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Pharmacokinetics and Ovarian-Stimulatory Effects of Equine and Human Chorionic Gonadotropins Administered Singly and in Combination in the Domestic Cat[1]

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ABSTRACT

Pregnancy success and embryo survival are low with the use of assisted reproduction in felids treated with exogenous gonadotropins. In this study, the pharmacokinetics and ovarian-stimulatory effects of eCG and hCG were evaluated in the domestic cat. Catheterized anestrual queens (n = 4 per treatment [Trt] group) were given 100 IU eCG i.v. (Trt 1), 100 IU eCG i.m. (Trt 2), 75 IU hCG i.v. (Trt 3), 75 IU hCG i.m. (Trt 4), or 100 IU eCG i.m. followed 80 h later by 75 IU hCG i.m. (Trt 5). Blood samples were collected at 0, 5, 30, and 60 min and 4, 8, 12, 24, 36, 48, 72, 96, 120, 144, and 168 h postinjection, and serum samples were analyzed for estradiol-17β, progesterone, eCG, and hCG. Pharmacokinetic traits (volume of distribution, Vd; elimination half-life, $t_{1/2\beta}$; clearance rate, Clr) were calculated for eCG and hCG. When i.v. and i.m. administration were compared, no differences ($p > 0.05$) were observed in follicle or corpus luteum (CL) number or hormone concentrations for queens receiving eCG or hCG alone. Number of mature ovarian follicles (≥ 2 mm diameter) observed at 168 h postinjection did not differ ($p > 0.05$) for eCG (mean ± SEM, 10.5 ± 2.0) vs. hCG (11.1 ± 3.0), indicating that these were equally effective in inducing follicular growth. In most queens (> 90%) given single gonadotropins (i.m. or i.v.), eCG and hCG persisted in circulation for at least 120 h and 96 h after injection, respectively, reflecting similar ($p > 0.05$) pharmacokinetic (i.v.) values for Vd (eCG, 91.4 ± 24.8 ml/kg; hCG, 59.1 ± 7.9 ml/kg), $t_{1/2\beta}$ (eCG, 23.0 ± 2.4 h; hCG, 22.9 ± 4.1 h), and Clr (eCG, 2.7 ± 0.5 ml/h per kg; hCG, 1.8 ± 0.1 ml/h per kg). Sequential treatment with eCG+hCG did not affect ($p > 0.05$) the $t_{1/2\beta}$ of individual gonadotropins. In summary, eCG and hCG have comparable pharmacokinetics and ovarian-stimulatory activity when administered alone to the domestic cat. These findings suggest that hCG promotes the ancillary follicle formation that is frequently observed after ovulation in cats treated with eCG+hCG regimens, possibly disrupting the maternal environment and decreasing fecundity following assisted reproductive procedures.

INTRODUCTION

Exogenous gonadotropin regimens, consisting of sequential treatment with eCG and hCG, are frequently used in domestic and nondomestic felids as one component of in vitro fertilization or artificial insemination procedures [1–8]. Typically, eCG is administered to stimulate ovarian follicular growth followed several days later by hCG to induce final follicular maturation and ovulation. Applied usage, however, has not consistently translated into high reproductive efficiency, as measured by embryo survival rates after embryo transfer or pregnancy success after artificial insemination [2–8]. Low fecundity after exogenous gonadotropin treatment possibly is related to a maternal environment unsuitable for supporting embryo development. It is well known that queens treated with eCG/hCG combinations typically develop ancillary ovarian follicles several days after induced ovulation and that these follicles subsequently form secondary corpora lutea (CL), possibly disrupting the maternal environment [2, 4, 9, 10]. Persistence of eCG after i.m. injection has been implicated in possibly contributing to postovulatory follicle growth; however, attempted neutralization of residual eCG was ineffective in preventing ancillary follicle and secondary CL development [10]. Therefore, perhaps hCG, rather than eCG, is responsible for ancillary follicle development after ovulation. This speculation is consistent with our earlier observations that hCG given to naturally estrual cats prolongs behavioral estrus [11] and may promote folliculogenic activity after natural mating [12].

Investigations of the normality of the cat maternal environment resulting from exogenous gonadotropin treatment have been hindered by two deficiencies. First, there has been no comprehensive, normative database describing embryogenesis and early maternal reproductive characteristics in naturally estrual, naturally mated cats—information that is essential for comparative purposes. Second, our understanding of the pharmacokinetic activity and ovarian-stimulatory effects of individual exogenous gonadotropins in cats is limited, and this limitation interferes with our ability to design effective ovarian stimulation protocols. To resolve the first shortcoming, we recently completed a series of studies characterizing developmental [13, 14], histological [15], and endocrine changes [16] associated with the periovulatory, conceptive, and preimplantation/early-implantation interval in the cat. From these studies, we now have a much broader understanding of what constitutes a "normal" maternal environment.

