

Effects of Equine Chorionic Gonadotropin, Human Chorionic Gonadotropin, and Laparoscopic Artificial Insemination on Embryo, Endocrine, and Luteal Characteristics in the Domestic Cat¹

Terri L. Roth,² Barbara A. Wolfe, Julie A. Long, JoGayle Howard, and David E. Wildt

Conservation & Research Center, National Zoological Park, Smithsonian Institution, Front Royal, Virginia 22630

ABSTRACT

The effects of gonadotropin treatment and laparoscopic artificial insemination (AI) on embryo quality, serum progesterone and estradiol concentrations, and luteal progesterone content were examined in the domestic cat. These data were compared to similar historical data reported for naturally estrual, mated queens. All queens in this study ($n = 32$) were treated with eCG followed by 1) natural breeding (eCG-NB), 2) NB and hCG (eCG-NB-hCG), 3) NB and a sham AI procedure (eCG-NB-sham AI), or 4) hCG and actual AI (eCG-hCG-AI). Queens ovulating in response to treatment were ovariectomized, and oviducts and uteri were flushed to collect embryos. Ovarian structures were recorded, corpora lutea (CL) were excised and evaluated for progesterone content, and serum was analyzed for estradiol-17 β and progesterone. Follicle and CL numbers ranged from 0 to 28 and 2 to 42 per cat, respectively, and treatment means did not differ ($p \geq 0.05$) among groups. Embryos were recovered from oviducts and uterine horns in all treatment groups, and recovery ranged from 60–96%. Mean embryo number per queen ranged from 8.2 ± 2.6 to 23.2 ± 3.8 and did not differ ($p \geq 0.05$) among groups. However, the proportions of unfertilized oocytes were greater ($p < 0.05$) for groups treated with hCG and/or artificially inseminated, and the proportion of blastocysts produced (31 of 107, 29.0%) was lower ($p < 0.05$) in the eCG-hCG-AI group than for any other treatment (range, 59 of 116 [50.9%] to 67 of 116 [57.8%]). Not all queens in each group produced good-quality embryos (eCG-NB, 5 of 5; eCG-NB-hCG, 5 of 8; eCG-NB-sham AI, 2 of 5; and eCG-hCG-AI, 3 of 6). Serum progesterone and estradiol-17 β , and total luteal progesterone per ovary did not differ ($p \geq 0.05$) among treatments. Compared to historical controls (naturally estrual, mated queens), eCG-NB queens produced > 4 times as many good-quality embryos and blastocysts. Similarly, eCG-hCG-AI-treated queens produced > 4 times the number of oocytes and embryos, although a high proportion of these were poor quality and did not develop to blastocysts. Together, these results indicate that queens treated with eCG are capable of consistently producing many good-quality embryos, at least half of which develop to blastocysts in culture. These data support the use of eCG in felids and suggest that other factors are responsible for reduced pregnancy success and small litter sizes following assisted reproduction.

INTRODUCTION

In 1992, domestic cats treated with eCG and hCG gave birth to kittens following laparoscopic artificial insemination (AI) [1]. Since then, similar protocols have been ap-

plied to several nondomestic felid species with the goal of eventually using assisted reproduction to facilitate conserving and managing endangered populations. On numerous occasions, these efforts have been rewarded by the birth of offspring [2–7]; however, pregnancies are not consistently produced by AI in most felid species. The cheetah and domestic cat are exceptions, with approximately 50% becoming pregnant after AI [1, 8]. However, resulting litters are smaller than those typically produced by naturally estrual, mated females, despite numerous fresh ovulation sites on the ovaries at AI.

In vitro fertilization (IVF) has proven useful for studying gamete function and the influence of exogenous gonadotropins among felids. IVF embryos have been produced using oocytes collected from gonadotropin-treated females of several felid species [9–14], and offspring occasionally have resulted after the transfer of these embryos [9, 11, 12, 14]. However, again, pregnancy rates are low following IVF and embryo transfer, and when pregnancies do result, litter sizes are small despite transferral of many embryos into recipients [9, 11, 12, 14]. These reports suggest that IVF-generated embryos are not all developmentally competent, an assertion partially supported by the failure of most IVF embryos to develop to the blastocyst stage in vitro [15–18]. This in vitro developmental block has not been overcome by modifying the culture system [15, 16, 18, 19] and is not demonstrated by embryos collected from naturally estrual, mated queens [17]. Together, AI and IVF/embryo transfer results have led us to question the quality of oocytes produced by felids treated with exogenous gonadotropins.

