Production of exogenous gonadotrophin-neutralizing immunoglobulins in cats after repeated eCG–hCG treatment and relevance for assisted reproduction in felids

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Combination regimens of equine chorionic gonadotrophin (eCG) and human chorionic gonadotrophin (hCG) are used in ovarian stimulation protocols for assisted reproduction of felids. In the present study, domestic cats exhibited decreased ovarian responsiveness after repeated administration of eCG–hCG and a possible immunological mechanism for this ovarian refractoriness was investigated. An ELISA was used to analyse sera from male, naive female and previously eCG–hCG-stimulated (1 ×, 3 × and 4 ×) female cats for the presence of immunoglobulins binding to eCG, hCG and pig FSH (pFSH). The sera of cats receiving multiple eCG–hCG injections, at intervals of 44–50 days, displayed greater eCG and hCG-binding than did the sera of male, naive female or female cats stimulated once, and demonstrated variable affinity for pFSH. In preovulatory and postovulatory ovarian stimulation assays, mice injected with an eCG–antisera mixture had lower ovary masses than did mice injected with eCG–saline and fewer ovulated oocytes compared with mice treated with eCG–naive sera. Treatment of queens that were refractory to eCG–hCG with a pFSH–hCG regimen caused a rebound in development of ovarian follicles but not in oocyte maturity. These studies indicated that repeated treatment of domestic cats with eCG and hCG may cause an immunologically mediated refractoriness to ovarian stimulation. Although alternative gonadotrophin regimens may alleviate this refractoriness, a preferable strategy might be the avoidance of potential immunological complications through the cautious use of eCG and hCG in domestic and endangered nondomestic felids.

Introduction

Exogenous gonadotrophins, such as eCG and hCG, have been used for stimulating development of ovarian follicles, and maturation and ovulation of follicles in a number of mammals including cattle (Boland et al., 1991), rhesus monkeys (Wolf et al., 1990), rabbits (Maurer et al., 1968) and mice (Edwards and Fowler, 1960). Combination regimens of eCG and hCG have been developed for use with domestic cats, as a component of successful in vitro fertilization (IVF) (Goodrowe et al., 1988; Johnston et al., 1991; Donoghue et al., 1992a; Swanson and Godke, 1994) and artificial insemination (Howard et al., 1992a) strategies. These ovarian stimulation protocols have also been extrapolated to studies of IVF and artificial insemination in a number of nondomestic cats, including tigers (Donoghue et al., 1990, 1993), pumas (Miller et al., 1990; Barone et al., 1994), cheetahs (Donoghue et al., 1992b; Howard et al., 1992b) and leopards (Goodrowe et al., 1989).

Although these assisted reproductive techniques may be of value for propagation of genetically valuable domestic and nondomestic felids (Wildt, 1990; Wildt et al., 1992; Howard et al., 1993), the potential immunological consequences of administering exogenous gonadotrophins must be addressed before these procedures are applied more widely. In several mammals, the injection of exogenous gonadotrophins, either once (Bavister et al., 1986) or several times (Lin and Bailey, 1965; Jainudeen et al., 1966; Land and McLaren, 1967; Maurer et al., 1968; Greenwald, 1970; Reel et al., 1976; Ottobre and Stouffer, 1985), has been associated with a decrease in responsiveness of the ovary on subsequent stimulation attempts. This ovarian refractoriness to repeated ovarian stimulation is mediated through a humoral immune response (Jainudeen et al., 1966; Greenwald, 1970; Reel et al., 1976; Ottobre and Stouffer, 1985; Bavister et al., 1986), with gonadotrophin-binding immunoglobulins attenuating the biological activity of these proteins.