The present study was designed to address the second deficiency and to provide additional insight into the development of gonadotropin treatments that will more consistently produce a physiologically normal maternal state. Our specific objectives were to 1) evaluate ovarian responses and the dynamics of circulating estradiol-17β and progesterone secretion in response to eCG and hCG, 2) characterize the pharmacokinetics of each gonadotropin after i.v. administration, and 3) assess the circulatory persistence of each gonadotropin alone and in combination following i.m. delivery.

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MATERIALS AND METHODS

Laparoscopy and Jugular Catheter Placement

Adult female cats (Liberty Research Inc., Waverly, NY) were housed in communal pens under controlled artificial lighting (12L:12D) and provided a commercial cat food diet (Purina Cat Chow; Ralston Purina, St. Louis, MO) and water ad libitum. Queens (n = 23) were evaluated daily for overt estrual behavior [13, 17] to identify the anestrual phase of the reproductive cycle before administration of gonadotropins. To verify ovarian inactivity, queens were anesthetized and both ovaries were examined for absence of mature follicles (≥ 2 mm in diameter, vesicular, raised above ovarian surface) and CL via laparoscopy [13, 18]. Only queens without mature follicles or CL were catheterized for blood sample collection. None of the study queens had been treated previously with gonadotropins.

For catheter placement, the ventral neck region of anesthetized queens was surgically prepared using 70% isopropyl alcohol and povidone-iodine solution (Betadine Surgical Scrub; The Purdue Frederick Company, Norwalk, CT), and a "through the needle" polymer resin catheter (Intracath Intravenous Catheter Placement Unit; Deseret Medical Inc., Parke, Davis and Company, Sandy, UT; 19 gauge, 20.3-cm length) was inserted percutaneously into the left or right external jugular vein. Each catheter was advanced (~6–8 cm) in the vein, the catheter hub and distal catheter (~4–6 cm) were excised, and the catheter needle was removed. A new catheter hub (Intramedic Luer Stub Adapter; Becton Dickinson and Co., Clay Adams Division, Franklin Lakes, NJ; 20 gauge), with attached PRN adapter (Becton Dickinson Vascular Access), was placed on the distal end of the catheter and flushed with sterile saline (1 ml) containing heparin sodium (30 U/ml; Lyphomed, Division of Fuijisawa USA, Inc., Deerfield, IL). The catheter was sutured in place, and the entry site was treated with antibiotic ointment (bacitracin zinc-neomycin sulfate-polymyxin B sulfate ointment; E. Fougera & Company, Melville, NY) and covered with sterile gauze. The external catheter was extended dorsolaterally to the dorsal aspect of the neck and thoroughly bandaged using stretch roll gauze, elastic bandage material (Vetrap Bandaging Tape; Animal Care Products, 3M Company, Saint Paul, MN), and elastic tape (Elastikon; Johnson and Johnson Medical Inc., Arlington, TX). All catheterized queens received s.c. penicillin (Flo-cillin, penicillin G benzathine, and penicillin G procaine; Fort Dodge Laboratories Inc., Fort Dodge, IA) prophylactically. After catheter placement, animals were weighed (± 0.01 g) and then housed singly for the next 3 days to reduce the likelihood of accidental catheter displacement.

Gonadotropin Administration and Blood Sampling

Lyophilized gonadotropins (eCG and hCG; Sigma Chemical Company, St. Louis, MO) were reconstituted in sterile water, immediately frozen (−80°C), and stored in individual-dose syringes until needed. Frozen, reconstituted gonadotropins were used within 3 mo of storage, and the same gonadotropin lots (eCG, lot no. 113H07851, 2620 IU/mg, nonbuffered; hCG, lot no. 63430321, ~3000 IU/mg, buffered with 0.01 M Na_2HPO_4) were used throughout the study. Catheterized, anestrual females were assigned randomly to one of five treatment (Trt) groups; and on the day after catheter placement, queens (n = 4 per Trt) were given either 100 IU eCG i.v. (Trt 1), 100 IU eCG i.m. (Trt 2), 75 IU hCG i.v. (Trt 3), 75 IU hCG i.m. (Trt 4), or 100 IU eCG i.m. followed 80 h later with 75 IU hCG i.m. (Trt 5). The latter (Trt 5) is the standard eCG/hCG combination regimen used with artificial insemination procedures in the domestic cat [4]. Gonadotropin preparations and standard dosages were chosen based on this current applied usage.

A blood sample (~2 ml) was collected from each unanesthetized queen via the jugular catheter immediately before gonadotropin injection (Time 0) and then at 5, 30, and 60 min and 4, 8, 12, 24, 36, 48, 72, 96, 120, 144, and 168 h after administration. Samples were centrifuged (1100 × *g*, 10 min), and recovered serum was stored at −80°C until analysis. For i.v. (Trt 1, 3) and i.m. (Trt 2, 4, 5) treatments, thawed gonadotropin doses were injected via the jugular catheter and into the caudal thigh muscles, respectively. After i.v. gonadotropin injection and each blood sample collection, catheters were flushed with heparinized saline (0.5–1.0-ml volume; 30 U heparin/ml). Jugular catheters were removed from all females after the 48-h time point, and subsequent blood samples were obtained from unanesthetized cats via jugular venipuncture. Concurrent with the final blood sampling at 168 h, queens were anesthetized and reevaluated laparoscopically for mature ovarian follicles or CL.

