Queens (n = 52) were evaluated daily for signs of behavioral estrus and, beginning on the second day of estrus, were mated three times daily at 3-h intervals for 2 consecutive days [1]. To determine whether or not ovulation had occurred in response to natural mating, queens were anesthetized and ovaries examined for CL via laparoscopy [14]. If CL were observed, queens (n = 47; ~90% ovulation response) were ovariohysterectomized immediately at one of six times after first copulation: 64 h (n = 7), 76 h (n = 11), 100 h (n = 8), 124 h (n = 7), 148 h (n = 6), 480 h (n = 8). Specific time intervals for ovariohysterectomy were selected based on the expectation that the 64-h interval would coincide with the earliest preimplantation stages (1- to 2-cell embryos) whereas 480 h would coincide with postimplantation stages. For cats in the preimplantation embryo groups (64–148 h), the reproductive tracts were flushed with tissue culture medium (Ham's F-10; Sigma Chemical Co., St. Louis, MO) to recover embryos and oocytes. Recovered embryos were assessed for developmental stage (1-cell to blastocyst) and assigned quality grades (grades 1, 2, or 3) [1]. For the postimplantation interval group (480 h), the number of implantation sites and fetuses was determined.

On the basis of embryo/implantation data, queens were classified as having "high" or "low" fertility. Queens with high fertility had ≥ two good-quality (grades 1 or 2) embryos and ≤ one degenerate (grade 3) embryo/unfertilized oocyte or had a disparity between implantation and CL number of ≤ 1. Queens with low fertility had ≤ one good-quality embryo and ≥ two degenerate embryos/unfertilized oocytes or had an implantation-to-CL disparity of ≥ 2.

Blood Sampling, Ovarian Examination, and CL Processing

Blood samples (3–4 ml) were collected via jugular venipuncture from anesthetized queens immediately before laparoscopy, and all serum was stored frozen at −20°C until analyzed. For each ovariohysterectomized queen, both ovaries were examined to determine the total number of large follicles (≥ 2 mm) and CL. The left ovary was assigned to a parallel histological study [13] and the right ovary was used in this endocrine study. If no CL were present on one ovary, the other ovary was bisected and one half was assigned to each study. For the endocrine evaluation, CL (total n = 129) were enucleated from the ovary, blotted dry, and bisected. The resulting hemi-CL were weighed, snap-frozen in liquid nitrogen, and stored frozen at −80°C until analyzed. Half of each hemi-CL pair was selected randomly and analyzed for progesterone concentration, and the other hemi-CL was assessed for LH receptor concentration (see below).

Serum Estradiol-17β and Progesterone Concentration

Estradiol-17β and progesterone concentrations were measured in unextracted serum through use of solid-phase ^{125}I RIA kits (Coat-a-Count; Diagnostic Products Corporation, Los Angeles, CA). Binding inhibition curves of serially diluted cat serum were parallel to the estradiol-17β and progesterone standard curves. Net recovery of estradiol-17β and progesterone added to cat serum was 102% ($y = 1.03x + 0.98$; $r = 0.99$) and 99% ($y = 0.99x + 0.02$; $r = 0.99$), respectively. All samples were evaluated simultaneously in a single RIA for each respective hormone. Assay sensitivities (based on 90% of maximum binding) for estradiol-17β and progesterone were 5.0 pg/ml and 0.05 ng/ml, respectively. The intraassay coefficients of variation were < 10% for both assays.

Luteal Progesterone and LH Receptor Concentrations

To quantify luteal progesterone concentrations (ng/mg), hemi-CL were individually homogenized in 1 ml PBS (0.01 M PO_4, 0.14 M NaCl, 0.01% thimerosal, pH 7.4) using a ground glass homogenizer. Homogenates were diluted with absolute ethanol to 5 ml total volume, boiled for 20 min, and centrifuged (500 × g, 20 min), and the supernatants were decanted. The residual luteal pellets were extracted a second time in ethanol (2 ml) and centrifuged, and the supernatants were combined. Supernatants were dried under air, resuspended in methanol (1 ml), diluted 1:200 with PBS, and stored frozen (−80°C). Thawed aliquots (100 μl) of diluted extract were analyzed through use of a ^{125}I solid-phase RIA as described for serum. Recovery of ^{14}C-progesterone (~2000 dpm), added to luteal tissue before extraction to monitor procedural losses, was > 95%. The binding inhibition curve of serially diluted luteal extract was parallel to the progesterone standard curve, and net recovery of unlabeled progesterone added to luteal homogenates was 100% ($y = 1.02x - 0.01$; $r = 0.99$).