In our laboratory, a standard eCG–hCG regimen (Johnston et al., 1991; Donoghue et al., 1992a) was used with domestic cats for the generation of IVF embryos (Swanson and Godke, 1994), but a pronounced decrease in ovarian responsiveness was observed when queens were stimulated repeatedly at short intervals (44–50 days). In the present study, a possible immunological mechanism for the ovarian refractoriness in these females was investigated. The specific objectives were
to: (1) determine whether eCG- and hCG-binding immunoglobulins are present in the sera of these queens that are refractory to eCG–hCG; (2) examine the affinity of these immunoglobulins for pFSH; (3) assess the biological effect of these immunoglobulins in vivo using mouse ovarian stimulation assays; and (4) evaluate the effectiveness of an alternative gonadotrophin regimen for ovarian stimulation of queens that are refractory to eCG–hCG.

Materials and Methods

Animals

Adult (estimated age range, 1–5 years) male (n = 2) and female (n = 16) domestic cats obtained from random sources were housed singly or in pairs in stainless steel cages (1 m × 1 m × 1 m) and maintained in a controlled ambient environment under a standard artificial illumination cycle (12 h light:12 h dark). A commercial dry cat food diet (Feline Maintenance Diet, Science Diet, Hills Pet Products, Topeka, KS) and water were provided ad libitum. Although the reproductive history of queens was unknown, previous treatment with exogenous gonadotrophins was considered highly unlikely and all queens were classified as naive for exogenous gonadotrophin exposure.

Ovarian stimulation

Gonadotrophin-induced ovarian stimulation and laparoscopy followed established procedures (Wildt et al., 1977; Goodrowe et al., 1988; Johnston et al., 1991). Briefly, queens were monitored every 1–3 days for signs typical of behavioural oestrus, such as lordosis, treading of the hind feet and increased vocalization (Michael, 1961). Anoestrous queens were injected i.m. with 150 μg eCG (Sigma Chemical Company, St Louis, MO) and 84 h later with 100 μg hCG (Sigma). At 24–27 h after administration of hCG, queens were anaesthetized with an i.m. injection (8 mg kg⁻¹ body mass) of telatamine–zolazepam hydrochloride (Telazol; A. H. Robbins Company, Richmond, VA), placed in dorsal recumbency and prepared for surgery. Queens were examined by laparoscopy (Wildt et al., 1977; Goodrowe et al., 1988) to determine the number of mature vesicular follicles (≥2 mm in diameter) present on the ovaries. Mature follicles were transabdominally aspirated (Goodrowe et al., 1988) to recover oocytes for use in IVF experiments and recovered oocytes were assessed for maturation status; mature oocytes possessed a distinct corona radiata and expanded cumulus cell mass and immature oocytes had a tightly compacted cumulus cell investment (Goodrowe et al., 1988; Johnston et al., 1991).

Queens (n = 8) that received multiple eCG–hCG injections were stimulated at intervals of 44–50 days for the first three procedures and at intervals of 80–141 days for the fourth procedure. Six cats in this group were treated with eCG–hCG for a fifth stimulation procedure at an interval of 102 days and then challenged 63 days later with a regimen of pFSH–hCG. These queens were injected once daily (s.c.) with 1.0 mg pFSH (FSH-P, Schering-Plough Animal Health Corporation, Kenilworth, NJ) for 5 consecutive days, administered (i.m.) 100 μg hCG on day 6 and subjected to laparoscopy 24–27 h later for evaluation of ovarian response and for follicular aspiration. Blood was collected via jugular venepuncture from queens at the time of laparoscopy after the third, fourth, fifth and sixth ovarian stimulation regimens (i.e., after decreased ovarian responsiveness was noted). Serum samples were not available from these eight queens before their first eCG–hCG treatment or at laparoscopy after the first or second treatments. For a comparative control population, blood was collected from naive female cats (n = 8), the same naive cats (n = 6) at laparoscopy after their initial eCG–hCG treatment and male cats (n = 2). All sera were stored frozen at −20°C until analysed by ELISA or used in mouse ovarian stimulation assays.