Gonadotropin and Steroid Hormone RIAs

Concentrations of eCG were measured in unextracted cat serum using a double-antibody RIA. This assay used a mouse monoclonal antibody against bovine LH (no. 518-B7; provided by Dr. Jan Roser, University of California, Davis), eCG standards (PM-230GB; provided by Dr. Harold Papkoff, University of California, San Francisco), and ^{125}I-labeled equine LH (E-263B; Dr. Harold Papkoff) in a phosphate buffer-based system (PBS; 0.01 M PO_4, 0.5% BSA, 2 mM EDTA, 0.9% NaCl, 0.01% thimerosal, pH 7.4). Duplicate standards (100 µl, 2.6–327.5 mIU/ml) and serum samples (100 µl; neat or diluted 1:1–1:40 in PBS) were incubated at room temperature (22°C) with antibody (1:500 000 in 100 µl) for 24 h; then tracer (20 000 c.p.m. in 100 µl) was added and incubation continued for an additional 24 h. Antibody-bound complexes were precipitated after a 1-h incubation with goat anti-mouse gamma globulin (1:200 in 1 ml containing 5% polyethylene glycol) and centrifugation for 30 min at 1500 × *g*. Radioactivity in the pellets was determined by gamma spectrometry. The antibody generally bound ~30% of the ^{125}I-equine LH with < 7% nonspecific binding. The assay was validated by 1) demonstrating parallelism between serial dilutions of cat serum pools and the standard curve and 2) significant recovery of exogenous eCG added to serum before analysis ($y = 1.01x - 0.282$; $r = 0.99$). Assay sensitivity was 2.6 mIU/ml at 90% of maximum binding, and the intra- and interassay coefficients of variation were < 10%, based on average variation within samples (n = 180) and internal controls (n = 2), respectively. The anti-bovine LH antibody cross-reacts ~39% with eCG and ~10% with hCG [19]. Because of hCG cross-reactivity, serum eCG concentrations for females receiving both gonadotropins (Trt 5) were adjusted by decreasing values by 10% of the measured hCG concentration (only for samples collected after hCG injection). Accordingly, these latter values should be considered estimates.

Concentrations of hCG were determined in unextracted serum using a double-antibody RIA (Diagnostic Products Corporation, Los Angeles, CA). This assay uses an antibody against the hCG ß subunit. Serum samples (100 µl;

neat or diluted 1:1–1:30 with pooled cat serum) or standards (3.9–250 mIU/ml diluted in cat serum) were coincubated with β-hCG antiserum (50 μl) for 30 min at 37°C followed by addition of ^{125}I-labeled hCG tracer (30 000 cpm in 50 μl) and further incubation at 37°C for 30 min. Antibody-bound complexes were precipitated by addition of 0.5 ml goat anti-rabbit gamma globulin and centrifugation for 30 min at 1500 × *g*. Cross-reactivity of hCG antisera with eCG and human LH is less than 0.1% and 0.2%, respectively. Serial dilutions of cat serum pools were parallel to the standard curve. Addition of exogenous hCG to cat serum resulted in significant net recovery ($y = 1.18x - 8.7$; $r = 0.99$). Assay sensitivity was 5 mIU/ml, and the intra- and interassay coefficients of variation were < 10% on the basis of average sample (n = 144) variation and internal controls (n = 2), respectively.

Estradiol-17β and progesterone concentrations were measured in unextracted serum using validated, solid-phase ^{125}I RIAs (Coat-a-Count; Diagnostic Products Corporation) as described previously [16]. Assay sensitivities for estradiol-17β and progesterone were 5.0 pg/ml and 0.05 ng/ml, respectively. Intra- and interassay coefficients of variation were < 10% on the basis of average sample (n = 300) variation and internal controls (n = 2), respectively.

Pharmacokinetic and Statistical Analysis

For i.v. gonadotropin treatment groups (Trt 1, 3), log serum eCG and hCG concentrations (mIU/ml) of individual animals were plotted against time and fitted to various compartmental models using a computer modeling program (SAAMII; SAAM Institute, University of Washington, Seattle, WA). After curve fitting to the most appropriate model, disappearance curves for each gonadotropin were subjected to linear regression analysis in a semilogarithmic scale to determine y intercepts (A and B) and slopes (α and β) of distribution and elimination components, respectively. For each gonadotropin, theoretical initial serum concentration (Co) was calculated by summing the y intercepts, and distribution ($t_{1/2\alpha}$) and elimination ($t_{1/2\beta}$) half-lives were calculated as $t_{1/2\alpha} = 0.693/\alpha$ and $t_{1/2\beta} = 0.693/\beta$, respectively. Area under the curve (AUC) for each disposition curve was determined using $AUC = A/\alpha + B/\beta$. Mean residence times (MRT) were calculated as MRT = AUMC/AUC, with the numerator $AUMC = A/\alpha^2 + B/\beta^2$. Volume of distribution (Vd) was determined using Vd = dose/(AUC)β, and clearance rate (Clr) was ascertained using Clr = β × Vd [20].