LH receptor concentrations (fmol/mg) were determined in crude homogenates through use of a standard curve technique as previously described [15–17]. Briefly, hemi-CL were homogenized in 1 ml cold (4°C) Tris-HCl buffer (0.01 M Tris-HCl, 0.005 M $MgCl_2$, 0.5% BSA, 0.1% NaN_3, 10% glycerol, pH 7.4, at 20°C) using a ground-glass homogenizer and stored at −80°C until analyzed. To determine total binding, triplicate aliquots (100 μl) of homogenate were incubated with ^{125}I-labeled hCG (100 μl) for 16 h at 20°C. Purified hCG (9800 IU/mg; Radio Systems Laboratories, Carson, CA) was iodinated by means of chloramine T (1 μg chloramine T/5 μg hCG) as previously described [17]. Specific activity of the ^{125}I-hCG was 40 μCi/μg as determined by self-displacement analysis. Nonspecific binding was determined in triplicate for each sample by addition of a saturating concentration of unlabeled hCG (1 μg/100 μl). After incubation, 3 ml of cold (4°C) PBS was added to each sample and, following

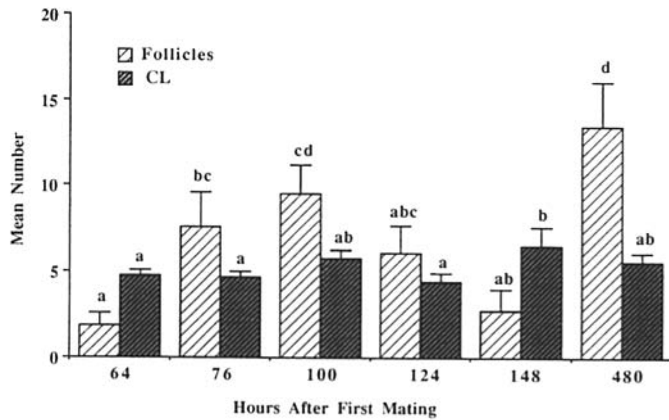

FIG. 1. Mean (± SEM) number of follicles (≥ 2 mm) and CL in the naturally estrous, mated domestic cat. Within follicle or CL groups, columns with different superscripts differ ($p < 0.05$).

centrifugation (3500 × g, 30 min), radioactivity in the pellets was determined by gamma spectrometry. To generate the standard curve, increasing amounts of a homogenized cat CL standard pool were incubated with a constant concentration of ^{125}I-hCG with or without saturating amounts of unlabeled hCG. LH receptor concentrations in unknown samples were determined by comparison with the standard curve. The number of unoccupied receptors for LH in the standard CL pool was quantified by Scatchard analysis of saturation curves [16]. Specific binding increased linearly with increasing concentration of cat CL homogenate. Binding of ^{125}I-hCG to CL tissue was specific and could be displaced > 70% by 100 ng unlabeled hCG, NIH-LH-S18, or NIH-LH-B10, but not by NIH-FSH-S8, NIH-GH-1003A, or NIH-P-S11. All samples were analyzed in the same assay, with an intraassay coefficient of variation < 15%.

Statistics

For each interval group, luteal content of progesterone (ng/CL) and LH receptors (fmol/CL) was calculated from CL mass and luteal progesterone and LH receptor concentration data. Mean (± SEM) values were determined for serum estradiol-17β and progesterone concentrations, follicle and CL number, luteal mass and luteal progesterone, and LH receptor concentration and content. Differences among interval groups were evaluated by analysis of variance [18] and least significant difference means comparison. Correlation coefficients were calculated between CL mass, serum progesterone, and luteal progesterone/LH receptor concentrations and between serum estradiol-17β concentration and follicle number [19]. For queens classified as having high or low fertility, differences between mean values for CL, serum progesterone, and luteal progesterone/LH receptor concentrations were analyzed using a Student's t-test [19].

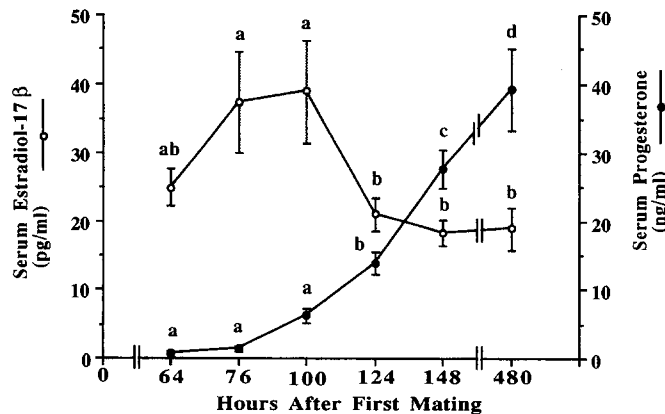

FIG. 2. Mean (± SEM) serum estradiol-17β and progesterone concentrations in domestic cats (n = 47) after natural mating and ovulation. Within each hormone, values with different superscripts differ ($p < 0.05$).

