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Histological Characteristics of the Uterine Endometrium and Corpus Luteum during Early Embryogenesis and the Relationship to Embryonic Mortality in the Domestic Cat[1]

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ABSTRACT

Pregnancy rates are low and litter sizes generally small when assisted reproduction techniques are used in gonadotropin-treated felid (cat) species. A prerequisite to determining whether or not abnormal morphological changes in the uterine lumen or corpus luteum (CL) are related to this reproductive failure is the documentation of normal histological kinetics during natural embryogenesis. This study characterized the histological changes of the endometrium and ovarian CL during the early stages of preimplantation embryonic development in the naturally estrous, mated queen. The purpose was to 1) develop a system for dating the cat endometrium and CL of early pregnancy; 2) document the frequency of abnormal uterine and CL histology under natural mating conditions; and 3) compare histological traits of queens producing good- vs. poor-quality embryos. Naturally estrous, mated queens were ovariohysterectomized at 64 h (n = 8), 76 h (n = 11), 100 h (n = 8), 124 h (n = 7), 148 h (n = 6), or 480 h (n = 8) after first copulation. Embryos collected from oviductal and uterine flushings were graded for quality, and uteri and ovaries were fixed in formalin. Fixed tissue sections were stained and multiple histological traits described for each uterine (endometrial height, endometrial vacuolation, percentage of glandular cells with subnuclear vacuoles, number of mitoses, nuclear-to-cytoplasmic ratio) and ovarian (presence of tertiary follicles, vacuolation of luteal cell cytoplasm, luteal cell shape) sample. Modest histological changes were observed at each time point, and these were documented in detail. The most prominent modifications occurred at 124 h after first copulation and included thickening of the endometrium, straightening of the glands, increased cytoplasmic vacuolation, and increased epithelial height. Of 15 queens failing to produce good-quality embryos, only 4 expressed unusual histological characteristics; and 3 of 25 queens producing only high-quality embryos exhibited abnormal uterine or CL cellular integrity. Therefore, aberrant histological changes are not primarily responsible for failure of the naturally estrous, mated queen to produce good-quality embryos. Furthermore, a normative database now is available to date the endometrium and CL of early pregnancy and to examine the impact of exogenous gonadotropins and assisted techniques on uterine/CL structure and function.

INTRODUCTION

The domestic cat (*Felis catus*) is a useful model for studying comparative biology of the Felidae family and for developing assisted reproductive techniques for propagating related, endangered species [1]. For example, an exogenous hormone regimen for inducing ovarian activity, IVF protocols, and a laparoscopic artificial insemination (AI) technique all were developed first in the domestic cat [2, 3]. Then gonadotropin therapy combined with IVF or AI was applied successfully to the cheetah (*Acinonyx jubatus*) [4, 5], tiger (*Panthera tigris*) [6, 7], puma (*Felis concolor*) [8], leopard cat (*Felis bengalensis*) [9], clouded leopard (*Neofelis nebulosa*) [10], ocelot (*Felis pardalis*) [11], and snow leopard (*Panthera uncia*) (Roth and Wildt, unpublished data). Despite these successes, pregnancy rates remain low and litter sizes small [2, 12, 13]. This reproductive inefficiency perhaps is attributable to fertilization failure and/or suboptimal embryo quality [14]. Alternatively, poor embryo survival may result from an abnormal uterine milieu or compromised corpus luteum (CL) function following administration of exogenous gonadotropins [7].

Before we can investigate whether unusual morphological changes occur in the uterus or CL of gonadotropin-treated cats, it is necessary to characterize uterine and CL histology in the naturally estrous, mated queen. Furthermore, we now know from recent studies that there is a ~30% disparity between the number of CL and the number of embryos/implantation sites after copulation, reflecting a remarkably high rate of oocyte/embryo mortality during natural reproduction [15]. Therefore, it is important to determine the frequency of irregular histological changes under natural mating conditions while ascertaining whether or not abnormal histology is a primary factor associated with this normal embryo loss. It is surprising that little histological data are available that describe uterine and ovarian changes during preimplantation pregnancy in a species as common and popular as the cat. Limited data date from the 1940s [16, 17]; although these are useful, the earliest stage of pregnancy examined was Day 4 postmating, and, most importantly, embryo quality was not measured to validate the existence of a successfully progressing early pregnancy.

Our general aim was to characterize in detail histological events occurring in the uterine lumen and ovarian CL at precisely timed intervals from fertilization through early implantation in the naturally estrous, mated queen. This is the

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third in a series of papers [15, 18] integrating crucial factors and events that influence early embyrogenesis in the domestic cat. Through adherence to a strict breeding/ovariohysterectomy regimen, our specific objectives were to 1) develop a system for histologically dating the endometrium and CL during early pregnancy; 2) document the frequency of abnormal uterine and CL histology in the naturally estrous, mated queen; and 3) compare histological traits of queens producing good-quality embryos and those producing unfertilized, developmentally retarded, or degenerate embryos, thereby determining whether or not there was a relationship between embryo quality and uterine or CL cellular structure/integrity. Lastly, one group of postimplantation queens was examined to determine whether embryo loss was associated with implantation failure.

MATERIALS AND METHODS

Animals

Adult (20 ± 2 mo), female domestic cats were housed in cages (1–2 queens per cage) or communal pens (2–8 queens per pen) and maintained in a controlled ambient environment under artificial fluorescent illumination (12L:12D) during the 1-yr study period. Two normospermic, proven breeder males were housed singly in separate pens. All cats were provided with a commercial feline diet (Purina Cat Chow; Ralston-Purina Co., St. Louis, MO) and water ad libitum.

Estrus Detection and Natural Breeding

Queens were checked daily for behavioral signs of estrus (treading of the hind feet, tail deflection, and lordosis posturing). Naturally estrous females were mated three times per day at 3-h intervals on the second and third days of estrus [19]. The two normospermic domestic cats were used on a rotating basis as natural breeding partners for females. Mated queens were assigned randomly to one of six time-interval groups: 64, 76, 100, 124, 148, and 480 h after the first copulation.