Solid-phase ELISA

ELISA procedures were similar to those described by Bavister et al. (1986) with slight modification. Equine CG, hCG and pFSH (Sigma) were diluted in 60 mmol carbonate–bicarbonate 1−1 buffer (pH 9.6) to 20 ng protein μl⁻¹. Aliquots (25 μl) of the respective gonadotrophin solutions were pipetted into the wells (500 ng protein per well) of 96-well flat bottom microtitre plates (Immunon I; Dynatech Laboratories, Inc., Alexandria, VA) and the plates were incubated for 3 h at 37°C. Plates were washed five times with 0.01 mol PBS 1−1−0.1% (v/v) Tween 20 (PBS–Tw) and blotted dry. Thawed serum samples were diluted (1:100, 1:200, 1:400) with PBS–Tw and aliquots (100 μl) of each added to wells in triplicate. Plates were incubated for 1 h at room temperature (22°C) and washed five times with PBS–Tw. Horseradish peroxidase (HRP)-conjugated, affinity-purified rabbit anti-cat IgG (Zymed Laboratories, Inc., South San Francisco, CA) was added (100 μl; 1:1000 dilution in PBS–Tw) to appropriate wells and incubated for 30 min at 37°C. The plates were washed and 100 μl of o-phenylenediamine solution (Sigma; 1 mg ml⁻¹ 0.05 mol citrate 1−1 buffer [pH 4.5]) and 0.05% (v/v) H₂O₂ was added to each appropriate well. Microtitre plates were incubated in the dark for 40 min at 22°C and 50 μl 2.5 mol H₂SO₄ 1−1 was added to each well to stop the colour reaction. The absorbance of wells was measured at 492 nm using an automated microplate reader (MR5000 model; Dynatech).

Each microtitre plate included serum samples from each treatment group in addition to substrate, serum and enzyme conjugate controls. For assessment of exogenous gonadotrophin binding for individual serum samples, mean absorbance values (± SD) were determined for the triplicate wells of each sample. Mean absorbance values above the mean value for the naive serum group + 3 SD were considered positive (+) for the presence of gonadotrophin-binding immunoglobulins.

Mouse ovarian stimulation assay

Two separate mouse ovarian assays, using modified protocols described for other rodents (Murphy et al., 1984; Bavister et al., 1986), were conducted to assess the preovulatory and postovulatory effects of eCG-binding immunoglobulins in vivo. For each assay, mice (ICR strain; 25–27 days of age) were weighed (± 0.1 g) and assigned to treatments to equalize their
Table 1. Number of ovarian follicles (≥ 2 mm) and proportion of recovered oocytes assessed as mature in individual queens after sequential treatments with eCG-hCG and pFSH-hCG

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<tr>
<th>Number of follicles observed with sequential eCG–hCG and pFSH–hCG treatments*</th>
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<td>Mean number (± SEM) of follicles per queen</td>
<td>17.4 ± 3.0b</td>
<td>17.8 ± 4.2b</td>
<td>4.8 ± 0.4c</td>
<td>1.3 ± 0.6d</td>
<td>4.0 ± 2.5ed</td>
<td>9.5 ± 2.1e</td>
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<td>Proportion (%) of recovered oocytes graded mature</td>
<td>70/84 (83-3)c</td>
<td>75/86 (87-2)b</td>
<td>6/29 (20-7)c</td>
<td>3/10 (30-0)d</td>
<td>0/14 (0)ed</td>
<td>3/53 (5-7)d</td>
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*Queens were stimulated with eCG–hCG at 44–50 day intervals for the first three treatments, at 80–141 day intervals for the fourth treatment and at a 102 day interval for the fifth treatment. For the sixth treatment, queens were injected with pFSH–hCG after a 63 day interval. With each treatment, queens were subjected to laparoscopy 24–27 h after administration of hCG and ovarian follicles were aspirated to recover oocytes.

b**Within rows, values with different superscripts are significantly different (P < 0.05).