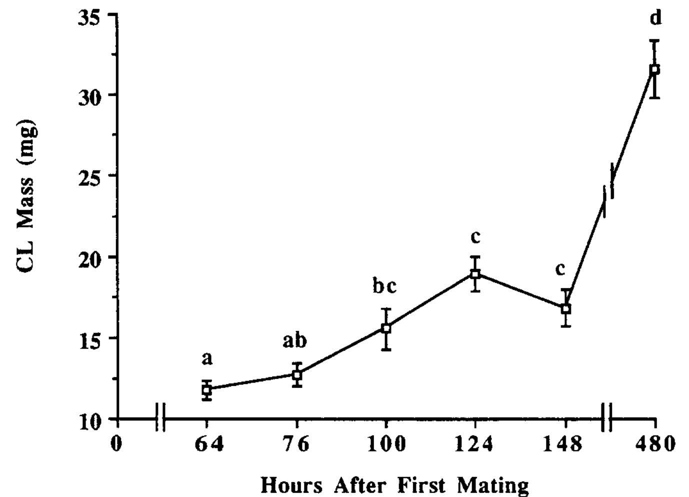

FIG. 3. Mean (± SEM) CL mass development (n = 129) in the naturally estrous, mated domestic cat. Values with different superscripts differ ($p < 0.05$).

RESULTS

Ovarian and Endocrine Characteristics

The mean number of large (≥ 2 mm) follicles was greater ($p < 0.05$) for queens in the 76-, 100-, and 480-h-interval groups than for queens in the 64-h group, and the 124- and 148-h groups were intermediate (Fig. 1). Average serum estradiol-17β concentrations were > 20 pg/ml for queens in the first three interval groups but decreased ($p < 0.05$) between 100 and 124 h after first mating, reaching close to 20 pg/ml by 148 and 480 h (Fig. 2). Across interval groups, serum estradiol-17β concentrations were unrelated to number of large follicles (r = -0.01; $p > 0.05$). CL number was similar ($p > 0.05$) among groups (mean range, 4.4 ± 0.5 to 6.5 ± 1.1) except that the 148-h group had more ($p < 0.05$) CL than the 64-, 76-, and 124-h groups (Fig. 1). Serum progesterone concentrations rose above 1 ng/ml between 64 and 76 h after first mating and then increased ($p < 0.05$) rapidly thereafter through 480 h (Fig. 2).

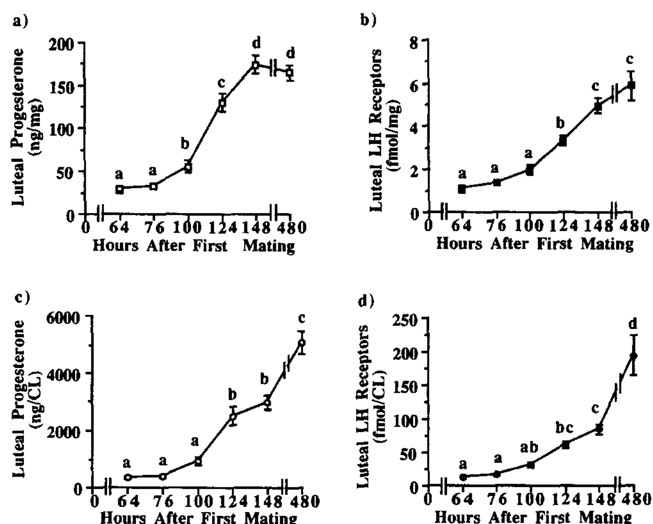

FIG. 4. Mean (± SEM) luteal progesterone and LH receptor concentrations (**a**,**b**) and content (**c**,**d**) for CL (n = 129) of naturally estrous, mated domestic cats. Within each parameter, values with different superscripts differ ($p < 0.05$).

During early embryogenesis, CL mass increased ($p < 0.05$) gradually between 64 and 100 h after the first mating, remained stable ($p > 0.05$) through 148 h, and then increased ($p < 0.05$) markedly through 480 h (Fig. 3). Luteal progesterone concentrations were unchanged ($p > 0.05$) between 64 and 76 h but then increased ($p < 0.05$) at each subsequent interval through 148 h, followed by a plateau (Fig. 4a). Similarly, luteal LH receptor concentrations remained unchanged ($p > 0.05$) for the first 100 h, increased ($p < 0.05$) at 124 and again at 148 h, and remained stable thereafter (Fig. 4b). Reflecting changes in both CL mass and luteal progesterone concentrations, luteal progesterone content was similar ($p > 0.05$) between 64 and 100 h but increased ($p < 0.05$) rapidly thereafter through 480 h (Fig. 4c). Similarly, luteal LH receptor content was unchanged ($p > 0.05$) from 64 to 100 h, increased ($p < 0.05$) gradually by 148 h, and then rose ($p < 0.05$) markedly (Fig. 4d). Across groups, individual serum progesterone concentrations were highly ($p < 0.01$) correlated with mean CL mass and luteal progesterone and LH receptor concentrations (Table 1). Similarly, luteal progesterone and LH receptor concentrations were highly ($p < 0.01$) correlated (Table 1).