Tissue Collection/Processing

Laparoscopy was performed to confirm that queens had ovulated after mating. Cats in each interval group (64 h, n = 8; 76 h, n = 11; 100 h, n = 8; 124 h, n = 7; 148 h, n = 6; 480 h, n = 8) were induced into a surgical plane of anesthesia with a ketamine hydrochloride (Vetalar; Parke-Davis, Morris Plains, NJ) plus acepromazine maleate (Ayerst Laboratories, Rouses Pt., NY) mixture (10:1 ratio; 10.0 mg/kg and 0.1 mg/kg BW, respectively, i.m.), and anesthesia was maintained by delivering isoflurane gas/oxygen via a face mask. At laparoscopy, a Verres needle was used to manipulate the ovaries to allow visualization of all aspects of both ovaries for the presence of fresh CL or corpora hemorrhagica (CH). All queens with fresh CL and/or CH were maintained under a surgical plane of anesthesia and ovariohysterectomized immediately via laparotomy. Initially, five females were assigned to each group, but if no embryos of "good" quality (as described below) were recovered from one or more of these cats, additional, randomly chosen females were assigned to the group until good-quality embryos were recovered from a total of five queens per interval.

For each queen, one ovary was processed for endocrine evaluations including LH receptor concentrations and progesterone content [20], and the other was trimmed free of fat and connective tissue and transferred into a 15-ml conical tube containing 10% buffered formalin. Arbitrarily, the left ovary was always chosen for histological examination, the exception being when no CL were present on one of the ovaries. In that case, the ovary with CL was bisected and one hemi-ovary was used for each of the histological and endocrine studies. Ovaries and hemi-ovaries for histological evaluation were placed in fixative within 10–30 min of excision.

After oviducts and ovaries were removed from the reproductive tracts, uteri were placed in an incubator and maintained for 10–30 min. During this time, oviducts were flushed repeatedly with 1–3 ml of sterile, warm (37°C) Ham's F-10 culture medium (Irvine Scientific; Santa Ana, CA), and the recovered fluid was examined for embryos. Embryos were evaluated for developmental stage and quality grade (grade 1 = dark, homogenous coloration and uniformly shaped blastomeres; grade 2 = lighter in color, some abnormally shaped blastomeres, slight vacuolation; grade 3 = degenerate, pale, fragmenting blastomeres) [15, 18]. If the number of embryos flushed from the oviducts matched the total number of ovarian CL, the uterus was fixed immediately in a 50-ml conical tube containing 10% buffered formalin. If all embryos were not recovered from the oviducts, each uterine horn was flushed several times with ~3 ml of warm (37°C), sterile Ham's F-10 before being placed in fixative. All embryos and oocytes were recovered from the oviducts in the 64- to 124-h-interval groups, and all but one embryo were recovered from the uterine horns in the 148-h group. Embryo developmental stage and quality grade have been described in detail previously [15]. For the 480-h group, the number and size (gestational sac diameter) of implantation sites within the uterine cornua were determined, and gestational sacs were incised to assess presence or absence of a fetus.

After all queens in each time interval were ovariohysterectomized, formalin-fixed tissues were processed for histological evaluation. Two transverse sections were sampled from each uterine horn. For queens in the 480-h group, transverse sections of two placental sites and two interplacentation zones were taken. Ovaries from all queens were sectioned through all visible CL and included adjacent ovarian parenchyma. All tissues were embedded in paraffin, sec-

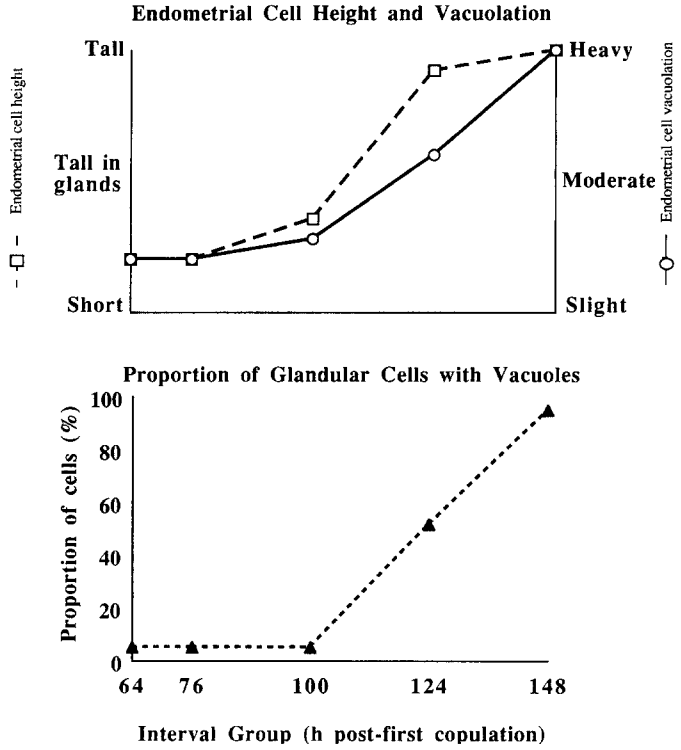

FIG. 1. Changes in three uterine histological traits during early embryogenesis in the cat. Naturally estrous, mated queens were ovariohysterectomized at 64, 76, 100, 124, and 148 h after first copulation. Data represent average traits for the five queens producing good-quality embryos in each interval group.

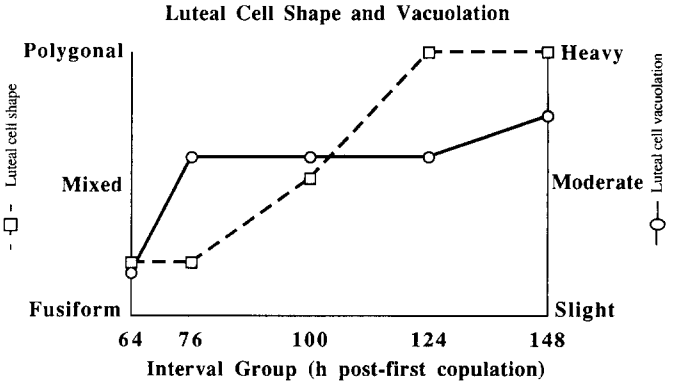

FIG. 2. Changes in two histological traits of the CL during early embryogenesis in the cat. Naturally estrous, mated queens were ovariohysterectomized at 64, 76, 100, 124, and 148 h after first copulation. Data represent average traits for the five queens producing good-quality embryos in each interval group.

tioned at 7 μm, and stained with hematoxylin and eosin. Ovarian and uterine tissue sections were evaluated without knowledge of the interval group. Uteri were evaluated for glandular and luminal epithelial morphology and the prevalence of mitoses. Sections for each queen were scored for the following traits: 1) endometrial height (short or tall in glands and lumen); 2) endometrial cytoplasmic vacuolation (slight, moderate, heavy); and 3) proportion of glandular cells with subnuclear vacuoles. Ovaries were surveyed for the presence of tertiary follicles and the morphology of cells within developing CL. Luteal cells were scored for vacuolation of cytoplasm (slight, moderate, heavy) and cell shape (fusiform, polygonal, mixed).