Follicular development can be stimulated in cats with exogenous FSH or eCG [9, 12, 20, 21], and ovulation can be induced with GnRH or hCG [22]. For nondomestic felids, eCG followed by hCG has become the regimen of choice to avoid animal stress associated with multiple FSH injections. Of concern, however, are reports that eCG treatment is associated with abnormal oocyte production [23–25], poor-quality embryos [26–28], and/or low pregnancy success [29, 30] in several domestic and laboratory species. Recent data in cattle suggest that single FSH injections, administered subcutaneously, are effective for inducing multiple ovulations [31, 32]. Therefore, FSH could perhaps substitute for eCG in felids, if in fact eCG is adversely affecting oocyte quality and/or the maternal milieu during early embryo development. However, eCG is not the only factor potentially affecting oocyte quality and embryo development. For example, high hCG dosages are associated with increased oocyte degeneration and decreased IVF success in the domestic cat [9]. It also is possible that anesthesia and/or the laparoscopic procedure itself may interfere with successful fertilization and embryo development in vivo.

It now is possible to study the effects of exogenous gonadotropins and laparoscopic AI on embryo, endocrine, lu-

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²Correspondence: Terri L. Roth, Center for Research of Endangered Wildlife, Cincinnati Zoo and Botanical Garden, 3400 Vine Street, Cincinnati, OH 45220. FAX: (513) 569–8213.

teal, and uterine characteristics in the domestic cat because comparative data are available in the naturally estrual, mated queen [17, 33–35]. The primary objective of this study was to determine the proportion of developmentally competent preimplantation embryos produced by eCG-treated cats. Additionally, the effects of eCG alone, the combined eCG-hCG regimen, and laparoscopic AI on embryo quality, circulating reproductive steroids, and luteal progesterone content were determined. These new data were compared to similar historical data reported for the naturally estrual, mated queen.

MATERIALS AND METHODS

Animals

Adult (1–3 yr old) female domestic cats ($n = 32$) were housed in stainless steel cages (1–2 cats per cage) or in communal pens (2–10 cats per pen). Two proven breeder males used in previous studies [17, 33–35] were housed singly in separate pens. All cats were provided a commercial feline diet (Purina Cat Chow; Ralston-Purina Co., St. Louis, MO) and water ad libitum and were maintained in a controlled ambient environment under artificial fluorescent illumination (12L:12D daily) during the ~2-yr study period.

Treatment Groups

Individual queens were monitored daily for signs of behavioral estrus [17, 34]. Females not showing estrus for ≥ 3 consecutive days were given an i.m. injection of 100 IU eCG (Sigma Chemical Company, St. Louis, MO) and were assigned randomly to one of four treatments: 1) natural breeding 80 h later (eCG-NB), 2) NB 79 h later with 75 IU hCG (Sigma) injected i.m. 1 h after the first copulation (eCG-NB-hCG), 3) NB 80 h later and sham AI (see below) 36–38 h after the first copulation (eCG-NB-sham AI), and 4) hCG 80 h later followed by AI 36–38 h after hCG (eCG-hCG-AI). To minimize the number of animals in the study, historical data reported for naturally estrual, mated queens served as a nontreated control data set [17, 33–35].

Natural Breeding and AI

The natural breeding regimen was one that has proven highly effective for inducing ovulation and producing embryos in naturally estrual cats [17, 33]. Each queen was mated three times a day at 3-h intervals for 2 days with two proven breeder males on a rotating basis. Laparoscopic AI was conducted according to previously described methods [1]. Briefly, 36–38 h after hCG injection or first copulation, queens were induced into a surgical plane of anesthesia with a ketamine hydrochloride (Vetalar; Parke-Davis, Morris Plains, NJ) plus acepromazine maleate (Ayerst Laboratories, Rouses Point, NY) mixture (10.0 mg/kg and 0.2 mg/kg BW, respectively, i.m.). Anesthesia was maintained by delivering isoflurane gas/oxygen via a face mask. A 7-mm laparoscope was inserted ~4 cm cranial to the umbilicus and used to examine ovaries for the presence of fresh corpora lutea (CL). Ovulating females were inseminated by inserting a 20-g indwelling catheter (Sherwood Medical, Tullamore, Ireland) percutaneously into the cranial portion of each uterine horn and depositing washed sperm (100 μ l/horn) directly into the uterine lumen [1, 2, 20]. Sham AI procedures were conducted in the same fashion except that Ham's F-10 medium (HF10) containing no sper-

matozoa was deposited into the uterine lumen (100 μ l/horn).