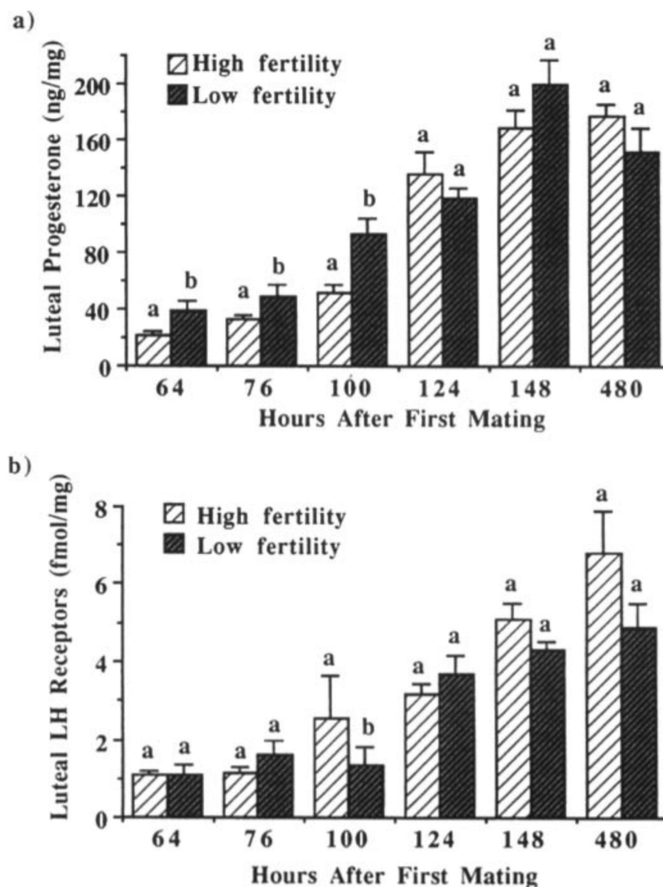

FIG. 5. Mean (± SEM) luteal progesterone (**a**) and luteal LH receptor (**b**) concentrations for queens with high (n = 29) or low (n = 14) fertility at different intervals after first mating. Within time periods, columns with different superscripts differ ($p < 0.05$).

Endocrine Traits and Fertility

On the basis of the fertility criteria provided in *Materials and Methods*, all but four queens (76 h, n = 2; 100 h, n = 2) could be classified clearly as having "high" or "low" fertility. One of these four females had 3 CL, but no embryos or oocytes were recovered; the other three had multiple ovulations (range, 5–9) but an approximately equal good-to-poor-quality embryo ratio. Of the remaining 43 queens, 29 (67.4%) were classified as having high fertility and 14 (32.6%) as having low fertility. Mean CL number was similar

TABLE 1. Correlation matrix for serum progesterone, luteal mass, and luteal progesterone/LH receptor concentration during early embryogenesis in the domestic cat.[a]

	Serum progesterone	Luteal progesterone concentration	Luteal LH receptor concentration
Luteal mass	0.69*	0.62*	0.67*
Luteal LH receptor concentration	0.78*	0.65*	
Luteal progesterone concentration	0.82*		

[a]Data represent serum samples (n = 47) and CL (n = 129) of ovulating queens (n = 47) ovariohysterectomized at 64, 76, 100, 124, 148, and 480 h after first copulation.
*$p < 0.01$.

($p > 0.05$) between queens with high (5.1 ± 0.3) and low (5.4 ± 0.3) fertility, and females with high or low fertility did not differ ($p > 0.05$) in mean endocrine or luteal traits (comparative data not shown) with the following exceptions. Within the 64-, 76-, and 100-h groups, queens with low fertility (64 h, n = 3; 76 h, n = 3; 100 h, n = 2) had higher ($p < 0.05$) luteal progesterone concentrations than females with high fertility (64 h, n = 4; 76 h, n = 6; 100 h, n = 4) (Fig. 5a). Additionally, in the 100-h group, queens with low fertility (n = 2) had a lower ($p < 0.05$) luteal LH receptor concentration than their high-fertility counterparts (n = 4) (Fig. 5b).

DISCUSSION

This study is the first to characterize and interrelate specific luteal characteristics in the cat to circulating gonadal steroids and fertility status based on embryo number and quality after natural mating. The lack of data on this subject likely is related to the difficulty (and expense) of conducting a controlled study involving large-scale, daily estrus monitoring, timed matings, and ovariohysterectomies of laboratory cats. This database needs to be established because the cat is an important companion species and also a useful research model for 1) studying various human genetic and metabolic anomalies [20, 21] and 2) applying assisted reproduction to endangered felid species [22, 23]. The present study clearly identified and characterized luteal growth and activity patterns with time. However, with the exception of CL progesterone, endocrine and luteal activity patterns during early embryogenesis were remarkably similar among queens. Further, fluctuations occurring over time appeared to be largely independent of subsequent number or quality of embryos or overall fertility status.