Statistical Analysis

Average values of each histological trait for the five queens producing good-quality (grades 1 or 2) embryos in each interval group were calculated to define normal changes during early embryogenesis. For descriptive traits, numerical values (1–3) were assigned to reflect the changes quantitatively and to enable plotting of the changes over time. Additionally, for each trait, a correlation coefficient was determined that compared queens that did not produce good embryos to those that did within each interval group. Likewise, a chi-square test was used to compare, across all groups, the proportion of queens producing good- and poor-quality embryos with abnormal histological traits. These analyses were used to determine whether abnormal histology was associated with failed embryogenesis.

RESULTS

The following summarizes uterine and ovarian CL histological characteristics and changes during the first 480 h after first copulation in the pregnant cat. Descriptions for each interval group were based upon evaluations of reproductive tracts collected from at least five queens producing good-quality embryos or implantation sites. Queens were consid-

TABLE 1. Number of queens in each preimplantation group producing good-quality or poor-quality (unfertilized, degenerate, none) embryos and exhibiting normal and abnormal histological characteristics of the uterine endometrium and/or ovarian CL.

		Queens producing good-quality embryos with:		Queens producing poor-quality embryos with:	
Group (h)	Number of queens	normal histology	abnormal histology	normal histology	abnormal histology
64	8	4	1[a]	1	2[a]
76	11	5	0	5	1[a]
100	8	4	1[b]	2	1[c]
124	7	4	1[a]	2	0
148	6	5	0	1	0
Totals[d]	40	22	3	11	4

[a] Queen(s) exhibited endometrial hyperplasia.
[b] Queen exhibited retarded histological development of the uterine endometrium.
[c] Queen exhibited retarded histological development of both uterine endometrium and CL.
[d] Proportion of queens with abnormal histological traits did not differ ($p < 0.01$) between good- and poor-quality embryo groups.

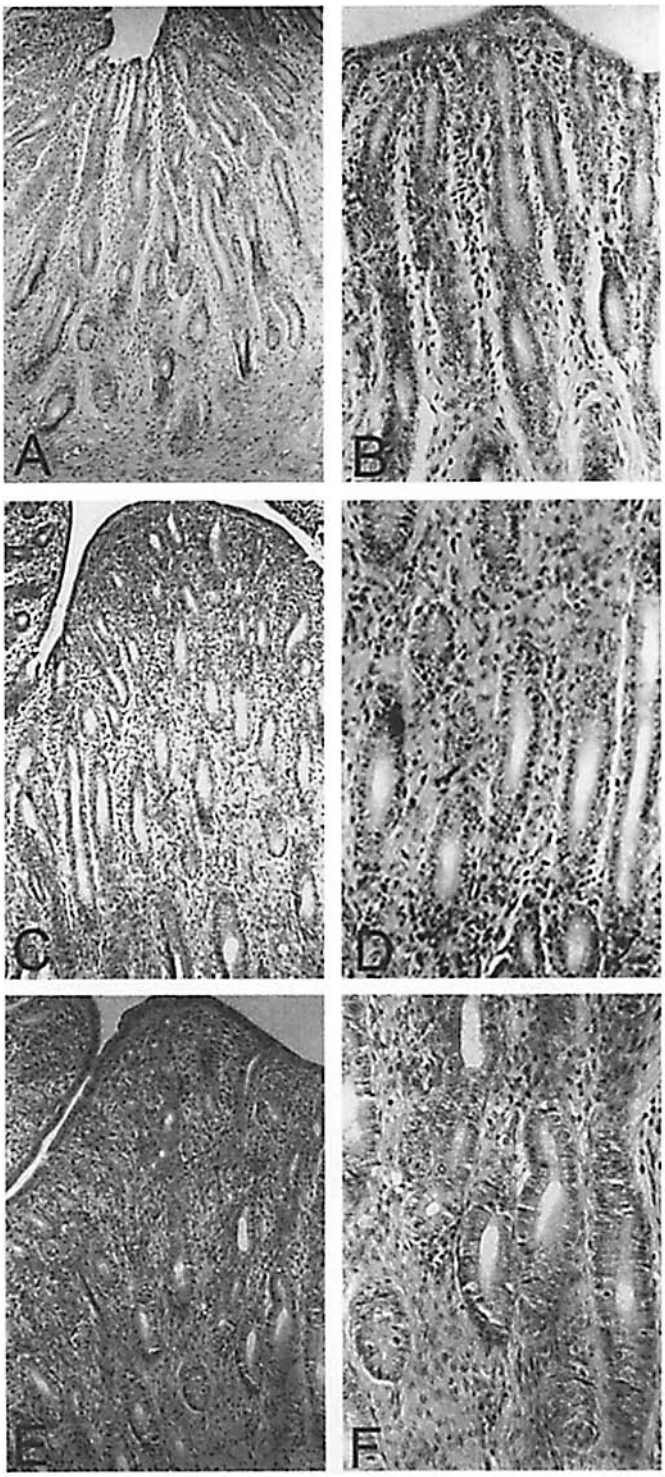

FIG. 3. Uterine endometrial histomorphology of naturally estrous, mated cats. **A)** At 64 h after first copulation. Luminal epithelium has a few shallow infoldings (×87). **B)** Endometrial glands are elongated and tortuous; cells are cuboidal; glandular epithelium is dense with pseudostratification (×174). **C)** At 76 h after first copulation. Endometrial glands are more elongated (×87). **D)** The glandular epithelium is more regular with less pseudostratification (×174). **E)** At 100 h after first copulation. Luminal epithelium continues to have only slight infoldings (×87). **F)** Glandular epithelium height and cytoplasmic vacuolation have increased (×174).