Semen Collection and Processing

The two males used for breeding also served as sperm donors for AI. These cats were induced into a surgical plane of anesthesia with tiletamine/zolazepan (Telazol; A.H. Robins Company, Richmond, VA; ~4 mg/kg BW, i.m.), and semen was collected by electroejaculation [36]. Semen was diluted with an equal volume of HF10 and centrifuged (150 $\times g$; 8 min). After removal of the supernatant, the sperm pellet was resuspended in 210 μ l of HF10 and maintained in room atmosphere protected from light for 1–3 h until used for AI. Immediately before AI, a 5- μ l aliquot was examined for percentage motile spermatozoa. Using this value along with the sperm concentration (determined using a hemacytometer) and sample volume, the total number of motile spermatozoa inseminated was calculated.

Ovariohysterectomy and Embryo Collection and Culture

A total of 26 ovulating queens were ovariohysterectomized 144–148 h after the first copulation or hCG injection at laparotomy under a surgical plane of anesthesia (as described above for AI). Within 30 min of excision, oviducts and uterine horns were flushed repeatedly with 1–5 ml of warm (38°C) HF10 supplemented with 5% fetal calf serum (Irvine Scientific, Princeton, NJ), 0.011 mg/ml pyruvate (Sigma), and 0.284 mg/ml glutamine (Sigma), and the recovered fluid was examined for embryos and oocytes. Uncleaved oocytes were classified as unfertilized and were discarded. Embryos were washed through two dishes containing 2 ml of HF10, 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), and cultured in 100- μ l HF10 drops under oil in a humidified incubator (38°C; 5% CO₂ and air). In the naturally estrual, mated cat, in vivo-produced embryos typically develop to the morula stage by 144–148 h after the first copulation [33]. Therefore, only embryos ≥ 9 –16 cells at recovery and classified as grade 1 or 2 were considered "good quality." Embryos were assessed every 24 h for up to 8 days (or until degeneration), and cleavage stage and blastocyst formation were recorded.

Serum Estradiol-17 β and Progesterone Concentration

Blood samples were collected via jugular venipuncture from anesthetized queens immediately before laparoscopy, and recovered serum was stored at -80°C until analyzed. Estradiol-17 β and progesterone concentrations were measured in unextracted serum using solid-phase ¹²⁵I RIA kits (Coat-a-Count; Diagnostic Products Corporation, Los Angeles, CA). This assay has been standardized and tested thoroughly for domestic cat serum [34]. All serum samples were evaluated simultaneously in a single RIA for each hormone. Assay sensitivities (based on 90% of maximum binding) for estradiol-17 β and progesterone were 5 pg/ml and 0.05 ng/ml, respectively. The intraassay coefficients of variation were $< 10\%$ for both assays.

Uterine and Ovarian Tissue Processing

For each queen, both ovaries were examined for the total number of follicles (≥ 2 mm diameter) and CL. Arbitrarily,

TABLE 1. Ovarian structures and embryo location and recovery from eCG-treated queens subjected to NB and/or AI, with or without hCG administration.

Treatment group	Cats (n)	Corpora lutea*	Follicles (≥ 2 mm)*	No. of cats with embryos in the			Recovery (%)†
				Oviducts	Uterus	Oviducts and uterus	
NB‡	6	6.5 \pm 1.1	2.6 \pm 1.6	1	5	0	56
eCG-NB	5	24.2 \pm 3.0	6.4 \pm 5.5	2	2	1	96
eCG-NB-hCG	8	20.9 \pm 1.7	7.5 \pm 1.6	2	3	3	70
eCG-NB-sham AI	5	13.6 \pm 4.1	4.4 \pm 2.5	1	3	1	60
eCG-hCG-AI	6	27.2 \pm 5.6	8.7 \pm 1.5	2	2	2	66

* Values are means \pm SEM.

† Recovery (%) = total number of oocytes + embryos/CL number \times 100.