The composite of serum estradiol-17β and progesterone analyses agreed with hormonal profiles published previously based on samples collected longitudinally from the same animals [3–5, 8, 9]. The cat is somewhat unique in that sexual receptivity is sustained even in the face of a transient decrease in estradiol-17β during mid-estrus [8]. Elevated serum estradiol-17β concentrations may then persist for several days postcoitum. In our study, circulating estradiol-17β was elevated at 64–100 h after onset of first mating, equivalent to 28–64 h after presumed ovulation [1]. Within individual queens, circulating estradiol-17β concentrations are dynamic, often changing markedly in a matter of hours. Although these fluctuations may, in part, reflect sporadic blood sampling protocols, this dynamic secretory activity also may partially explain the lack of correlation between serum estradiol-17β concentrations and the presence of distinct ovarian follicles at ovariohysterectomy that was observed in the present study. However, the latter finding is consistent with our previous assertions that ovarian follicles observed during the luteal phase do not always produce measurable quantities of estradiol-17β (i.e., these follicles appear nonfunctional in terms of estrogen secretion) [8, 24].

Similar to previous results [4, 8, 9], circulating progesterone did not rise above baseline until ~76 h after first mating or ~40 h postovulation, on the basis of our estimate that ovulation occurs 30–36 h after first mating [1]. This temporal relationship between serum estradiol-17β and progesterone concentrations logically corresponds with our recent documentation of a relatively prolonged oviductal transport time for cat embryos during the preimplantation interval [1]. Administering exogenous estrogen is known to retard cat embryo transport through the oviduct into the uterine cornua [25]. With the new hormonal kinetics data, we now can presume that, during the initial 100 h after first mating, the sustained elevation in estradiol-17β concentrations and the delayed onset of luteal progesterone production likely delayed embryonic movement through the uterotubal junction. Beginning 100–124 h after first mating (64–88 h after presumed ovulation), the decline in circulating estradiol-17β and the simultaneous rapid increase in progesterone likely promoted the oviductal-to-uterine embryo transition that normally occurs 124–148 h after first copulation [1].

A gradual increase was measured in CL mass over time; this occurred concurrently with incremental increases in serum progesterone. Changes in CL mass corresponded to measured luteal diameters previously observed at laparoscopy [8]. In that study, CL initially were 2.7 mm in diameter 2–3 days after first copulation, grew only slightly over the next 48 h, and peaked at 3.6-mm diameter about 10 days later. It is interesting that although CL are well organized grossly (this study) and histologically [13] and are easily enucleated from the ovary by 64 h after first copulation, CL remain small in mass until ~100 h. CL mass increased slowly during the early preimplantation period but doubled in size between 148 and 480 h, an increase that corresponded to a 40% increase in mean serum progesterone concentration.

Our strategy was to measure the number of unoccupied LH receptors in cat CL, a fairly accurate estimation of total LH receptors because few of these receptors typically are occupied [26, 27]. Temporal patterns in CL progesterone and unoccupied LH receptor concentrations were similar to the CL mass profile through the first 76–100 h after first copulation. As a consequence, luteal progesterone and LH receptor content was generally static for the first 100 h after mating onset. However, although CL mass increased only moderately during the next 48 h, both CL progesterone and LH receptor concentration increased dramatically to parallel the increasingly rapid rise in serum progesterone. In contrast, while luteal progesterone and LH receptor concentrations were unchanged from 148 through 480 h, CL mass increased substantially during this time. These sequential changes in luteal progesterone and LH receptor concentrations and in CL mass accounted for the pronounced rise in luteal progesterone and LH receptor content observed be-

tween 100 and 480 h. These results also indicated that the rapid increase in serum progesterone concentrations beginning 76–100 h after first mating reflected accelerated progesterone synthesis within the CL and not simply CL growth. In contrast, it is probable that the greater serum progesterone concentrations detected at 148 and 480 h did not result from intensified luteal progesterone secretion but that they were due primarily to increasing CL mass. A similar temporal relationship has been described for circulating progesterone, CL mass, and LH binding capacity during the bovine luteal phase [28].

Strong positive correlation between serum progesterone, luteal mass, luteal progesterone, and LH receptors supports the contention that these characteristics are tightly linked by secretory mechanisms within the growing CL and suggests that LH has a luteotropic role in CL function in the cat, as in other species [29–31]. Several studies have examined LH surge profiles associated with natural mating and copulatory-induced ovulation in queens [6, 7, 32], but none have specifically investigated potential luteotropic functions of LH. After the ovulatory LH surge(s), serum LH concentrations remain near baseline, with only slight, sporadic fluctuations occurring throughout pregnancy [9]. However, similar low LH levels are known to be sufficient for supporting luteal function in the sheep [26], cow [28] and mare [33]. In these species, affinity and number of available LH receptors have proven more critical in regulating LH activity than absolute levels of circulating LH. Although preliminary evidence suggests that prolactin may play a luteotropic role after midgestation in the cat [34], virtually no information is available on putative luteotropic or luteolytic agents during early pregnancy. Additionally, although FSH is known to induce LH receptor formation in other species [35, 36], basic FSH secretory patterns and other possible mechanisms of LH receptor induction and regulation remain unstudied in the cat. It is hoped that the present study will provide some impetus for examining the nature and regulatory mechanisms of cat luteal function.