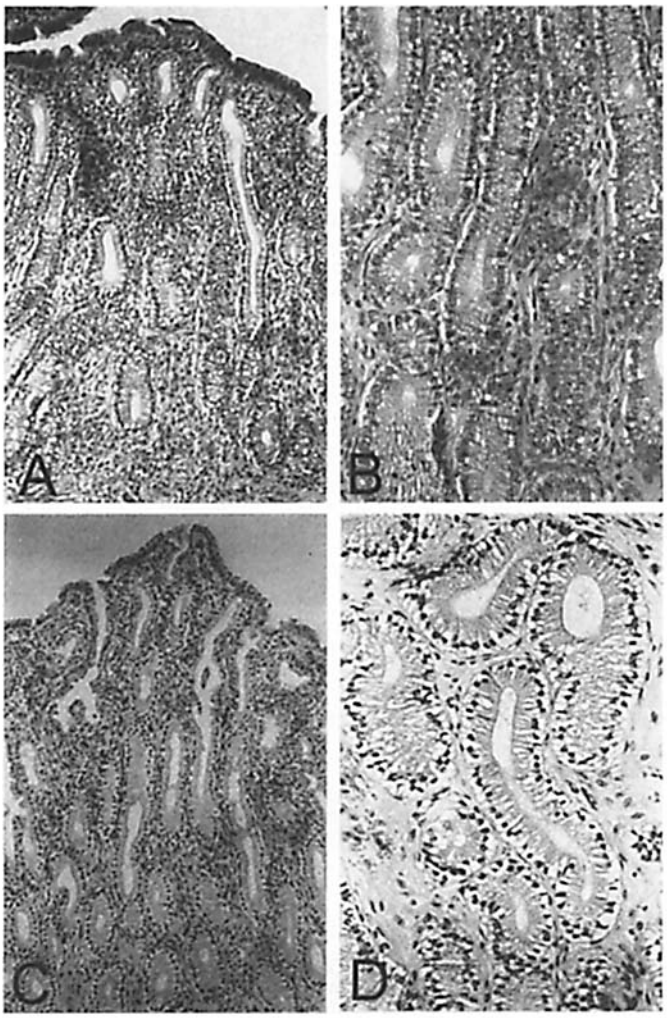

FIG. 4. Uterine endometrial histomorphology of naturally estrous, mated cats. **A)** At 124 h after first copulation. Depth and number of intraluminal infoldings have increased appreciably (×87). **B)** Glands are straighter and are lined by tall columnar epithelium with increased cytoplasmic vacuolation (×174). **C)** At 148 h after first copulation. The luminal epithelium has abundant, deep, even surface infoldings (×87). **D)** Glands now are highly elongated with an epithelium composed of tall columnar cells with abundant, vacuolated cytoplasm (×174).

ered to have abnormal histology if uterine and/or CL traits differed from those of the five queens representing that interval group (i.e., advanced or retarded histological development) or if they exhibited uterine pathology (i.e., endometrial hyperplasia). Changes in specific uterine and CL traits from 64 to 148 h are summarized graphically along a timeline in Figures 1 and 2, respectively. Histological characteristics of those queens producing only unfertilized oocytes (n = 3), degenerate embryos (n = 10), or no embryos (n = 1) also are described. Within each group, there was no correlation (range, $r = 0.30–0.65$; $p > 0.05$) between abnormal uterine or CL histological traits and embryo quality. Likewise, the overall proportion of queens with abnormal histological traits did not differ ($p > 0.05$) between groups that produced poor- and good-quality embryos (Table 1).

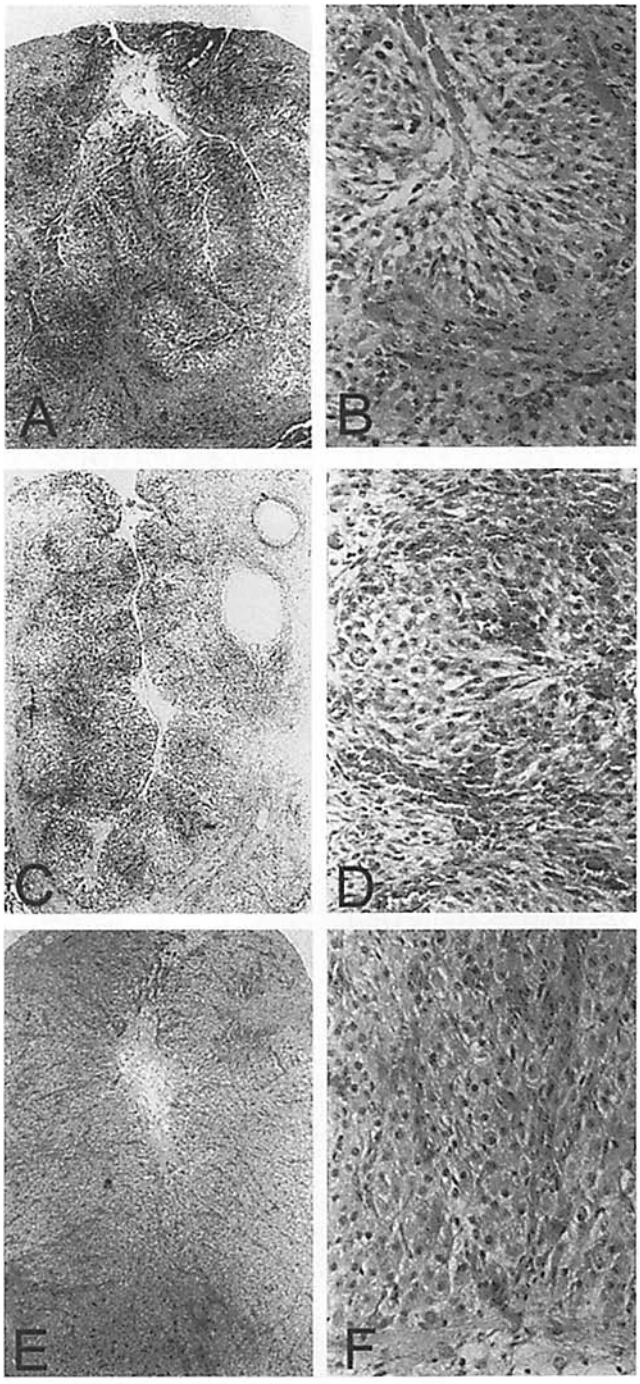

FIG. 5. CL histomorphology of naturally estrous, mated cats. **A)** At 64 h after first copulation. CH with central cavity. CH margins are infolded from collapse of the Graafian follicle (×35). **B)** Early luteinization is most prominent in the periphery. Cells are fusiform with orientation toward the central cavity. Vasculature is prominent and dilated, and capillaries run perpendicular to and extend from the theca externa to the CH lumen (×174). **C)** At 76 h after first copulation. CH with central cavitation still present (×35). **D)** Luteal cells are still fusiform and oriented perpendicular to the CL margins. Peripheral cells exhibit a slight increase in cytoplasmic vacuolation (×174). **E)** At 100 h after first copulation. CL with irregular border and persistent central cavity (×35). **F)** Luteal cells still are predominantly fusiform, but there are more polygonal cells with cytoplasmic vacuolation in the CL periphery (×174).