For i.m. treatment groups (Trt 2, 4, 5), log serum eCG and hCG concentrations (mIU/ml) of individual animals were plotted against time and slopes (β) of the postabsorptive elimination curves determined using linear regression analysis in a semilogarithmic scale. Elimination half-lives ($t_{1/2\beta}$) were calculated from $t_{1/2\beta} = 0.693/\beta$, and AUCs were calculated by the log trapezoidal method, extrapolated to infinity. Absolute bioavailability (F) for each gonadotropin was determined as $F = AUC_{i.m.}/AUC_{i.v.}$, using mean values for $AUC_{i.m}$ and $AUC_{i.v.}$.

For each treatment group, serum estradiol-17β concentrations over time were assessed by calculating AUC using the trapezoidal method, extrapolated to infinity [20]. Mean (± SEM) values were determined for pharmacokinetic parameters (Co, A, B, $t_{1/2\alpha}$, $t_{1/2\beta}$, MRT, AUC, Vd, Clr), estradiol concentrations over time (AUC), progesterone concentrations (for anovulatory and ovulatory queens), and number of ovarian follicles and total ovarian structures (mature

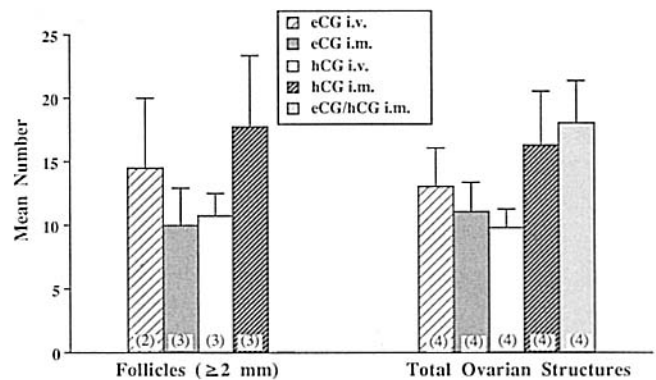

FIG. 1. Mean (± SEM) number of follicles (≥ 2 mm) in anovulatory queens and total ovarian structures (follicles + CL) in all queens observed via laparoscopy at 168 h after gonadotropin injection. Anestrual queens received either 100 IU eCG (i.v. or i.m.), 75 IU hCG (i.v. or i.m.), or 100 IU eCG i.m. followed 80 h later with 75 IU hCG i.m. Numbers in parentheses indicate the number of queens included in each mean value calculation.

follicles + CL) at the 168-h time point. Values for dosage-independent ($t_{1/2\alpha}$, $t_{1/2\beta}$, MRT, Vd, Clr) and dosage-dependent (AUC) pharmacokinetic parameters were compared between gonadotropins and/or routes of administration using a Student's *t*-test [21], when appropriate. Across treatment groups, mean values for estradiol concentrations, number of ovarian follicles, and total ovarian structures were compared using analysis of variance [22]. Correlation coefficients were calculated between estradiol (AUC) and total number of ovarian structures [21].

RESULTS

Ovarian and Endocrine Characteristics

On the basis of behavioral and laparoscopic assessments, study females were classified as anestrual on 24 occasions and subjected to jugular catheterization. On four occasions, catheters could not be placed appropriately or were inserted properly but later found to have malfunctioned. Following i.v. or i.m. administration of eCG or hCG, most females (95%) exhibited behavioral estrus within 2–3 days, with queens receiving i.v. injections entering estrus ~1 day earlier ($p < 0.05$) than queens injected i.m. Laparoscopy at 168 h revealed that 4 of 4 females receiving the combination eCG/hCG treatment ovulated (based on presence of ovarian CL). However, distinct ovulatory responses also were observed in some females given single injections of eCG (i.v.: 2 of 4, 50%; i.m.: 1 of 4, 25%) or hCG (i.v.: 1 of 4, 25%; i.m.: 1 of 4, 25%), regardless of route of administration. Based on laparoscopic evaluations at 168 h, the mean number of mature ovarian follicles (range, 10.0 ± 2.9–17.7 ± 5.6) in anovulatory queens and the number of total ovarian structures (follicles + CL; range, 9.8 ± 1.5–18.0 ± 3.4) in all queens did not differ ($p > 0.05$) among treatment groups (Fig. 1).