‡ Historical data on naturally estrual, mated queens adapted from previous reports [17, 33]; data not included in statistical analyses.

the right ovary was chosen for CL progesterone content analysis. CL were excised from the ovary and bisected. Each hemi-CL was weighed, snap-frozen in liquid nitrogen, and stored at -80°C until analyzed. To quantify luteal progesterone concentrations (ng/mg), hemi-CL were processed according to a previously described protocol [34]. Briefly, CL were individually homogenized in 1 ml PBS. Homogenates were diluted with ethanol to 5 ml total volume, boiled for 20 min, and centrifuged ($500 \times g$, 20 min). After the supernatants were decanted, residual luteal pellets were extracted a second time in ethanol (2 ml) and centrifuged. The supernatants were combined and dried under air. Finally, extracts were resuspended in 1 ml methanol, diluted 1:400 with PBS, and stored at -80°C . Aliquots (100 μl) of these diluted extracts were analyzed using a ^{125}I solid-phase RIA as described for serum. This assay has been validated for quantifying cat luteal progesterone [34].

Uterine endometrial hyperplasia is associated with poor embryo quality in domestic cats [35]. To ensure that it was not a factor associated with poor embryo quality in this study, uteri from all queens were examined histologically. After each uterine horn was flushed with warm (38°C), sterile HF10 to recover embryos, uteri were placed in 50-ml conical tubes containing 10% buffered formalin and later processed for histological evaluation. Two transverse sections were sampled from each uterine horn, embedded in paraffin, sectioned at 7 μm , and stained with hematoxylin and eosin. Data for queens diagnosed with endometrial hyperplasia were discarded from the data set.

Statistical Analysis

Average values are reported as means \pm SEM. Numbers of ovarian structures (follicles and CL), oocytes and embryos collected, blastocysts produced, and all endocrine data were compared among treatment groups by analysis of variance and least significant difference mean comparisons [37]. The proportions of unfertilized oocytes, good-quality embryos, and blastocysts produced per total embryos collected for each treatment group were compared by chi-square. Correlation coefficients were calculated between follicle number and serum estradiol-17 β concentrations. Additionally, CL number was correlated with total luteal progesterone content and serum progesterone concentrations. Finally, correlation coefficients were calculated for serum endocrine and luteal traits in cats producing good-versus poor-quality embryos.

RESULTS

Breeding, Ovarian Characteristics, and Embryo Recovery

Of the original 32 queens, eight were excluded from further analysis for the following reasons: five estrual queens

scheduled for natural breeding refused to mate with males; one failed to ovulate; one was diagnosed histologically with severe endometrial hyperplasia; and the eighth was inseminated with $< 2 \times 10^6$ motile spermatozoa, far fewer than the number received by all other cats in the eCG-hCG-AI group (range, 8–14 $\times 10^6$).

Therefore, data were analyzed from a total of 24 queens with a minimum of five per treatment group as follows: eCG-NB, $n = 5$; eCG-NB-hCG, $n = 8$; eCG-NB-sham AI, $n = 5$; eCG-hCG-AI, $n = 6$. For these groups, numbers of follicles (≥ 2 mm) and CL at ovariohysterectomy ranged from 0 to 28 and 2 to 42 per cat, respectively, and treatment means did not differ ($p \geq 0.05$) among groups (Table 1). Embryo recovery (total number of oocytes plus embryos divided by CL number and multiplied by 100) ranged from 60% to 96%, and embryos were recovered from the oviducts and uterine horns in all treatment groups (Table 1). Embryo recovery and location were similar to those reported for naturally estrual, mated queens in a previous study [33].

Embryo Number, Quality, and Development to Blastocysts

Mean numbers of oocytes plus embryos recovered per female did not differ ($p \geq 0.05$) among treatment groups (Table 2), primarily because of extreme variation among cats within groups. For example, naturally bred eCG-treated cats produced from 15 to 36 embryos and oocytes; cats treated with the eCG-hCG combined regimen and artificially inseminated produced from 4 to 40 embryos and oocytes. For all treatment groups, the number of embryos plus oocytes recovered was more than twice that reported for naturally estrual, mated queens [33].