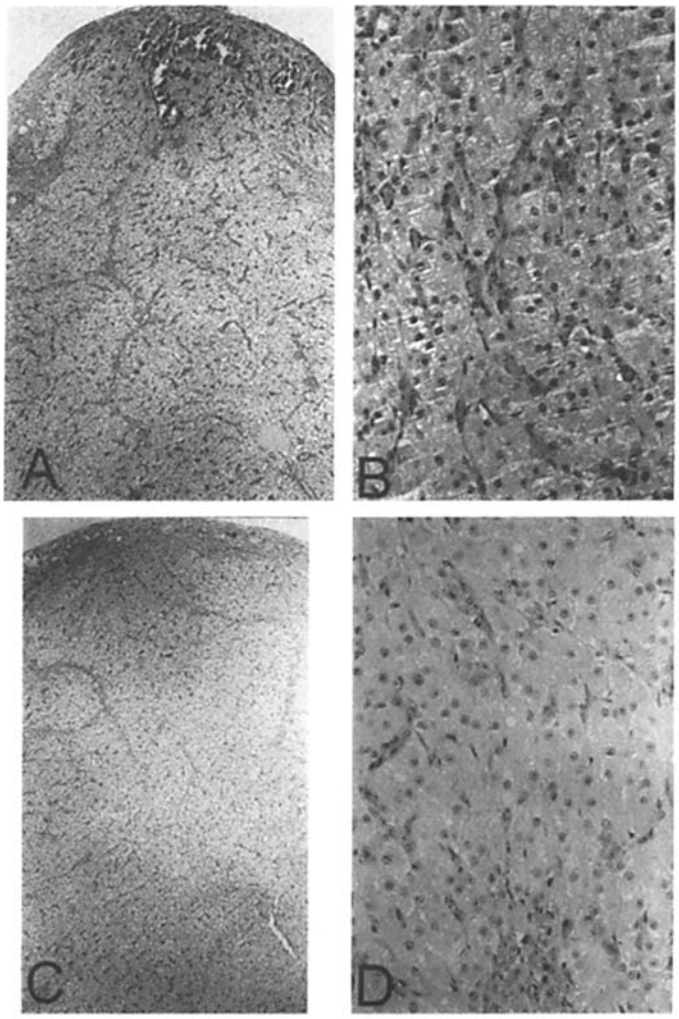

FIG. 6. CL histomorphology of naturally estrous, mated cats. **A)** At 124 h after first copulation. CL margins are more rounded and the cavity is filling with fibrosis (×35). **B)** More cells are polygonal with increased cytoplasmic vacuolation. Vasculature is congested and no longer oriented perpendicular to the CL margin (×174). **C)** At 148 h after first copulation. CL has expanded, rounded margins (×35). **D)** Most luteal cells are polygonal with abundant cytoplasmic vacuolation. Vasculature is less prominent (×174).

Histological Changes of the Uterine Lumen

At 64 h (Fig. 3, A and B), endometrial glands were elongated and slightly tortuous. The glandular epithelium was quite dense with pseudostratification. Cells were cuboidal with a nuclear/cytoplasmic (N/C) ratio of approximately 1:1. Some deep glands had dilated lumina and contained proteinaceous secretions. Subnuclear vacuoles were rare or absent. The superficial (luminal) epithelium had a few shallow infoldings. Cells were low cuboidal-shaped with microvilli and an N/C ratio of 1:0.5. Few mitoses, 0–1 per high-power field (0–1/HPF), were present.

At 76 h (Fig. 3, C and D), the endometrial glands were more elongated and showed increased branching. The deep glandular epithelium was more regular with less cellular

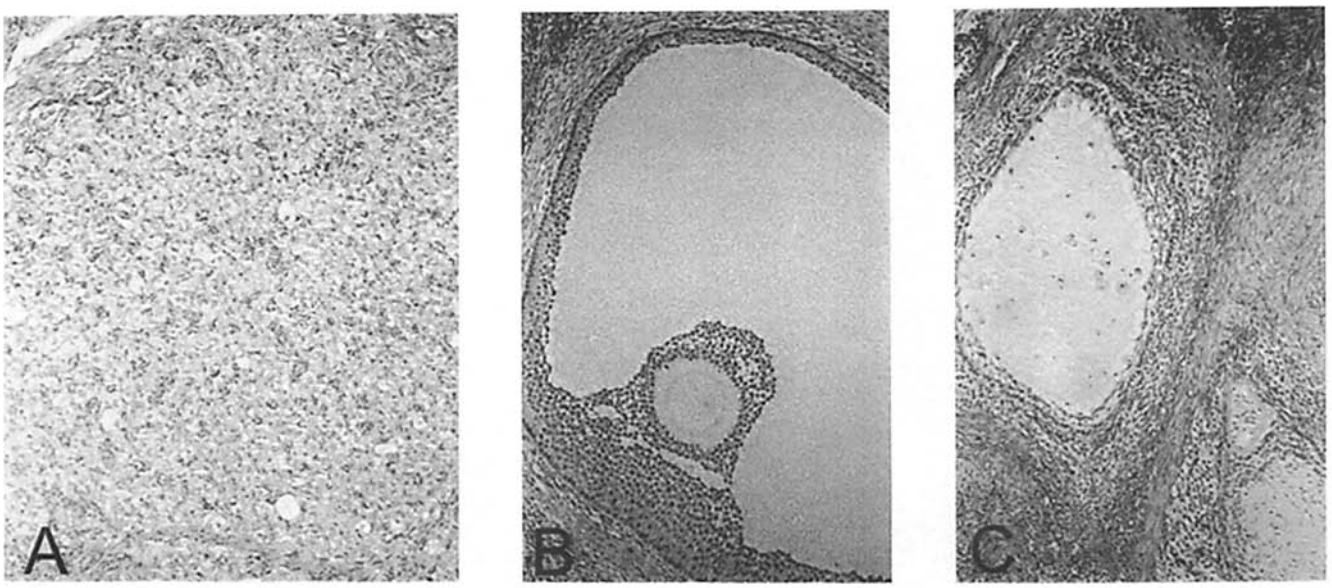

FIG. 7. Several ovarian structures were present in addition to fresh CL and CH. **A)** Regressing CL from a previous cycle. Vasculature still is mature and relatively prominent. Many cells have large lipid vacuoles (×100). **B)** Tertiary follicle. Most ovaries contained tertiary follicles of varying sizes (×100). **C)** Tertiary follicle undergoing atresia as noted by collapse and fibrosis of the lumen, hypertrophy of theca interna, and absence of ovum (×100).

pseudostratification. Glandular epithelial cells remained cuboidal, but the N/C ratio was 1:3. The luminal epithelium had the same infolding and pseudostratification observed at 64 h, and the cells remained low cuboidal-shaped, but the N/C ratio increased slightly to 1:0.8. Mitotic rates in glandular and luminal epithelia remained low (0–1/HPF).

