The effect of pre-ovulatory anaesthesia on ovulation in laparoscopically inseminated domestic cats

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Summary. Laparoscopic intrauterine artificial insemination (AI) of electroejaculated spermatozoa was used to compare embryo development and conception rates in domestic cats inseminated either before or after ovulation. Females were given a single (100 iu) injection of pregnant mares' serum gonadotrophin (PMSG) followed by either 75 or 100 iu human chorionic gonadotrophin (hCG) 80 h later. Cats were anaesthetized (injectable ketamine HCl/acepromazine plus gaseous halothane) 25–50 h after administration of hCG for laparoscopic assessment of ovarian activity and for transabdominal AI into the proximal aspect of the uterine lumen. At the time of AI, 23 cats were pre-ovulatory (25–33 h after hCG injection) and 30 were post-ovulatory (31–50 h after hCG injection). Pre-ovulatory females produced 10.5 ± 1.1 follicles and no corpora lutea compared with 1.9 ± 0.5 follicles and 7.5 ± 0.9 corpora lutea for the post-ovulatory group ($P < 0.05$). Six days later, the ovaries of nine pre-ovulatory and 12 post-ovulatory females were re-examined and the reproductive tracts flushed. On this day, pre-ovulatory cats produced fewer corpora lutea (2.8 ± 1.5 ; $P < 0.05$) and embryos (0.4 ± 0.3 ; $P < 0.05$) than post-ovulatory females (18.9 ± 3.3 corpora lutea; 4.6 ± 1.2 embryos). Two of the 14 cats (14.3%) inseminated before ovulation and not flushed became pregnant compared with 9 of 18 cats (50.0%) inseminated after ovulation and up to 41 h after hCG injection ($P < 0.05$). These results indicate that ovulation in cats is compromised by pre-ovulatory ketamine HCl/acepromazine/halothane or laparoscopy or by both and that electroejaculated spermatozoa deposited by laparoscopy *in utero*, after ovulation, result in a relatively high incidence of pregnancy. Because ovulation usually occurs 25–27 h after injection of hCG, the lifespan for fertilization of the ovulated ovum appears to be at least 14 h *in vivo* in cats.

Keywords: ovulation; anaesthesia; artificial insemination; laparoscopy; cat

Introduction

The domestic cat is important in biomedical research, serving as an animal model for 36 anomalies and metabolic defects homologous to human diseases (Migaki, 1982). The cat is also a valuable model for conservation biology studies because virtually all wild felids are at risk of extinction (Wildt, 1991). Reproductive performance of laboratory cats is poor, especially in populations that have genetic diseases contributing to mortality at an early age or that compromise reproductive behaviour or copulatory ability. Artificial insemination (AI) could be beneficial for (i) individuals that fail to mate because of sexual incompatibility or physical/medical handicaps; (ii) increasing the genetic diversity within a population and expanding the gene pool through breeding of selected individuals and (iii) safely distributing semen between different geographical locations without the risk and expense of transporting live animals. The development of AI techniques in domestic cats may also accelerate the use of artificial breeding in rare, nondomestic felids.

Pregnancies have been achieved in domestic cats after vaginal deposition of semen into anaesthetized queens, but the incidence of pregnancy was only 10.6% (Platz *et al.*, 1978). This

low conception rate may be related to improper site of deposition of spermatozoa resulting in compromised sperm transport, or timing of insemination or both with respect to ovulation. We determined that intrauterine insemination using a laparoscopic transabdominal approach was effective for overcoming problems of transport of spermatozoa in common ferrets (Wildt *et al.*, 1989). Vaginal deposition of spermatozoa into ferrets failed to result in pregnancies, whereas depositing spermatozoa *in utero* resulted in high pregnancy rates (70%) using fresh (Wildt *et al.*, 1989) or frozen-thawed (Howard *et al.*, 1991a) semen.

The aims of this present study were to (i) assess the efficacy of laparoscopic intrauterine AI in the domestic cat; and (ii) determine the relationship between onset of ovulation (as potentially influenced by anaesthesia/laparoscopy) and the deposition of spermatozoa into the reproductive tract. Specific objectives were to evaluate the influence of dose of human chorionic gonadotrophin (hCG) on ovarian activity, ovulation and embryo production and compare fertilization, embryo development and pregnancy rate in females inseminated either before or after ovulation. To determine the likelihood that cats were either pre-ovulatory or post-ovulatory, we used the following information. Ovulation in domestic cats occurs only after mating or a comparable artificial stimulus (Wildt & Seager, 1978; Wildt *et al.*, 1980). Ovarian follicular activity can be stimulated by a single injection of pregnant mares' serum gonadotrophin (PMSG) (Cline *et al.*, 1980). Mature ovarian follicles ovulate approximately 25–27 h after the administration of hCG (Hamner *et al.*, 1970; Sojka *et al.*, 1970).