Proportions of unfertilized oocytes were greater ($p < 0.05$) for cats given hCG and/or subjected to AI than for those naturally bred (Fig. 1). The proportion of degenerate embryos was greater ($p < 0.05$) in cats artificially inseminated after eCG-hCG administration than in those that were naturally bred after eCG treatment (Fig. 1). Cats in the hCG-AI group also produced the fewest ($p < 0.05$) morulae (Fig. 1). Furthermore, the proportions of good-quality embryos produced by eCG-treated, naturally bred queens (with or without hCG) were greater ($p < 0.05$) than for cats given eCG and hCG and then artificially inseminated (Table 2). Although oocytes and/or embryos were collected from all 24 eCG-treated queens, not all cats in each group produced good-quality embryos except those that were naturally bred without hCG administration (5 of 5). In contrast, 5 of 8, 2 of 5, and 3 of 6 cats in the eCG-NB-hCG, eCG-NB-sham AI, and eCG-hCG-AI groups, respectively, produced good-quality embryos. Mean numbers

TABLE 2. Embryo quality and development after collection from eCG-treated queens subjected to NB and/or AI, with or without hCG administration.

Treatment group	No. embryos recovered/cat*	Good-quality embryos/total collected (%)†	No. embryos developing to blastocysts*	Blastocysts formed/total embryos collected (%)
NB*	3.7 ± 0.7	18/22 (81.8)	2.2 ± 0.7	13/22 (59.1)
eCG-NB	23.2 ± 3.8	81/116 (69.8) ^a	11.8 ± 1.8	59/116 (50.9) ^a
eCG-NB-hCG	14.5 ± 3.3	73/116 (62.9) ^a	8.4 ± 3.2	67/116 (57.8) ^a
eCG-NB-sham AI	8.2 ± 2.6	22/41 (53.7) ^{a,b}	4.4 ± 2.9	22/41 (53.7) ^a
eCG-hCG-AI	17.8 ± 5.6	37/107 (34.6) ^b	4.8 ± 3.2	31/107 (29.0) ^b

* Values are means ± SEM.

† Only embryos ≥9–16 cells at recovery and classified as grade 1 or 2 were assigned “good-quality” status.

* Historical data on naturally estrual, mated queens adapted from previous reports [17, 33]; data not included in statistical analyses.

^{a,b} Within columns, values with different superscripts differ ($p < 0.05$).

of embryos developing to blastocysts after collection did not differ among groups ($p < 0.05$), but proportionally fewer ($p < 0.05$) blastocysts were produced in the eCG-hCG-AI group compared to any other group (Table 2). Still, even in this group, the mean number of blastocysts was twice that reported for naturally estrual, mated controls [17, 33].

Endocrine Characteristics

Serum progesterone and estradiol-17 β concentrations did not differ ($p \geq 0.05$) among treatments (Table 3), again largely due to variation among individuals, and were not unlike those reported previously for naturally estrual, mated queens [34]. Luteal progesterone concentration (ng/mg) was less ($p < 0.05$) for cats naturally bred and given hCG than for the other three groups. However, luteal mass also was high in these individuals, so the total luteal progesterone per ovary did not differ ($p \geq 0.05$) among groups. When data were analyzed for cats across all treatments, CL number was positively correlated ($p < 0.01$) with serum progesterone concentration ($r = 0.77$) and total luteal progesterone ($r = 0.81$). Similarly, serum progesterone concentration was highly correlated ($p < 0.01$) with total luteal progesterone ($r = 0.89$). There was no significant relationship ($p \geq 0.05$) between serum estradiol-17 β concentration

and follicle number. Across all groups, there were no correlations ($p \geq 0.05$) between serum endocrine or luteal traits and queens producing poor-quality embryos.

DISCUSSION

The general hypothesis of this study was that the exogenous gonadotropin eCG is responsible for recruiting poor-quality oocytes and/or producing poor-quality embryos that ultimately result in reduced pregnancy success and small litter sizes following assisted reproduction in the domestic cat. Results clearly allowed rejecting this hypothesis. Queens that were treated with eCG and naturally bred consistently produced many good-quality embryos, at least half of which were capable of developing into blastocysts in vitro. These findings were comparable to those reported in an extensive recent study that examined embryo development and quality in naturally estrual cats (no eCG) mated using the same copulatory regimen described here [17, 33–35].