At 100 h (Fig. 3, E and F), epithelium height had increased in the deep glands with most cells having N/C ratios of 1:4. Correspondingly, epithelial cytoplasmic vacuolation had increased, presumably reflecting increased secretory activity. Glandular epithelial cells still were pseudostratified and undergoing mitosis (1–2/HPF). The luminal epithelium continued to have only slight infoldings. The N/C ratio was 1:0.8, with the cells pseudostratified with nuclear overlap.

At 124 h (Fig. 4, A and B), there was notable increased evenness of the glandular epithelium and an overall thickening of the endometrium compared to what was observed at 100 h. Glands were straighter and were lined by tall columnar epithelium with increased cytoplasmic vacuolation. Epithelial height and degree of vacuolation were greater in the deep than in the superficial glands. Still, the N/C ratio was ~1:4, and mitoses were evident in many glandular cells (1–3/HPF). Approximately half of the glandular epithelial cells had a subnuclear glycogen vacuole. Depth and number of intraluminal infoldings of the surface epithelium had increased appreciably, and there was less pseudostratification of luminal epithelial cells (N/C ratio, 1:1).

At 148 h (Fig. 4, C and D), glands were now highly elongated with an even epithelium composed of tall columnar cells with abundant, vacuolated cytoplasm. The N/C ratio ranged from 1:6 to 1:8, and almost all cells contained a subnuclear glycogen vacuole. The luminal epithelium had abundant, even surface infoldings that were deeper than at 124 h. The N/C ratio was approximately 1:4, and the low columnar cells remained stacked. Cells had fine microvilli, and cell nuclei were separated from the basal membrane by a subnuclear vacuole. Mitoses were rare.

Histological Changes of the Ovary and CL

At 64 h (Fig. 5, A and B), almost all ovaries had CH with varying amounts of blood within the central cavity. Margins of the CH were infolded from collapse of the Graafian follicle. There was early vacuolation (luteinization) of granulosa and theca interna cell cytoplasm that was most prominent in the periphery (presumably thecal cells). The luteal cell N/C was ~1:3. Most cells were fusiform with polar orientation toward the CH central cavity. Vasculature was very prominent and dilated. Capillaries ran perpendicular to and extended from the theca externa to the CH lumen. Orientation of the luteal cells and vasculature suggested growth inward toward the central cavity.

At 76 h (Fig. 5, C and D), most ovaries still had CH and/or CL with central cavitation, although many CL had immature fibrous tissue within the central cavity. Luteal cells remained fusiform and oriented perpendicular to the CL margins. Peripheral cells (of presumed thecal origin) had slightly more cytoplasmic vacuolation than at 64 h and an N/C ratio of 1:3 to 1:4.

At 100 h (Fig. 5, E and F), only a few ovaries had CH, and most contained at least one CL with an irregular border and a persistent central cavity, although generally these CL had some fibrosis within the cavity. Luteal cells remained

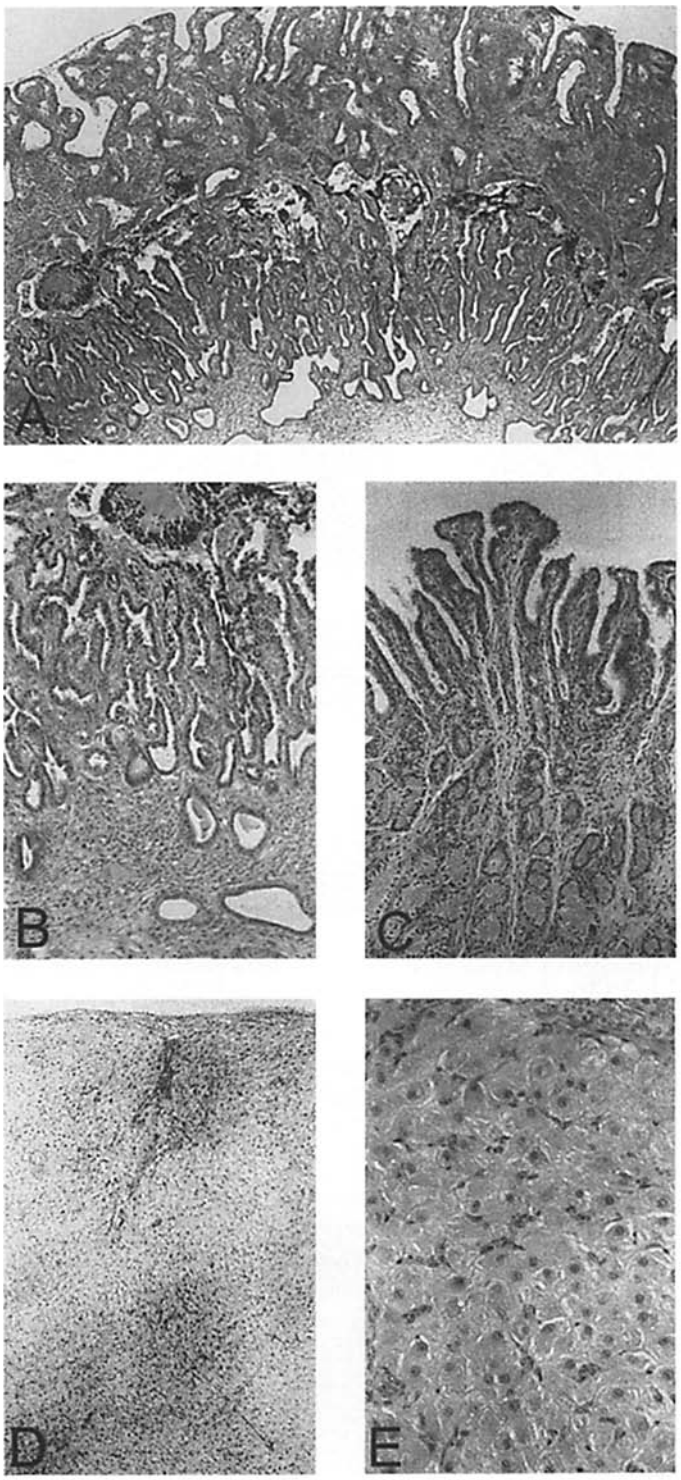

FIG. 8. Histomorphology of the uterine endometrium, CL, and placenta of naturally estrous, mated cats at 480 h after first copulation. **A**) The upper third of the endometrium is in contact with the fetal placenta and is eroded. Large syncytia are present (×34). **B**) Glands beneath the placenta are complex and branching. The deepest glands are dilated and contain abundant proteinaceous fluid (×84). **C**) Between two closely apposed placentas are deep infoldings resulting in the appearance of papillary fronds of superficial endometrium extending into the uterine lumen (×84). **D**) CL are large and expansile with even, rounded edges and no central cavities (×84). **E**) All cells are polygonal with abundant, finely vacuolated cytoplasm (×168).

predominantly fusiform, but more polygonal luteal cells with cytoplasmic vacuolation were noted at the CL periphery. The N/C ratio of luteal cells remained at 1:3 to 1:4, but vasculature was less prominent than at 76 h.