Females also exhibited similar temporal patterns in serum estradiol concentrations, with values typically increasing above basal levels (≤ 20 pg/ml) 24–72 h after gonadotropin injection, reaching peak values by 120–144 h, and then declining to baseline by 168 h (Fig. 2). Across treatments, there were no differences ($p > 0.05$) in mean estradiol values over time based on AUC calculations (mean range, 1936.6 ± 203.5–4909.6 ± 573.0 pg/h per ml). Estradiol concentrations over time were positively correlated

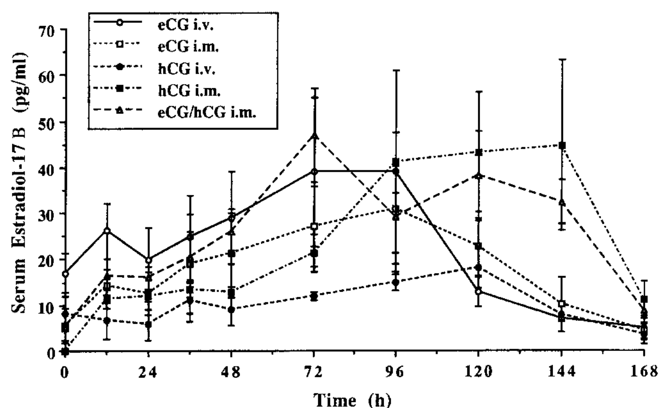

FIG. 2. Mean (± SEM) serum estradiol-17β concentrations in anestrual queens administered either 100 IU eCG (i.v. or i.m.), 75 IU hCG (i.v. or i.m.), or 100 IU eCG i.m. followed 80 h later with 75 IU hCG i.m.

($r = 0.80$; $p < 0.01$) with number of total ovarian structures at 168 h.

Serum progesterone concentrations typically remained basal (≤ 2 ng/ml) in anovulatory queens but rose above baseline in ovulatory, eCG or hCG only-treated females beginning ~96 h after injection and in ovulatory eCG+hCG-treated queens beginning ~120–144 h after eCG injection. In ovulatory females, mean progesterone increased ($p < 0.01$) from a nadir of 1.2 ± 0.4 ng/ml (Time 0) to 9.6 ± 1.7 ng/ml at 168 h postinjection. Despite eCG+hCG-treated queens having more ($p < 0.05$) CL (mean, 15.0 ± 3.3) than ovulatory females given eCG or hCG only (5.4 ± 1.4), progesterone concentrations at 168 h did not differ ($p > 0.05$) between the two groups (12.8 ± 2.2 and 7.1 ± 1.9 ng/ml, respectively).

Pharmacokinetics

Pharmacokinetic analysis revealed that eCG and hCG disappearance curves after i.v. delivery best fit a two-compartment, pharmacokinetic model, typified by a rapid distribution phase and a slow elimination phase (Fig. 3). Pharmacokinetic values were similar for the two gonadotropins (Table 1), with no differences ($p > 0.05$) between any dosage-independent parameter ($t_{1/2\alpha}$, $t_{1/2\beta}$, MRT, Vd, Clr).

TABLE 1. Pharmacokinetic parameters (mean ± SEM) of eCG and hCG following bolus injection in domestic cats.[a]

Parameter[b]	eCG	hCG
Co (mIU/ml)	1948.9 ± 1028.7	1697.2 ± 169.9
A (mIU/ml)	1558.4 ± 943.4	1435.5 ± 154.5
B (mIU/ml)	390.6 ± 101.9	261.7 ± 50.4
$t_{1/2\alpha}$ (h)	0.9 ± 0.7	3.0 ± 0.5
$t_{1/2\beta}$ (h)	23.0 ± 2.4	22.9 ± 4.1
MRT (h)	32.1 ± 3.7	20.5 ± 3.3
AUC (mIU-h/ml)	12571.4 ± 2508.8	14476.7 ± 2231.2
Vd (ml/kg)	91.4 ± 24.8	59.1 ± 7.9
Clr (ml/h/kg)	2.7 ± 0.5	1.8 ± 0.1
BW (kg)	3.4 ± 0.1	3.0 ± 0.3

[a] Anestrual queens (n = 4 per Trt) were given either eCG (100 IU/dose) or hCG (75 IU/dose) via i.v. catheters, and serial blood samples were collected over the next 168 h for determination of serum gonadotropin concentrations.

[b] Co, theoretical initial serum concentration; A, zero intercept rapid distribution curve; B, zero intercept elimination curve; $t_{1/2\alpha}$, half-life of rapid distribution; $t_{1/2\beta}$, half-life of elimination; MRT, mean residence time; AUC, area under the curve; Vd, volume of distribution; Clr, total clearance rate; BW, body weight.

Concentration-time curves for eCG and hCG after i.m. injection revealed a variable absorptive phase and a prolonged postabsorptive elimination phase (Fig. 4). When i.m. and i.v. delivery were compared, elimination half-lives were similar ($p > 0.05$) for eCG (27.1 ± 1.7 vs. 23.0 ± 2.4 h) and hCG (26.1 ± 3.1 vs. 22.9 ± 4.1 h), and the AUC (eCG, 6601.9 ± 1076.9 vs. 12571.4 ± 2508.8 mIU/h per ml; hCG, 8873.2 ± 1221.2 vs. 14476.7 ± 2231.2 mIU/h per ml) was not different ($p > 0.05$). Both gonadotropins demonstrated comparable absolute bioavailability (eCG, 52.5%; hCG, 61.3%) after i.m. injection. After i.v. or i.m. delivery of individual gonadotropins, eCG persisted in circulation in all queens for at least 120 h, whereas hCG was detectable in 7 of 8 females for at least 96 h. In one female given hCG i.v., hCG was undetectable after 72 h. For the eCG+hCG combination, initial absorption and elimination curves for eCG after i.m. injection (Fig. 5) were qualitatively similar to that of eCG administered alone, and the elimination half-lives of eCG (23.7 ± 1.2 h) and hCG (23.2 ± 2.5 h) administered in sequential combination did not differ ($p > 0.05$) from that of individual gonadotropins