weights between groups. Sera with low (− sera; from naive cats) and high (+ sera; from cats stimulated three times) eCG-binding immunoglobulin contents (as indicated by ELISA) were each pooled, mixed with equal volumes of eCG (100 i.u. ml⁻¹ saline) and incubated for 15 min at 37°C. Mice (n = 40, assay 1; n = 48, assay 2) received i.p. injections (100 μl) of either eCG (5 i.u.)/saline, eCG (5 i.u.)− sera, eCG (5 i.u.)/+ sera or saline alone. For the first, or preovulatory, assay mice were killed by cervical dislocation 48 h after i.p. injection and the paired ovaries from each mouse were cleaned of extraneous tissue, blotted dry and weighed (± 0.01 mg). For the second, or postovulatory, assay; all mice received an injection (i.p.) of hCG (5 i.u.) 47 h after the initial treatment and the reproductive tracts were harvested 20 h later. Paired ovaries were weighed as in the first assay and the oviducts of each mouse were flushed with PBS containing 1% (v/v) fetal calf serum to allow determination of the number of ovulated oocytes.

**Results**

**Repeated ovarian stimulation**

Queens repeatedly stimulated with eCG–hCG at short intervals (44–50 days) demonstrated a significant decrease (P < 0.05) in ovarian responsiveness (as measured by the number of mature follicles) by the third stimulation attempt (Table 1). A fourth series of eCG–hCG injections resulted in a further decrease (P < 0.05) in ovarian follicle development, despite extending the stimulation interval from 44–50 days to 80–141 days. Queens (n = 6) subjected to a fifth stimulation attempt at a 102 day interval exhibited a similar (P > 0.05) mean number of mature follicles to that observed with the third and fourth stimulation procedures. Oocyte maturation demonstrated a parallel decline to number of follicles, with a lower proportion (P < 0.05) of oocytes recovered at the third, fourth and fifth stimulations exhibiting morphological characteristics typical of mature oocytes. However, treatment of these same six queens 63 days later, using a pFSH–hCG regimen, resulted in a significant increase (P < 0.05) in the mean number of follicles (9.5 ± 2.1) relative to the third, fourth and fifth stimulation attempts, but the proportion of mature oocytes (3 of 53, 5.7%) was not different (P > 0.05) from that observed with the fourth and fifth stimulations.

**Development of an ELISA**

In the development of the ELISA for this study, optimal dilutions of serum samples (1:100, 1:200, 1:400) and HRP-conjugated anti-cat immunoglobulin (1:1000) were determined to produce absorbance values for most samples within the quantifiable range of the microplate reader (absorbance < 2.0) and within the linear portion of the absorbance curve for serum.
Table 2. ELISA binding activity for exogenous gonadotrophins (eCG, hCG, pFSH) of sera from individual queens that had received three (3×) or four (4×) treatments with eCG–hCG

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<tr>
<th>Treatment group</th>
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*Absorbance values of serum samples > and ≤ absorbance value (+3 SD) of naive sera were classified as positive (+) or negative (−), respectively.

serial dilutions. The concentration of eCG, hCG and pFSH in wells (500 ng) was chosen on the basis of the optimal antigen concentration reported by Bavister et al. (1986). For each gonadotrophin, mean absorbance values of naive sera +3 SD were chosen as threshold values to provide conservative determinants of positive binding activity. Nonspecific binding was minimized by the use of a relatively high concentration (0.1% v/v) of Tween 20 in PBS for sample and enzyme conjugate dilution and for plate washing. Blocking plates with 0.5% (w/v) BSA before adding the serum sample had no appreciable effect on absorbance values for any tested sample (data not shown), so this step was omitted.