At 124 h (Fig. 6, A and B), CH were no longer evident. Degree of CL cavitation varied considerably within and among queens; some CL maintained large central cavities, whereas others were filled by central fibrosis or had no cavity. CL margins were more rounded and luteal cells more consistently polygonal with more cytoplasmic vacuolation than at 100 h. Vasculature was congested but was no longer oriented perpendicular to the CL margin.

At 148 h (Fig. 6, C and D), CL had expanded and rounded margins. Most luteal cells were polygonal with abundant vacuolation of the cytoplasm. Vasculature was less prominent than at 124 h. At least one CL in four of six queens still had a central cavity.

In addition to these fresh CH/CL, regressing CL (aged, from previous cycles) were noted in some ovaries in all groups (Fig. 7A). These CL were small and had irregular margins. Cells had low N/C ratios (1:3), yet the vasculature still was mature and relatively prominent. Many cells had large lipid vacuoles. Most ovaries also contained tertiary follicles of varying sizes (Fig. 7B), some of which were undergoing early atresia (Fig. 7C) as noted by collapse, luminal fibrosis, hypertrophy of theca interna cells, and absence of an ovum.

Histology of the Uterus, CL, and Placenta during Early Implantation

At 480 h (Fig. 8, A and B), the upper third of the endometrium in contact with the fetal placenta was eroded. Large syncytia were present at this junction that may have been endometrial or trophoblastic in origin. Glands beneath the placenta were highly complex with branching and fusion of papillary infoldings. The deepest glands were dilated and contained abundant proteinaceous fluid. The N/C ratio in most glands was 1:1.

The interplacental endometrium had differing morphologies. Between two closely apposed placentas, there were deep infoldings that extended into the upper 50% of the endometrium, resulting in the appearance of papillary fronds of superficial endometrium extending into the uterine lumen (Fig. 8C). Glandular lumens in the deep endometrium contained proteinaceous fluid and expressed an N/C ratio of 1:5 for the low columnar cells with a microvillar surface. The second morphology was observed distal to placental sites. Here, the upper 50% of the endometrium had complex papillary extensions. Cells were cuboidal with an N/C ratio of ~1:0.5 and a microvillous surface. The deep half of the endometrium contained convoluted, simple-to-slightly-branched glands with minimal luminal protein. The N/C ratio of deep gland epithelium was approximately 1:4, and these cells had abundant vacuolation.

The CL (Fig. 8, D and E) were large and expansile with even, rounded edges and no central cavities. All luteal cells were polygonal and had abundant, finely vacuolated cytoplasm. Tertiary follicles were present in all ovaries.

Histological Characteristics of Queens Failing to Produce Good-Quality Embryos or Implantations

Three queens in the 64-h group failed to produce good-quality embryos. Of these, one had histological characteristics that mimicked those of the five queens producing good-quality embryos. A second queen was similar in all characteristics except that she expressed severe endometrial hyperplasia. The third queen also had moderate endometrial hyperplasia but additionally had tall columnar cells in the deep endometrial glands and increased endometrial and luteal cell cytoplasmic vacuolation (resembling a typical cat at 100 h after first copulation).

Of 11 queens ovariohysterectomized at 76 h after first copulation, 6 failed to produce good-quality embryos. Five of these exhibited no unusual histological characteristics that might explain the poor embryo production. However, one queen producing no embryos had mild endometrial hyperplasia and a notable lack of luteal cell cytoplasmic vacuolation.

At 100 h, of the three queens failing to produce good embryos, two had histological characteristics similar to those of the five queens that produced high-quality embryos. CL histology of the third queen revealed only slight luteal cell vacuolation, similar to that observed at 64 h after first copulation.

Two queens ovariohysterectomized at 124 h failed to produce good embryos and, histologically, appeared similar to the other five in this interval group. There were no unusual uterine or ovarian histological characteristics.

Only one queen at 148 h failed to produce good embryos, but the uterine and ovarian histology was similar to that of the other five queens in this group.

All eight queens ovariohysterectomized at 480 h had at least two implantation sites. Two queens were classified as having poor embryo survival because their ovaries contained a total of seven and six CL, but each had only two implantation sites. Histologically, both females appeared similar to the other six queens in the interval group.

DISCUSSION

We have documented, in detail, the histological events occurring at the level of the uterine endometrium and CL during early embryogenesis in the domestic cat. Within most interval groups, females had similar histological characteristics, with modest and gradual variation occurring over time as a result of natural developmental processes. Only within the 100- and 124-h groups was there slight variation among queens, especially in the context of endometrial cell height and vacuolation and luteal cell shape and vacuolation. In contrast, Dawson and Kosters [16] reported considerable variation in the rate of endometrium modification during the first 5 days postcopulation. It is likely that this variability was due to uncontrolled factors including age and parity of queens and the time of year (season) when queens were mated. Our study eliminated these potentially confounding factors by using a select population of queens that were young, mostly nulliparous adults maintained under a controlled light cycle year-round. Still, the most probable cause for the variation observed by Dawson and Kosters [16] was the random mating of queens on various days of estrus. It now is known that the day of estrus and number of copulations markedly influence the magnitude of the LH surge and ovulation success [21–23]. Therefore, a random mating regimen is likely to result in asynchronous ovulation among queens. In our study, queens were checked daily for behavioral estrus so that all controlled matings occurred on Days 2 and 3 of sexual receptivity, thereby achieving relatively synchronous ovulation. Additionally, the same two males were used for all breedings, and each female was mated to both males, thereby avoiding any potential male effect on ovulation, fertilization, or embryo quality. Finally, to ensure that the histological assessments were accurately illustrating normal kinetics, embryo quality was used as the criterion for choosing five females representing each time interval group. Validation of successful embryogenesis has never been reported in other histological evaluations of the uterus or CL in the early pregnant cat. Data from queens producing unfertilized, degenerate, retarded, or no embryos were not used for determining the typical changes at each time interval. Rather, these data were compared to the normal data for each interval to identify any relationship between abnormal histology and failed embryogenesis.