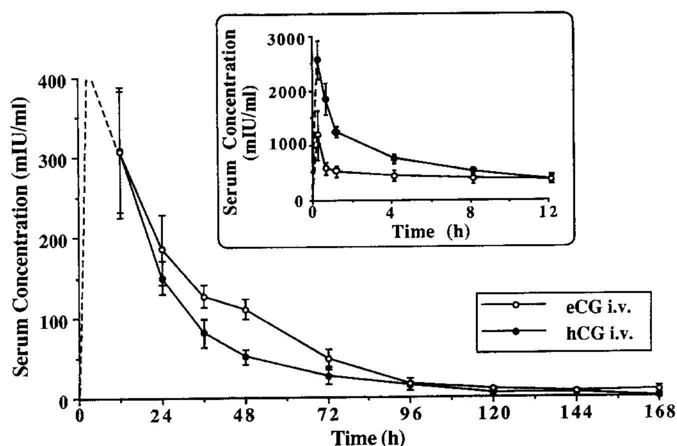

FIG. 3. Disappearance curve of eCG and hCG following bolus i.v. injection in the domestic cat. Anestrual queens (n = 4 per Trt) received either 100 IU eCG i.v. or 75 IU hCG i.v., and serial blood samples were collected via jugular catheters and venipuncture for the next 168 h. Values are expressed as means (± SEM). The inset graph depicts the distribution phase.

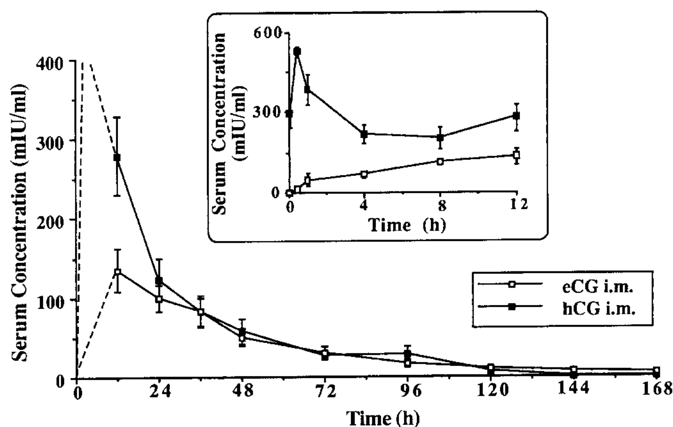

FIG. 4. Disappearance curve of eCG and hCG following i.m. injection in the domestic cat. Anestrual queens (n = 4 per Trt) received either 100 IU eCG i.m. or 75 IU hCG i.m., and serial blood samples were collected via jugular catheters and venipuncture for the next 168 h. Values are expressed as means (± SEM). The inset graph depicts the absorptive and distribution phases.

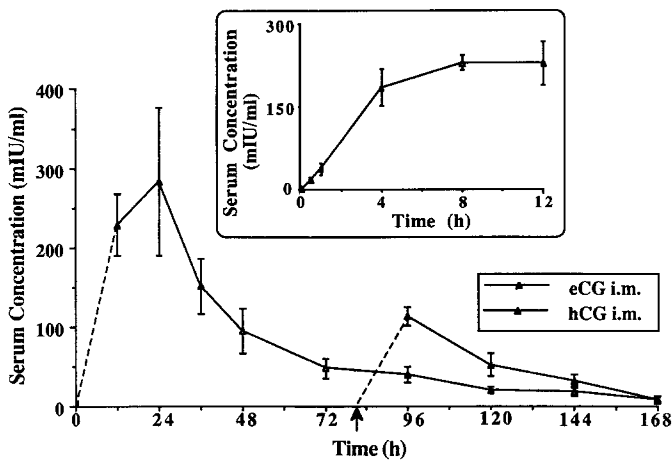

FIG. 5. Disappearance curve of eCG and hCG following sequential i.m. injection in the domestic cat. Anestrual queens (n = 4) received 100 IU eCG i.m. followed 80 h later by 75 IU hCG i.m., and serial blood samples were collected via jugular catheters and venipuncture during a 168-h period. The arrow indicates time of hCG injection. Values are expressed as means (± SEM). The inset graph depicts the absorptive and distribution phases of eCG.

after i.m. delivery. In all females given the combination regimen, eCG was detectable in serum samples for at least 168 h after eCG injection.

DISCUSSION

This study represents the first objective assessment of gonadotropin pharmacokinetics and their relation to the stimulatory effects of gonadotropins alone or in combination in the domestic cat. Although of basic comparative value for mammalian species in general, these data are particularly important for the development of effective ovarian stimulation protocols for endangered felid species. Especially significant were the observations of hCG folliculogenic effects and persistence in circulation, findings that suggest the need to modify existing gonadotropin therapies.