Affinity of immunoglobulins for eCG, hCG and pFSH

ELISA data for eCG binding indicated that sera obtained from naive queens (n = 8) and diluted 1:100, 1:200, and 1:400 had mean absorbance values (± SD) of 0.199 ± 0.098, 0.118 ± 0.056 and 0.066 ± 0.029, respectively. Absorbance values of individual serum samples above the naive sera means +3 SD (1:100, 0.493; 1:200, 0.286; 1:400, 0.153) were considered positive (+) for eCG binding immunoglobulins. While none of the males (n = 2), naive queens (n = 8) or queens stimulated once with eCG–hCG (n = 6) had positive (+) sera, seven of eight queens stimulated three times and six of eight queens stimulated four times with eCG–hCG had positive (+) sera for eCG-binding (Table 2).

ELISA data for hCG binding indicated that diluted (1:100, 1:200, 1:400) serum from naive queens had mean absorbance values (± SD) of 0.050 ± 0.021, 0.031 ± 0.011 and 0.020 ± 0.006, respectively. Individual serum samples were classified as positive (+) for hCG binding if mean absorbance values exceeded naive sera means +3 SD (1:100, 0.113; 1:200, 0.064; 1:400, 0.038). Sera from male cats and all naive queens were negative for hCG binding, while one queen treated once with eCG–hCG had a positive titre for hCG only. Five of eight queens treated three and four times with eCG–hCG were positive for hCG binding (Table 2). Naive sera, diluted 1:100, 1:200 and 1:400, was also assessed for pFSH binding activity, and mean absorbance values (± SD) were 0.329 ± 0.235, 0.216 ± 0.131 and 0.151 ± 0.069, respectively. Individual serum samples were considered positive (+) if the mean absorbance value was greater than the naive sera mean +3 SD (1:100, 1.034; 1:200, 0.609; 1:400, 0.358). Sera from male cats and all naive queens were negative for pFSH binding, while one queen treated once with eCG–hCG had a positive titre for pFSH only. Sera from two of eight queens stimulated three times, and three of eight queens stimulated four times, with eCG–hCG were positive for pFSH-binding activity (Table 2).

Mouse ovarian stimulation assay

In the preovulatory mouse ovarian stimulation assay, mice injected with eCG/ + sera had lower (P < 0.05) ovary masses than did mice injected with eCG–saline, but similar (P > 0.05) ovary masses to mice injected with saline alone (Fig. 1). In the postovulatory mouse ovary stimulation assay, ovary mass differed only (P < 0.05) among treatments for the saline control group (Fig. 2a). Mice injected with eCG/ + sera had fewer (P < 0.05) ovulated oocytes than did mice injected with eCG/– sera, but this value did not differ (P > 0.05) from mice injected with eCG–saline (Fig. 2b). Within both assays, mean mouse mass did not differ (P > 0.05) between treatment groups.
ovarian short in populations able Values eCG/saline injection (Fig. 2. intervals, with eCG-hCG paired (D). Preovulatory Domestic follicular 0) c 1 E 10 14 1 from (n = 10 per treatment) 48 h after injection with saline (b), eCG/ + sera (■), eCG/ - sera (■) or eCG/saline (□). Values with different superscripts are significantly different (P < 0.05).

Values of ovarian mass (mg) recovered from these follicles. Data from ELISA and mouse bioassays indicated that this quantitative and qualitative deterioration in gonadotrophin-induced folliculogenesis was a probable consequence of immunological interference with the bioactivity of eCG, hCG or both hormones.

Queens were initially stimulated with eCG–hCG at 44–50 day intervals to approximate the normal interoestrous interval and to simulate a natural pattern of ovarian cyclicity. Gonadotrophin-stimulated queens form functional corpora lutea after follicular aspiration (Goodrowe et al., 1988; Donoghue et al., 1992a) and these have a comparable secretory lifespan to the corpora lutea formed after natural mating with vasectomized males (Goodrowe et al., 1988). After sterile mating, queens typically exhibit a luteal phase (pseudo-pregnancy) of 36–38 days duration and an interoestrous interval of 40–50 days (Paape et al., 1975; Wildt et al., 1981). Although the stimulation interval mimicked natural reproductive processes, queens exhibited a pronounced decrease in ovarian responsiveness after the third eCG–hCG treatment, and this reduced responsiveness was not alleviated by lengthening the treatment interval. Because colony husbandry (caging, lighting, diet) was strictly controlled and the study time frame was relatively short (about 1 year), environmental and age-related factors were discounted as causes. Given the physicochemical properties of eCG and hCG, an immunological basis was suspected as the primary cause of this ovarian refractoriness.