Mean CL and embryo numbers for all treatment groups were at least twice those reported for naturally estrual, mated queens ovariectomized at the same stage of pregnancy in a previous study [33]. Additionally, more blastocysts were produced in all treatment groups as compared to the number reported for the naturally estrual, mated cat

FIG. 1. Within treatment groups, percentage of recovered embryos at each developmental stage. Different superscripts denote differences among treatments within developmental stage ($p < 0.05$)

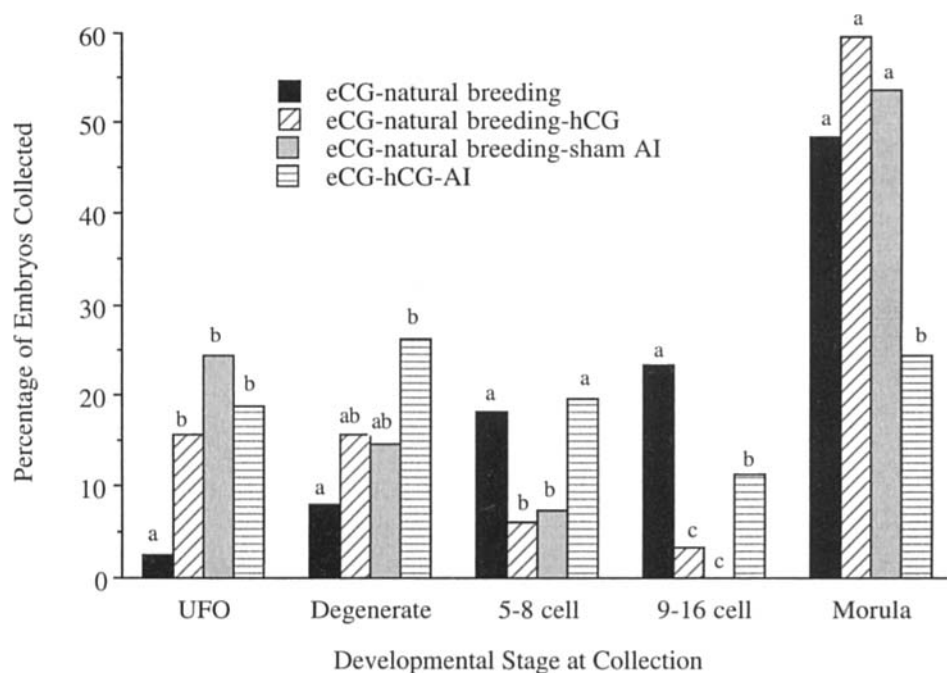


TABLE 3. Serum and luteal endocrine traits for eCG-treated queens subjected to NB and/or AI, with or without hCG administration (means \pm SEM).

Treatment group	Serum progesterone (ng/ml)	Serum estradiol (pg/ml)	Luteal progesterone/CL (ng/mg)	Luteal mass (mg)	Total luteal progesterone/ovary (mg)
NB*	27.7 \pm 2.8	18.3 \pm 1.8	174.6 \pm 11.0	16.8 \pm 1.1	29.8 \pm 2.6
eCG-NB	46.4 \pm 12.2	7.3 \pm 1.5	157.5 \pm 8.8 ^a	15.4 \pm 0.9 ^a	23.2 \pm 5.4
eCG-NB-hCG	32.9 \pm 2.9	23.9 \pm 9.4	92.5 \pm 4.9 ^b	19.0 \pm 0.9 ^b	16.4 \pm 2.9
eCG-NB-sham AI	25.8 \pm 5.2	5.7 \pm 0.4	140.4 \pm 11.6 ^a	16.7 \pm 1.2 ^{a,b}	16.6 \pm 3.6
eCG-hCG-AI	51.3 \pm 12.7	18.1 \pm 2.3	153.3 \pm 7.0 ^a	15.3 \pm 0.8 ^a	36.2 \pm 10.8

* Historical data on naturally estrual, mated queens adapted from a previous report [34]; data not included in statistical analyses.

^{a,b} Within columns, values with different superscripts differ ($p < 0.05$).

[17, 33]. The greatest difference was observed in the eCG-NB group; queens in this group produced > 4 times as many blastocysts as the untreated, historical controls. However, in some species, the negative impact of exogenous gonadotropins becomes most apparent later in pregnancy. For example, eCG is associated with implantation failure and retarded fetal growth in mice and hamsters, ultimately resulting in few live fetuses [27, 38].