When given alone, eCG and hCG had comparable effectiveness in inducing ovarian follicular development, each stimulating the production of more than 10 mature follicles per queen. In contrast, naturally cyclic females develop about 5 mature follicles during a typical estrus [23]. Folliculogenic and luteotrophic activities of eCG have been well documented in vivo and in vitro [24, 25]. In cats, eCG traditionally has been used to induce follicular growth [26, 27], although high dosages or multiple injections also are known to induce ovulation [27]. In contrast, hCG is known for its luteotrophic bioactivity [28], but intrinsic folliculogenic activity has been reported in the hamster [29] and rat [30, 31]. In the cat, hCG has a strong affinity for LH receptors [16] and has been used for almost two decades to induce ovulation in naturally estrual and eCG-treated females [4, 32].

Earlier observations [11, 12] combined with the present results support the assertion that hCG has luteotrophic and folliculogenic effects in the cat, including the capacity to stimulate growth and maturation of smaller antral follicles (< 2 mm in diameter). Domestic cat ovaries, regardless of female reproductive status, contain diverse, asynchronous cohorts of developing preantral and antral follicles [33–35] that likely differ markedly in responsiveness to individual gonadotropins. Among polytocous species, naturally cyclic and eCG-stimulated pigs also exhibit ovarian follicular heterogeneity as demonstrated by variable follicular size, gonadotropin-binding capacity, and steroid content [36, 37]. Similarly, hamster ovaries contain a heterologous follicle population with variable FSH and LH receptor expression and steroidogenic capacity [38, 39]. Although eCG is predominantly folliculogenic and hCG luteotrophic in the cat, each exhibits duality and thus can mimic the other's primary action. Some evidence has suggested, in fact, that dual activity of administered gonadotropins is required in the cat, since highly purified human FSH administered alone ineffectively promotes folliculogenesis [40]. Furthermore, the superovulatory potential of eCG and hCG is related to a capacity to "rescue" preantral and early antral follicles from atresia [29, 41], and the domestic cat ovary is composed of a large follicle population in various stages of atresia [35].

In about 30% of queens, immature follicles underwent final maturation and ovulation within 2–3 days of a single eCG or hCG injection, as evidenced by distinct CL formation and elevated circulating progesterone. Ovulation may reflect the innate ovulatory (LH) properties of each gonadotropin, or, alternatively, each promoted follicular growth and ovulation occurred "spontaneously." Although domestic cats usually are considered induced ovulators, spontaneous ovulation has been observed in naturally estrual, unmated females [42]. Sporadic spontaneous ovulation probably also represents another impediment to inducing consistent ovarian responses in the cyclic felid, especially in nondomestic cats that are intractable and difficult to assess for estrual status [43, 44]. The present findings reinforce our previous assertions [7, 43] that pharmaceutical down-regulation of pituitary activity may be a prerequisite to improving ovarian responses to exogenous gonadotropin treatments in felids.

In a previous study [10], the elimination half-life of eCG in the cat was estimated at ~45 h, based on i.m. delivery and a brief sampling period (i.e., 12–84 h). In the present expanded study involving i.v. administration, extensive blood sampling, and more sophisticated analysis, we determined that the eCG disposition curve best fits a two-compartment model, characterized by distinct exponential distribution and elimination phases. The half-life of eCG elimination was ~23 h, substantially shorter than our earlier estimation. Compared to pharmacokinetic eCG values reported for the sheep [45, 46], cow [47], and rat [48], values for elimination half-life, volume of distribution, and clearance rate in the cat generally fell within an intermediate range. Elimination kinetics in these other species also best fit a two-compartment model, with elimination half-lives ranging from 6 (rat) to 120 (cattle) h. Clearance rates and volumes of distribution are more similar among species, ranging from 1.3 (sheep) to 2.1 (rat) ml/h per kg and from ~50 (rat) to 94.5 (sheep) ml/kg, respectively. In cattle and sheep, eCG reportedly persists in circulation for 120–240 h after injection [46, 47]. In cats, eCG was detectable in blood for at least 120 h, an observation consistent with its proven capacity to promote folliculogenesis after a single injection in multiple felid species [2, 3, 5, 7, 8].

Although hCG has a lower molecular mass than eCG (~39 kDa vs. ~64 kDa) and a lower percentage of carbohydrate (33% vs. 45%) [24, 49, 50], it demonstrated very similar pharmacokinetic properties after i.v. injection. Similar to findings in the human and monkey [51, 52], hCG elimination in the cat best fits a biexponential model. Injected hCG in humans has a slower clearance rate and a longer elimination half-life (~36 h) [51] than observed in

the cat. By contrast, the clearance rate and elimination half-life of hCG for the cat were relatively prolonged compared to values for the monkey [52] and rat [53]. Both eCG and hCG had limited volumes of distribution relative to the measured plasma volume of the cat (46.8 ml/kg) [54], possibly reflecting the large molecular size and low lipid permeability of both glycoproteins [46]. Also related to these physicochemical properties, both gonadotropins exhibited reduced bioavailability after i.m. injection, with only 50–60% of injected gonadotropin entering circulation. Despite similar pharmacokinetic traits, hCG persistence in circulation (~96 h) was generally less than that of eCG, possibly due to differences in total injected dosage (75 IU vs. 100 IU) and sensitivities of the respective RIAs. However, given that ovulation occurs approximately 30 h after hCG administration [4, 55], this persistence indicates that hCG is present in circulation for ~66 h postovulation, likely promoting postovulatory follicular growth and affecting luteinization of ovulated and/or unovulated follicles.