Because of the close association between endocrine changes and histological modifications of the uterus and CL, it is appropriate that tissue cellular structure be discussed in the context of hormonal dynamics. In the cat, circulating estradiol-17β remains elevated for 2–4 days postcoitum [19, 24, 25]. Therefore, at 64 h postcopulation, the uterus still reflected an estrogen influence characterized by tortuous endometrial glands and dense cellular pseudostratification, similar to that reported for the estrous cat [16]. The uterus responds to increasing circulating progesterone with enhanced proliferation and secretory activity [24]. These changes occurred slowly at first, with only a few discernible changes at 76 h, more notable changes at 100 h, and very apparent changes by 124 h after the first copulation. By then, there was an evenness of the glandular epithelium, a thickening of the endometrium, and an increase in cytoplasmic vacuolation of epithelial cells and within the deep epithelial glands. These results are in accordance both with what is already known about the timing of ovulation and

normal onset of progesterone secretion in the naturally mated queen, and with endocrine data for queens in the present studies [20]. When subjected to the mating regimen described here, cats ovulate 30–36 h after the first copulation [15], and endocrine profiles demonstrate slight increases in serum progesterone as early as 1–2 days after ovulation (3–4 days postcopulation) [19, 26].

Histological changes of the CL also are in accordance with endocrine dynamics of the mated queen and support the chronology of uterine modifications observed in this study. As early as 64 h after first copulation, vacuolation (luteinization) of granulosa and theca interna cell cytoplasm was evident within fresh CH, indicating onset of progesterone secretion. CL vacuolation increased slightly by 76 h and then became more apparent by 100 and 124 h as cells changed from a fusiform to a polygonal morphology. By 148 h after first copulation, most luteal cells were polygonal with abundant cytoplasmic vacuolation. These changes correspond perfectly with endocrine data for these queens [20] and also support previous data demonstrating a very rapid increase in serum progesterone beginning on Day 4 after the first copulation [19, 25]. Interestingly, even at 64 h, almost all ovaries contained CH with varying amounts of blood within the central cavity. CH generally were transformed by 100 h after copulation onset. These observations are different from those reported by Dawson [17], who examined ruptured follicles at 29–36 h postcopulation and reported no hemorrhage into the follicular lumen.

This report provides the necessary database for dating (within 24–48 h) the reproductive tract of queens during early pregnancy. It is unlikely that histological dating can be more accurate. In humans, a more comprehensive system has been established for determining the day of the reproductive cycle, and, even with data from approximately 8000 endometrial biopsies, dating is only 86% accurate ($\pm$ 2 days) [27]. It is noteworthy that the most significant histological changes in both uterine endometrium and CL occurred 124–148 h after the first copulation in the present study. This coincides with embryo compaction and migration from the oviducts into the uterine cornuae [15]. It appears that this time interval represents one of the most important, dynamic periods during early pregnancy in the cat, a time marked by significant, simultaneous changes in histological, endocrine, and embryonic events.

Uteri of the eight queens ovariohysterectomized at 480 h revealed no histological evidence of implantation failure and only one fetal absorption. These results were not surprising in light of our previous observation that the number of good-quality preimplantation embryos recovered from the uterus at 64–148 h postcopulation was similar to the number of implantation sites at 480 h [15]. Those data suggest that most embryo loss is occurring before implantation, and our histological data support this assertion. In this respect, the cat appears different from the pig or the human, two species that are affected by a high rate of embryo loss in the periimplantation interval [28, 29].

Of the 15 queens failing to produce good-quality embryos, only four exhibited unusual histological characteristics, and three of these expressed some degree of endometrial hyperplasia. Therefore, aberrant uterine or CL histology does not appear useful for predicting embryo quality in the naturally estrous, mated queen. However, because cat embryos remain in the oviducts for 124 h before migrating into the uterine horns, it is possible that oviductal histology more accurately reflects early embryo quality. For this study, oviducts were flushed with medium several times, massaged, and sometimes dissected to recover embryos, rendering them useless for histological assessment. Even so, we expect that conditions in the maternal milieu affecting oviductal histology would similarly be reflected in uterine histology. It is important to note that five of the 48 queens in the study demonstrated signs of endometrial hyperplasia. Of these, three (one each with mild, moderate, and severe hyperplasia) failed to produce good-quality embryos, while good embryos were recovered from the remaining two (both with only mild hyperplasia). This suggested that queens with endometrial hyperplasia were less likely to produce good-quality preimplantation embryos. However, atypical uterine or CL histology is not the primary factor associated with naturally occurring embryo loss in this species.

We examined the uterine endometrium and CL only for histological changes. It is possible that a detailed ultrastructural or immunohistochemical examination of uterine or CL tissues would reveal more subtle characteristics that are indicative of embryo quality. For example, uterine cell cytoplasmic or nuclear hormone receptor concentrations deserve some attention. There are considerable data available describing the impact of estradiol, progesterone, and steroidal interactions on cell receptor concentrations in the cat uterus and CL [24, 30–32]. Additionally, uterine secretions should be considered potentially useful for determining the status of the uterine milieu during early pregnancy in the cat. Two recently identified endometrial proteins possibly important for implantation in the cat are cathepsin-L [33–35] and insulin-like growth factor binding protein-1 [36]. To-date, no studies have been conducted that compare these uterine secretions or hormone receptor concentrations with embryo quality or between naturally estrous and gonadotropin-treated queens.

Although no relationship was found between abnormal uterine or CL histology and poor embryo quality in the naturally estrous, mated cat, this normative database will be important in future studies for comparing histological changes in gonadotropin-treated queens used for assisted reproduction. Already, results from one study suggest slight histological differences between CL from gonadotropin-treated cats subjected to follicular aspiration and those from

naturally mated queens [37]. More comparative studies like these are needed to determine why success rates are not higher after AI or embryo transfer in felids treated with exogenous hormones.

In summary, we have documented uterine and CL histological changes at very specific intervals during successfully progressing early (preimplantation) pregnancy in the cat. In so doing, we have produced a large-scale, normative database useful for dating the endometrium or CL of early pregnancy in this species. We also have established that aberrant histological changes do not appear to be associated with failure of the naturally estrous, mated queen to produce good-quality embryos. However, we cannot exclude the possibility that abnormal endometrial dynamics and/or aberrant CL function contribute to poor embryo survival in the gonadotropin-treated queen following AI or embryo transfer. In this context, data reported here will serve an important comparative function for future studies that examine uterine and CL histology of gonadotropin-treated felids.

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