The temporal changes in cat endocrine and luteal traits generally agree with patterns measured in pregnant and nonpregnant luteal phases in other species [26–28, 33, 37] but are in marked contrast to those in another carnivore, the dog [38]. In the latter species, serum progesterone concentrations increase prior to ovulation as a result of premature follicular luteinization [39, 40], and CL LH receptor concentrations remain low and unchanged after ovulation and during diestrus in nonmated females [38]. This is interesting because the dog and cat share a number of similarities in 1) luteal activity (extended nonpregnant luteal phase, probable CL dependence throughout pregnancy) [8, 9, 34, 41] and 2) the chronology of in vivo embryogenesis (prolonged period of embryonic transport through the oviduct, advanced embryonic development at the uterine transition, intrauterine embryo migration before implantation) [1]. Although comparative luteal LH receptor data are lacking for pregnant bitches, it appears that mechanisms controlling functional luteinization may be qualitatively different between these two species.

In examining possible associations between hormonal/luteal traits and embryonic mortality, we noted that queens with high and low fertility expressed few differences in serum hormones, CL weight, and luteal progesterone and LH receptor concentration, with one exception. There were consistently elevated luteal progesterone concentrations at 64, 76, and 100 h after the first mating in cats producing the poorest-quality embryos. This could indicate that these females experienced premature luteinization or other aberrant follicular dynamics that may have compromised oocyte fertilizability directly or altered the endocrine milieu locally, to an extent sufficient to disrupt oviductal hospitality and eventually decrease embryonic viability. Of the 14 queens classified as having poor fertility, 8 (circa 57%) were in these three time-interval groups. Moreover, it is possible that the remaining queens designated as having poor fertility (in the 124–480 h groups) also had abnormal early luteal progesterone content that went unmeasured because these cats were ovariohysterectomized later in the study. In any case, we have other evidence consistent with this theory because oocytes recovered from queens previously treated with exogenous gonadotropins exhibit decreased fertilizability in vitro if any ovarian follicles ovulate prior to oocyte recovery [42]. We have interpreted this to mean that even subtle, local elevations in progesterone secretion as a result of premature follicular luteinization or ovulation can negatively affect the quality of remaining follicular oocytes. Several other cat studies appear to affirm this relationship. For example, there is a clear association between poor pregnancy or embryonic survival rates and the production of an accelerated progesterone increase or higher peak serum progesterone concentrations in queens treated with exogenous gonadotropins [10, 11, 24]

Decreased fertility of queens with high CL progesterone in the early-interval groups suggested that most embryonic mortality occurred during the earliest days after mating. This assumption is consistent with our previous observation that the number of good-quality embryos recovered from queens during the early preimplantation intervals (64–100 h) did not differ from the number during the later preimplantation intervals (124–148 h) or from the number of gestational sacs observed in queens in the postimplantation period [1]. However, although abnormal luteal progesterone concentrations were measured in most queens with poor fertility, a cause-and-effect relationship is still unproven. Other, as yet unidentified, nonluteal factors also may be responsible for the substantial embryonic mortality naturally occurring in this species.

Integrating the present results with our previous findings on in vivo embryogenesis, embryo migration, embryonic

mortality, and in vitro developmental competence of in vivo-produced embryos [1, 12] and our concurrent examination of luteal/uterine histology [13] has permitted the compilation of a comprehensive characterization of early-pregnancy events in the domestic cat. For the near future, this information clearly has the most relevance for investigating mechanisms related to embryonic mortality after either natural or assisted reproduction. For example, poor embryo survival and pregnancy rates after artificial insemination or embryonic transfer into gonadotropin-treated domestic [10, 11, 43–45] and nondomestic [45–47] felid species may be a consequence of an abnormal maternal environment. Recently, we determined that gonadotropin-treated domestic cats subjected to ovarian follicular aspiration had irregular CL histology [24] and, compared with that in naturally produced CL in the present study, abnormal luteal progesterone content. Our new database of developmental, endocrine, and histological norms will be useful for systematically improving exogenous gonadotropin therapies for the purpose of identifying a protocol most likely to mimic a normal maternal environment. Finally, we suspect that this basic approach, using the laboratory cat as a model, will have broad applications to further refining reproductive technologies suitable for helping to manage and propagate related, rare felid species.

ACKNOWLEDGMENTS

The authors thank Beth Jennette, Kathy Cooper, and Jon Anderson for technical assistance.