Importantly, pharmacokinetic patterns in cats given eCG+hCG were not appreciably different from those obtained with eCG or hCG administered alone. Because gonadotropins influence FSH and LH receptor expression [56, 57], the pharmacokinetics of gonadotropins administered in sequential combination could be affected by increased or decreased receptor induction and receptor-ligand interaction. In other species, the circulatory clearance of eCG and hCG is primarily mediated through renal, hepatic, and plasma-associated metabolism, with the ovary playing a limited role [58, 59]. Hepatic and renal function in gonadotropin clearance has not been investigated in the cat, but humoral immune responses to these foreign proteins can affect in vivo bioactivity, presumably by decreasing circulatory persistence [60, 61]. Because of the naive immunological status of study females and the expected natural lag time associated with primary immune responses [61], it is unlikely that immunoglobulin production significantly influenced clearance rates reported in the present study.

Persistence of hCG postinjection and its demonstrated folliculogenic effects when given alone suggest that it is at least partially responsible for ancillary follicle development after ovulation in the cat. However, eCG, when given prior to hCG, likely stimulates some additional follicle growth and potentiates the responsiveness of early antral follicles to the intrinsic folliculogenic and luteotrophic activity of hCG. A similar relationship probably occurs with FSH+hCG regimens, because anestrual cats treated with multiple injections of FSH followed by hCG also develop ancillary follicles several days after ovulation [27]. A preeminent role for hCG in promoting postovulatory follicle growth is supported by our recent observation that neutralizing residual eCG at the time of follicular aspiration is ineffective in preventing formation of ancillary follicles [10]. Furthermore, the persistence of hCG in circulation and its prolonged luteotrophic effects may explain the luteinization of ancillary follicles to form secondary CL [10].

The disruptive significance of postovulatory ancillary follicles in the cat is unknown. Ancillary follicles forming after eCG injection and ovulation appear to be functional in the cow, as evidenced by elevated plasma estradiol concentrations detected using frequent blood sampling [62, 63]. However, in contrast to the cat, the cow usually is not treated with hCG but is allowed to generate an endogenous LH surge. It is possible that ancillary follicles in cats are not producing significant estradiol because prolonged exposure to the LH-like signal of hCG combined with rising progesterone may disengage follicular aromatase activity [64, 65]. Additionally, estradiol release in the cat is highly pulsatile, complicating accurate longitudinal assessments [23]. Finally, sustained elevations in serum estradiol that persist for several days after ovulation in eCG+hCG-treated cats also may represent a relatively slow transition from estradiol to progesterone secretion in ovulated follicles [2, 9].

Recently, fecal hormone metabolite monitoring has been used to compare estradiol production between naturally estrual, mated tigers (*Panthera tigris*) and tigers treated with eCG+hCG [66]. In that study, mean fecal estradiol values in the early postovulatory period were significantly greater in gonadotropin-treated compared to "natural" females, remaining elevated for 15 days after administration of hCG. Several of these females also produced a pronounced fecal estradiol spike 1–2 wk after hCG, suggesting the formation of ancillary follicles, previously documented laparoscopically [3], that are contributing to abnormal estradiol patterns. Similar fecal metabolite patterns are observed in the eCG+hCG-treated snow leopard (*Uncia uncia*) but are absent in the clouded leopard (*Neofelis nebulosa*) and cheetah (*Acinonyx jubatus*) [43, 44, 67]. Because cats exhibit profound species-specific differences in responsiveness to exogenous gonadotropins [7, 8], these variations in postovulatory estradiol production are likely related to natural differences in gonadotropin sensitivities among species.

The persistence of hCG and associated ancillary follicle formation could be managed by several approaches, including the use of hCG-specific antisera to neutralize residual hCG after ovulation induction [28]. An alternative approach might be to alter the ovulatory signal by perhaps substituting exogenous GnRH for hCG. Naturally estrual cats readily ovulate in response to GnRH injection [12, 68], but studies in progress in our laboratory are revealing that GnRH used in combination with eCG rarely elicits ovulation in the cat, possibly due to poor timing of LH release relative to follicular maturity and the comparatively short duration of the LH surge. The challenge is to identify an ovulation induction protocol that provides a sufficiently prolonged LH-like signal that is terminated with the onset of ovulation, ideally similar to that measured in naturally mated queens [23]. This strategy presumably would produce a more physiologically normal maternal environment, which we suspect would translate into higher fertility and pregnancy success with the use of assisted reproductive procedures in felids.

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