When studying eCG effects on pregnancy success in the cat, it may be more appropriate to examine the proportion of queens producing blastocysts than the overall average number of blastocysts produced. All of the eCG-treated cats that were naturally mated produced embryos that developed into blastocysts, in contrast to all other groups, in which at least 40% of females failed to produce blastocysts. Of the cats treated with the standardized AI protocol (eCG+hCG+AI), half produced blastocysts—exactly the incidence of pregnancy reported after laparoscopic AI in this species [1]. Individual variation in response to exogenous gonadotropins is not surprising, since timing of gonadotropin administration relative to natural, cyclic fluctuations in ovarian activity can markedly alter follicular and ovulatory response [26, 39]. In most species, variation is minimized by synchronizing the estrous cycle prior to gonadotropin treatment, but luteal regulation is poorly understood in the cat and conventional estrous synchronization protocols have not been developed. In any case, ovarian responses of queens given eCG and then naturally bred were consistently high, suggesting that some other factor(s) must be responsible for ovarian/embryo variations observed across treatment groups. Candidates include hCG, anesthesia, and/or the AI procedure itself.

The impact of hCG on embryo quality and/or pregnancy success appears to be species specific. In humans, hCG has been reported to increase the number of oocytes, transferable embryos, and resulting pregnancies [40]. However, compared to naturally mated mice, hCG-treated counterparts produce more abnormal embryos and experience higher postimplantation mortality [27]. In the rabbit, an induced ovulator like the cat, hCG increases the proportion of poor-quality and degenerate or fragmenting embryos [41, 42]. Information in the cat is limited, but in one study, high hCG dosages were associated with increased numbers of degenerate oocytes [9]. In the present study, eCG-treated queens that were naturally bred and given hCG produced more unfertilized oocytes than naturally bred queens receiving no hCG. Nonetheless, hCG treatment had no negative effect on the overall number of morulae produced. Furthermore, more than half of these embryos developed to blastocysts in culture, almost double the number of blastocysts produced by either of the two groups subjected to AI. Therefore, these data did not suggest that hCG is pri-

marily responsible for poor embryo quality in gonadotropin-treated queens.

Of the queens subjected to sham or actual AI, only $\sim 45\%$ (5 of 11) produced embryos that formed blastocysts compared to $\sim 77\%$ (10 of 13) of naturally bred cats. These data suggested that the AI procedure itself may have adversely affected embryo quality. Laparoscopic AI was developed in cats because transcervical AI is relatively unsuccessful, presumably due to compromised sperm transport [1]. Furthermore, postovulatory laparoscopic intrauterine AI is necessary in the cat because anesthesia inhibits ovulation [1]. To confirm the presence of fresh CL, a Verres needle is used to elevate and gently remove the fimbria from the ovarian surface before AI commences. Manipulating the reproductive tract at this sensitive time in the periovulatory interval could disrupt oocyte capture by the fimbria. In the rat, ovarian bursa displacement results in significant loss of oocytes [43]. Additionally, laparoscopic intrauterine AI in the ewe has been suspected of adversely affecting subsequent embryo quality and recovery [44], but primarily as a result of inappropriate timing relative to ovulation [44, 45] and not directly related to "procedure." The actual disruption of ovum fertilizability resulting from laparoscopic AI seems unlikely considering that similar techniques are routinely successful ($> 70\%$ pregnancies) in ferrets and deer [46, 47].

It also is useful to consider the effects of the AI procedure in the context of hCG treatment. Previous studies in the naturally estrual cat have demonstrated that an endogenous LH surge peaks ~ 9 h after the first of a series of matings [48]. However, there is considerable individual variation, and ovulation can occur 24–64 h after first copulation [33, 48, 49]. The breeding schedule used in the present study ensured that sperm were present in the reproductive tract 28 h before ovulation began, with the last natural insemination occurring after (or as many as 34 h before) ovulation. In eCG-treated cats that were naturally mated, follicular development was initiated by eCG, whereas final oocyte maturation and ovulation presumably were caused by mating-induced endogenous LH surges. It seems logical that the sequence of events in these females (LH surge, ovulation, fertilization) was similar to that for naturally estrual, mated queens [33, 48, 49], thereby explaining the similarity in good-quality embryo production. Superimposing a sham AI procedure on the eCG-NB regimen resulted in more unfertilized oocytes but a comparable proportion of recovered morulae. The increased number of unfertilized oocytes perhaps resulted from anesthesia and reproductive tract manipulation during the final hours of ovulation, just as fertilization commenced.