REFERENCES

1. Swanson WF, Roth TL, Wildt DE. In vivo embryogenesis, embryo migration and embryonic mortality in the domestic cat. Biol Reprod 1994; 51:452–464.
2. Paape SR, Shille VM, Seto H, Stabenfeldt GH. Luteal activity in the pseudopregnant cat. Biol Reprod 1975; 13:470–474.
3. Verhage JG, Beamer NB, Brenner RN. Plasma levels of estradiol and progesterone in the cat during polyestrus, pregnancy and pseudopregnancy. Biol Reprod 1976; 14:579–585.
4. Shille VM, Stabenfeldt GH. Luteal function in the domestic cat during pseudopregnancy and after treatment with prostaglandin $F_{2\alpha}$. Biol Reprod 1979; 21:1217–1223.
5. Shille VM, Lundstrom KE, Stabenfeldt GH. Follicular function in the domestic cat as determined by estradiol-17β concentrations in plasma: relation to estrous behavior and cornification of exfoliated vaginal epithelium. Biol Reprod 1979; 21:953–963.
6. Concannon P, Hodgson B, Lein D. Reflex LH release in estrous cats following single and multiple copulations. Biol Reprod 1980; 23:111–117.
7. Wildt DE, Seager SWJ, Chakraborty PK. Effect of copulatory stimuli on incidence of ovulation and on serum luteinizing hormone in the cat. Endocrinology 1980; 107:1212–1217.
8. Wildt DE, Chan SYW, Seager SWJ, Chakraborty PK. Ovarian activity, circulating hormones and sexual behavior in the cat. I. Relationships during the coitus-induced luteal phase and the estrous period without mating. Biol Reprod 1981; 25:15–28.
9. Schmidt PM, Chakraborty PK, Wildt DE. Ovarian activity, circulating hormones and sexual behavior in the cat. II. Relationships during pregnancy, parturition, lactation and the postpartum estrus. Biol Reprod 1983; 28:657–671.
10. Goodrowe KL, Wall RJ, O'Brien SJ, Schmidt PM, Wildt DE. Developmental competence of domestic cat follicular oocytes after fertilization in vitro. Biol Reprod 1988; 39:355–372.
11. Goodrowe KL, Howard JG, Wildt DE. Comparison of embryo recovery, embryo quality, oestradiol-17β and progesterone profiles in domestic cats (*Felis catus*) at natural or induced oestrus. J Reprod Fertil 1988; 82:553–561.
12. Roth TL, Swanson WF, Wildt DE. Developmental competence of domestic cat embryos fertilized in vivo versus in vitro. Biol Reprod 1994; 51:441–451.
13. Roth TL, Munson L, Swanson WF, Wildt DE. Histological characteristics of the uterine endometrium and corpus luteum during early embryogenesis and the relationship to embryonic mortality in the domestic cat. Biol Reprod 1995; 53:1012-1021.
14. Wildt DE, Kinney GM, Seager SWJ. Laparoscopy for direct observation of internal organs in the domestic cat and dog. Am J Vet Res 1977; 38:1429–1432.
15. Nett TM, Crowder ME, Moss GE, Duello TM. GnRH-receptor interaction. V. Down-regulation of pituitary receptors for GnRH in ovariectomized ewes by infusion of homologous hormone. Biol Reprod 1981; 24:1145–1155.
16. Brown JL, Stuart LD, Chakraborty PK. Endocrine profiles, testicular gonadotropin receptors and sperm production in hemi-castrated ram lambs. J Anim Sci 1987; 65:1563–1570.
17. Brown JL, Bush M, Packer C, Pusey AE, Monfort SL, O'Brien SJ, Jansen DL, Wildt DE. Developmental changes in pituitary-gonadal function in free-ranging lions (*Panthera leo*) of the Serengeti Plains and Ngorongoro Crater. J Reprod Fertil 1991; 91:29–40.
18. SAS. SAS User's Guide. Cary, NC: Statistical Analysis Systems Institute, Inc.; 1984.
19. Steel RD, Torrie JH. Principles and Procedures of Statistics, New York: McGraw-Hill; 1960.
20. Migaki G. Compendium of inherited metabolic diseases in animals. In: Migaki G, Desnick RJ, Patterson DF (eds.), Animal Models of Inherited Metabolic Diseases. New York: Alan R Liss, Inc; 1982: 473–501.
21. O'Brien SJ. Genetic map of the domestic cat (*Felis catus*). In: O'Brien SJ (ed.), Genetic Maps, Volume 6. Cold Spring Harbor, NY: Cold Spring Harbor Press; 1993: 4.258–4.261.
22. Wildt DE, Schiewe MC, Schmidt PM, Goodrowe KL, Howard JG, Phillips LG, O'Brien SJ, Bush M. Developing animal model systems for embryo technologies in rare and endangered wildlife species. Theriogenology 1986; 25:33–51.
23. Wildt DE, Monfort SL, Donoghue AM, Johnston LA, Howard JG. Embryogenesis in conservation biology—or how to make an endangered species embryo. Theriogenology 1992; 37:161–184.
24. Donoghue AM, Johnston LA, Munson L, Brown JL, Wildt DE. Influence of gonadotropin treatment interval on follicular maturation, in vitro fertilization, circulating steroid concentrations and subsequent luteal function in the domestic cat. Biol Reprod 1992; 46:972–980.
25. Herron MA, Sis RF. Ovum transport in the cat and the effect of estrogen administration. Am J Vet Res 1974; 35:1277–1279.
26. Diekman MA, O'Callaghan P, Nett TM, Niswender GD. Validation of methods and quantification of luteal receptors for LH throughout the estrous cycle and early pregnancy in ewes. Biol Reprod 1978; 19:999–1009.
27. Ziecik A, Shaw HJ, Flint APF. Luteal LH receptors during the oestrous cycle and early pregnancy in the pig. J Reprod Fertil 1980; 60:129–137.
28. Spicer LJ, Ireland JJ, Roche JF. Changes in serum LH, progesterone and specific binding of ^{125}I-hCG to luteal cells during regression and development of bovine corpora lutea. Biol Reprod 1981; 25:832–841.
29. Niswender GD, Menon KMJ, Jaffe RB. Regulation of the corpus luteum during the menstrual cycle and early pregnancy. Fertil Steril 1972; 23:432–442.
30. Hansel W, Concannon PW, Lukaszewska JH. Corpora lutea of the large domestic animals. Biol Reprod 1973; 8:222–245.
31. Concannon P. Effects of hypophysectomy and of LH administration on luteal phase plasma progesterone levels in the beagle bitch. J Reprod Fertil 1980; 58:407–410.
32. Glover TE, Watson PF, Bonney RC. Observations on variability in LH release and fertility during oestrus in the domestic cat (*Felis catus*). J Reprod Fertil 1985; 75:145–152.
33. Roser JF, Evans JW. Luteal luteinizing hormone receptors during the postovulatory period in the mare. Biol Reprod 1983; 29:499–510.
34. Verstegen JP, Onclin K, Silva LDM, Wouters-Ballman P, Delahaut P, Ectors F. Regulation of progesterone during pregnancy in the cat: studies on the roles of corpora lutea, placenta and prolactin secretion. J Reprod Fertil Suppl 1993; 47:165–173.
35. Zeleznik AJ, Midgley AR Jr, Reichart LE Jr. Granulosa cell maturation in the rat: increased binding of human chorionic gonadotropin following treatment with follicle-stimulating hormone in vivo. Endocrinology 1974; 95:818–825.
36. May JV, McCarty K Jr, Reichert L Jr, Schomberg DW. Follicle-stimulating hormone-mediated induction of functional luteinizing hormone/human chorionic gonadotropin receptors during monolayer culture of porcine granulosa cells. Endocrinology 1980; 107:1041–1049.
37. Cheng KW. Changes in rat ovaries of specific binding for LH, FSH and prolactin during the oestrus cycle and pregnancy. J Reprod Fertil 1976; 48:129–135.
38. Fernandes PA, Bowen RA, Kostas AC, Sawyer HR, Nett TM, Olson PN. Luteal function in the bitch: changes during diestrus in pituitary concentration of and the number of luteal receptors for luteinizing hormone and prolactin. Biol Reprod 1987; 37:804–811.
39. Concannon P, Hansel W, McEntee K. Changes in LH, progesterone and sexual behavior associated with preovulatory luteinization in the bitch. Biol Reprod 1977; 17:604–613.
40. Wildt DE, Panko WB, Chakraborty PK, Seager SWJ. Relationship of serum estrone, estradiol-17β and progesterone to LH, sexual behavior and time of ovulation in the bitch. Biol Reprod 1979; 20:648–658.
41. Concannon PW, Hansel W, Visek WJ. The ovarian cycle of the bitch: plasma estrogen, LH and progesterone. Biol Reprod 1975; 13:112–121.