Materials and Methods

Animals and facilities

Adult cats from random sources (6 males; 54 females) were maintained at the National Institutes of Health (NIH) Animal Center in Poolesville, MD. Males were maintained separately and females were housed in groups of two in stainless-steel cages (83 cm high $\times$ 78 cm wide $\times$ 91 cm deep). Dry, commercial cat food (Purina Cat Chow, Ralston Purina Co., St Louis, MO, USA) and water were available *ad libitum*.

Induction of follicular development and ovulation

Females that did not demonstrate oestrous behaviour (Wildt *et al.*, 1978) were given a single (100 iu) intramuscular (i.m.) injection of PMSG (Sigma Chemical Co., St Louis, MO, USA) to induce ovarian follicular development. Eighty hours later, an injection of either 75 ($n = 26$ females) or 100 ($n = 28$ females) iu hCG (Sigma Chemical Co.) was administered i.m. to induce ovulation. Females were anaesthetized for laparoscopy and AI 25–50 h after hCG injection.

Anaesthesia

Food and water were withheld from males for 12 h and females for 24 h before scheduled anaesthesia. For semen collection, anaesthesia was induced with an i.m. injection of ketamine hydrochloride (HCl) (Vetalar, Parke-Davis, Morris Plains, NJ, USA; 25.0 mg kg⁻¹). For laparoscopy of females, a surgical plane of anaesthesia was induced with a combination of ketamine HCl (20.0 mg kg⁻¹; i.m.) and acepromazine (Ayerst Labs, Rouses PT., NY, USA; 0.18 mg kg⁻¹; i.m.) and maintained with halothane gas (1–2%) administered via a face mask.

Semen collection and processing

Semen was recovered by electroejaculation from six proven-breeder males (nine ejaculations per male) and evaluated as described previously (Wildt *et al.*, 1983; Howard *et al.*, 1986, 1990). An aliquot of the ejaculate was examined immediately at 250 $\times$ for subjective estimates of the percentage of motile sperm (0 to 100%) and sperm progressive motility on a 0 to 5 scale (0 = no movement; 5 = rapid forward progression). The number of spermatozoa per ml of ejaculate was determined using a haemocytometer. Sperm morphology was assessed by fixing ejaculate aliquots (5 μ l) in 1% glutaraldehyde, followed by phase-contrast microscopic examination of 200 sperm per aliquot at 1000 $\times$. Semen samples containing at least 50% sperm motility and 60% structurally normal spermatozoa were transferred into a sterile 1.5 ml conical tube (Sarstedt Inc., Princeton, NJ, USA) and slowly diluted with an equal volume of Ham's F10 medium containing 5% fetal calf serum (Johnston *et al.*, 1991; Irvine Scientific, Irvine, CA,

USA). Diluted semen was centrifuged (300 *g* for 10 min), the supernatant discarded and the sperm pellet resuspended gently in 210 μ l of fresh medium. The diluted suspension was assessed for concentration, motility and progressive motility of spermatozoa. Samples were maintained at room temperature (23°C) and shielded from light until used for AI. A total of 200 μ l of the sperm suspension was used for AI of each female (100 μ l per horn). The total number of motile spermatozoa per AI was calculated by multiplying the concentration of spermatozoa by the inseminate volume by sperm % motility.

Laparoscopic AI

Each female was subjected to laparoscopy as described earlier (Wildt, 1980). In brief, an intra-abdominal pneumoperitoneum was produced using a Verres needle (inserted lateral to the midline) attached to an automatic insufflator (Richard Wolf Medical Instruments Corp., Rosemont, IL, USA) containing 100% CO₂. A 7 mm diameter trocar cannula was inserted through a 1 cm skin incision made 3 cm cranial to the umbilicus. The trocar was removed and replaced with a rigid, 7 mm diameter laparoscope (Richard Wolf Medical Instruments Corp.) attached to a light source (Fig. 1a). All aspects of each ovary were examined for pre-ovulatory follicles and post-ovulatory corpora lutea using previously published criteria (Wildt & Seager, 1978). In brief, pre-ovulatory vesicular follicles (2–4 mm in diameter) were clear in appearance, spherical in shape and generally flattened or only slightly raised above the ovarian surface. In contrast, the early developing post-ovulatory corpora lutea (each approximately 4 mm in diameter) were opaque, dark red and prominently raised 2–3 mm above the ovarian surface. Cats demonstrating one or more pre-ovulatory follicles and no corpora lutea were classified as pre-ovulatory, whereas, females exhibiting at least one corpus luteum were classified as post-ovulatory regardless of the number of follicles present.