Queens given hCG after the onset of natural breeding again produced more unfertilized oocytes than queens that

mated but did not receive hCG. These results may be explained, in part, by the recent confirmation that hCG alone induces both follicular development and ovulation in the cat [50]. Recovered embryos likely originated from eCG-induced follicles that ovulated in response to hCG and/or NB, whereas the unfertilized oocytes possibly were produced by hCG-induced secondary follicles that may have ovulated later but failed to fertilize in the absence of viable sperm. This theory is supported by the lower luteal progesterone/CL in this group; CL formed from the secondary ovulating follicles would have been less mature at ovariectomy.

Finally, cats given eCG and hCG and then artificially inseminated produced a high proportion of unfertilized oocytes and fragmenting or degenerate embryos. For this group, the timing of ovulation relative to laparoscopy and sperm deposition is critical. It is possible that 1) some of the queens ovulated early and there were no sperm present to fertilize the oocytes; 2) queens ovulated at the time of AI and oocytes were somehow disrupted during manipulation of the fimbria and reproductive tract; or 3) some of the follicles did not rupture prior to AI, and anesthesia delayed ovulation of these follicles beyond the functional longevity of in vitro-processed, inseminated sperm. Although we have not determined exactly why queens in the eCG-hCG-AI group produced many poor-quality embryos, it seems reasonable that the problem stems from an inability to consistently ensure that fertilizable oocytes and fully functional sperm are present in the reproductive tract simultaneously.

Aberrant serum estradiol levels in rats and cats following exogenous gonadotropin treatment have been implicated in poor embryo recovery and development, respectively [51, 52]. Although there were numerous follicles (≥ 2 mm) on the ovaries of queens in all groups, circulating estradiol concentrations at ovariectomy were within the range of those reported for naturally estrual, mated queens at the same stage of pregnancy [34]. Percentage embryo recovery (based upon CL number) was equivalent to, or better than, the 56% recovery rate reported for naturally estrual, mated queens [33]. These two findings are important for the following reasons. First, recovery of 70–96% of embryos produced by queens in the eCG-hCG-NB and eCG-NB groups, respectively, provided strong evidence against the hypothesis that follicular luteinization in the absence of oocyte release occurred, a finding reported after gonadotropin treatment in other species [53, 54]. Second, these findings clearly indicated that embryos were not being lost due to accelerated transport through the reproductive tracts of gonadotropin-treated females, a phenomenon reported in rats [51]. In fact, the time of ovariectomy was specifically chosen because it coincided with embryo transport through the uterotubal junction in naturally estrual and mated cats [33]. Because we found embryos in the oviducts and uterine horns in all treatment groups, it appeared that the various treatments were allowing embryo progression through the reproductive tract at a rate mimicking that for naturally estrual, mated queens.

Luteal insufficiency following gonadotropin treatment is recognized as one cause of pregnancy failure in humans after assisted reproduction [55]. In contrast, hyper-elevated circulating progesterone has been associated with poor embryo quality in cats [34]. Serum progesterone concentrations for queens after all four treatments reported here were variable but generally similar to those for naturally estrual, mated queens [34]. Furthermore, mean luteal mass and pro-

gesterone concentrations were similar to those reported for the naturally estrual, mated queen [34], and no aberrant serum endocrine or luteal traits were found associated with queens producing poor-quality embryos. Therefore, it appeared that luteal function, at least during preimplantation embryo development, was not compromised in queens receiving eCG alone or in conjunction with hCG. Rather, domestic cat embryos appeared relatively tolerant to a variety of endocrine environments, at least during the early preimplantation stages of development.

Overall, these results were important for two primary reasons. First, data from eCG-treated cats that were naturally bred with or without receiving supplemental hCG produced fertilizable oocytes that were developmentally competent to the blastocyst stage. Therefore, the partial morula-to-blastocyst developmental block routinely encountered in this species after IVF [15–18] is likely the result of inappropriate culture conditions during fertilization or early embryo development. Second, these results suggested that some other factor, perhaps the timing of insemination or an unknown component of the AI technique, is responsible for reduced pregnancy success and small litter sizes following AI. In any case, these data support the use of eCG as an ovarian-stimulating gonadotropin for assisted reproduction in felids.

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