42. Roth TL, Donoghue AM, Byers AP, Munson L, Wildt DE. Influence of oviductal cell monolayer co-culture and the presence of corpora hemorrhagica at the time of oocyte aspiration on gamete interaction in vitro in the domestic cat. J Assist Reprod Gen 1994; 10:523–529.
43. Howard JG, Barone MA, Donoghue AM, Wildt DE. Preovulatory anesthesia compromises ovulation in laparoscopically-inseminated domestic cats. J Reprod Fertil 1992; 96:175–186.
44. Swanson WF, Godke RA. Transcervical embryo transfer in the domestic cat. Lab Anim Sci 1994; 44:288–291.
45. Pope CE, Keller GL, Dresser BL. In vitro fertilization in domestic and non-domestic cats including sequences of early nuclear events, development in vitro, cryopreservation and successful intra- and interspecies embryo transfer. J Reprod Fertil Suppl 1993; 47:189–201.
46. Howard JG, Donoghue AM, Barone MA, Goodrowe KL, Blumer ES, Snodgrass K, Starnes D, Tucker M, Bush M, Wildt DE. Successful induction of ovarian activity and laparoscopic intrauterine artificial insemination in the cheetah (*Acinonyx jubatus*). J Zoo Wildl Med 1992; 23:288–300.
47. Donoghue AM, Johnston LA, Seal US, Armstrong DL, Tilson RL, Wolff P, Petrini K, Simmons LG, Gross T, Wildt DE. In vitro fertilization and embryo development in vitro and in vivo in the tiger (*Panthera tigris*). Biol Reprod 1990; 43:733–744.