On the basis of the ELISA data, most queens treated multiple times with eCG–hCG generated eCG- or hCG-binding immunoglobulins or both. As this study was partially retrospective, naive serum samples were not available from these queens and we cannot be certain that these females had negative anti-gonadotrophin titres before eCG–hCG treatment. However, the presence of pre-existing titres are unlikely; all naïve male and female cats in the comparative population had negative titres and pre-study exposure of queens to exogenous gonadotrophins was highly improbable.

This presence of both eCG- and hCG-binding immunoglobulins is not surprising since both gonadotrophins are large (eCG, 45–65 kDa molecular mass; hCG, about 37 kDa molecular mass), immunologically complex proteins. Both glycoproteins comprise noncovalently linked alpha and beta subunits, and the major difference in bioactivity is attributed to the nonhomologous portions (about 30%) of the beta subunit (Birken and Canfield, 1978; Pierce and Parsons, 1981). While it appears that eCG was more consistently immunogenic after a few injections, both eCG and hCG produced pronounced increases in immunoglobulin titres. Given the homologous nature of these gonadotrophins, some immunoglobulins may have expressed affinity for common epitopes shared by eCG and hCG.

The crossreactivity of specific sera for different gonadotrophins also reflected individual variability in the immune responses of stimulated queens. Sera that were positive for eCG binding were crossreactive for hCG or pFSH binding but not for both gonadotrophins. The selective crossreactivity of immunoglobulins to shared epitopes between eCG and hCG (possibly) or eCG and pFSH demonstrates that the immune systems of individual queens may exhibit variable recognition of the multiple antigenic sites found on exogenous

**Fig. 1.** Preovulatory mouse ovarian stimulation assay. Mean ( ± SEM) paired ovarian masses of mice (n = 10 per treatment) 48 h after injection with saline (■), eCG/ + sera (■), eCG/ - sera (■) or eCG/saline (□).

**Fig. 2.** Postovulatory mouse ovarian stimulation assay. (a) Mean ( ± SEM) paired ovarian masses of mice (n = 12 per treatment) 20 h after injection with hCG (5 µg). Mice were treated 47 h before hCG injection with saline (■), eCG/ + sera (■), eCG/ - sera (■) or eCG/saline (□). (b) Mean ( ± SEM) number of ovulated oocytes recovered from mice (n = 12 per treatment) 20 h after hCG injection. Values with different superscripts are significantly different (P < 0.05).

**Discussion**

While eCG–hCG combination regimens have proven invaluable for the application of assisted reproductive technology to populations of endangered cats, the results of the present study have shown that the repeated administration of these gonadotrophins may induce undesirable immunological consequences in cats. Domestic cats repeatedly stimulated with eCG–hCG, at short intervals, demonstrated a significant decrease in both ovarian follicular development and the maturity of oocytes recovered from these follicles. Data from ELISA and mouse bioassays indicated that this quantitative and qualitative deterioration in gonadotrophin-induced folliculogenesis was a probable consequence of immunological interference with the bioactivity of eCG, hCG or both hormones.

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gonadotrophins. This variability may be one factor in developing approaches to mitigate the development of ovarian refractoriness in individual queens.

The capacity of these gonadotrophin-binding immunoglobulins to affect exogenous gonadotrophin activity in vivo was evaluated in pre- and postovulatory mouse ovarian stimulation assays. Collectively, the results of these assays indicated an overall decreased responsiveness of mice treated with eCG mixed with eCG–hCG antisera. This observation is important since exogenous gonadotrophins may have separate biological and immunological active sites (Christakos and Bahl, 1979) and mere binding of immunoglobulins does not always neutralize biological activity (Louveit et al., 1974). In retrospect, a more pronounced difference between treatment groups may have resulted if the volume of positive sera relative to the amount of injected eCG (10 µl per iu eCG) was increased to approximate the physiological concentrations seen in immunorefractory domestic cats more closely (about 1000 µl sera per iu of injected eCG; based on estimated blood volume in cats and the standard eCG dosage).

Treatment of queens that were refractory to eCG–hCG with an alternative ovarian stimulation protocol, using pFSH, demonstrated that the ovaries of these queens are still responsive to exogenous folliculogenic stimuli. These queens were selected for pFSH treatment because their sera lacked high titres of anti-pFSH immunoglobulins and, consequently, they developed significantly more follicles than observed during the three previous eCG–hCG stimulation procedures. The maturity of recovered oocytes was still decreased, possibly due to impairment in hCG bioactivity, since five of these six queens had positive sera for hCG-binding immunoglobulins. Because control queens (i.e., nonrefractory) were not stimulated simultaneously, definite conclusions about oocyte maturity are not possible. However, a similar pFSH–hCG regimen (Pope et al., 1993) was assessed in an IVF protocol for domestic cats and 97% of recovered oocytes were classified as mature. Substitution of pLH for hCG in the present study may have resulted in higher percentages of mature oocytes. The responsiveness of refractory queens to pFSH supports the assertion that immunological interference inhibits ovarian responsiveness to eCG–hCG, but also indicates that alternative gonadotrophin stimulation protocols might be used in refractory queens, provided immunological crossreactivity is minimal. This finding is critically important for nondomestic felids, since individual cats may be irreplacable from a genetic perspective (potential founders, for example) (Foosie, 1983) but, owing to physiological or logistical constraints, may require intensive management for assisted reproduction purposes.

Natural cyclicity did not appear to be inhibited by the presence of eCG–hCG neutralizing immunoglobulins. One to two months after the fourth stimulation attempt, signs of behavioural oestrus were detected in seven of eight immunorefractory queens, and laparoscopy of four of these oestrus queens revealed the presence of multiple developing follicles on their ovaries (data not shown). The occurrence of natural cyclicity, despite the presence of gonadotrophin-neutralizing immunoglobulins, is consistent with findings in other species (Lin and Bailey, 1965; Jairudeen et al., 1966; Land and McLaren, 1967; Greenwald, 1970; Reel et al., 1976; Bavister et al., 1986). Immunoglobulins directed against exogenous gonadotrophins are frequently specific for epitopes unique to those proteins, with minimal crossreactivity for endogenous gonadotrophins (FSH, LH). In the present study, endogenous FSH bioactivity appeared unaffected, but the impact of circulating immunoglobulins on endogenous LH function and ovulation induction after natural mating was not assessed. Particularly for endangered nondomestic species, it is essential that assisted reproductive techniques do not compromise the future potential of animals to bear offspring naturally.

Although studies in other species have also reported ovarian refractoriness to repeated exogenous gonadotrophin administration, few of these studies have attempted to investigate systematically the nature of the immunological response and examine its specific impact on gonadotrophin-induced reproductive function. Furthermore, these immunological consequences have not been investigated in cats, despite the increasing use of eCG–hCG combination regimens in domestic and endangered nondomestic cats, and the potential implications for these species. The advantages of these exogenous gonadotrophins, in inducing desirable physiological responses (after a single injection), make their continued use of importance for the future reproductive management of genetically valuable or endangered cat populations. However, immunological factors dictate that these gonadotrophin combinations should be used judiciously in cats and that the suitability of alternative ovarian stimulation protocols should be investigated further.

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