Fig. 1. Laparoscopic intrauterine insemination in domestic cats. (a) The laparoscope was initially used to identify the reproductive tract and (b) accessory forceps were used to grasp the uterine horn. (c) The horn then was elevated and a catheter punctured through the ventral abdominal wall and inserted into the uterine lumen. (d) The stylette was withdrawn from the catheter and tubing containing spermatozoa was guided through the catheter into the uterine lumen for deposition of spermatozoa.

Intrauterine AI was facilitated by inserting an accessory forceps (Semm's Atraumatic Grasping Forceps, no. 8383.14, Richard Wolf Medical Instruments Corp.) 3 cm caudal and 4 cm lateral to the umbilicus to stabilize the uterus (Fig. 1b). Each uterine horn was elevated toward the ventral abdominal wall and cannulated for deposition of spermatozoa *in utero* using a sterile, feline indwelling catheter (Sovereign, Sherwood Medical, St Louis, MO, USA; 20 gauge, 5 cm long). The catheter was inserted percutaneously into the proximal third of the uterine lumen under laparoscopic observation (Figs 1c, 2). After penetrating the lumen, the stylette was withdrawn slightly within the catheter and the latter advanced. The stylette then was withdrawn completely and replaced with sterile, polyethylene tubing (PE-10, Intramedic, Clay Adams, Parsippany, NJ, USA) attached to a 30 gauge needle and a 1 ml syringe containing 100 μ l sperm suspension (Fig. 1d). Tubing was advanced through the catheter into the uterine lumen for AI. After depositing a second 100 μ l sperm sample into the contralateral horn, the catheter, tubing and laparoscopic instruments were removed and the incision sites sutured. The entire anaesthesia/laparoscopy interval (from anaesthesia induction through suturing) was 30–45 min. Additionally, semen was deposited into the uterus of each cat within 30 min to 1 h of ejaculate processing.

Fig. 2. Laparoscopic insemination of a domestic cat using a grasping forceps (arrow) to stabilize the left uterine horn (UH) and a catheter (arrowhead) inserted percutaneously into the uterine lumen for deposition of spermatozoa.

Embryo collection and evaluation

Six days after anaesthesia/AI, 21 females classified as either pre-ovulatory ($n = 9$) or post-ovulatory ($n = 12$) were subjected to ovariectomy for re-examination of ovarian activity and embryo collection. Of the 21 cats, 10 (4 pre-ovulatory and 6 post-ovulatory) and 11 (5 pre-ovulatory and 6 post-ovulatory) females had received 75 and 100 iu hCG, respectively. In each female, ovaries were assessed for the number of follicles and corpora lutea, and the reproductive tract was flushed from the oviduct to the uterine bifurcation for embryo recovery. The number of unfertilized ova and the number and developmental stage of embryos were determined (Goodrowe *et al.*, 1988a). Percentage fertilization was calculated as the number of embryos divided by the total number of unfertilized ova plus embryos recovered $\times 100$. A quality grade (QG) of 1, 2, 3 or 4 was assigned to embryos of excellent, good, fair or degenerate quality, respectively (Schiewe *et al.*, 1987; Goodrowe *et al.*, 1988a). Embryos classified as QG 1 or QG 2 contained blastomeres that were spherical (or only slightly asymmetrical) in shape and uniformly dark. QG 3 embryos contained blastomeres that were asymmetrical, pyknotic or pale, and QG 4 embryos contained blastomeres that were degenerate or lysed (Fig. 3).

Pregnancy determination

The remaining 32 females, which were inseminated and classified as either pre- ($n = 14$) or post- ($n = 18$) ovulatory, were monitored for pregnancy and allowed to complete gestation. Of these females, 16 (6 pre-ovulatory and 10 post-ovulatory) and 16 (8 pre-ovulatory and 8 post-ovulatory) had received 75 and 100 iu hCG, respectively.

Fig. 3. Embryos of domestic cats recovered 6 days after AI were assigned a quality grade (QG) of 1 (excellent), 2 (good), 3 (fair) or 4 (degenerate). (a) Two-cell embryo, classified as QG 3 owing to the presence of pyknotic blastomeres; (b) eight-cell embryo, classified as QG 3 owing to asymmetrical blastomeres; (c) morula, classified as QG 2; and (d) expanded blastocyst, classified as QG 1.

Gestation length (defined as the interval from day of AI to day of parturition) and the number of kittens produced per litter were recorded.

Analysis of data

Values are reported as means $\pm$ standard error of the means (SEM). Differences in the mean number of follicles, corpora lutea, motile spermatozoa inseminated, embryos recovered, gestation interval and litter size between the pre-ovulatory and post-ovulatory groups of cats and the influence of dose of hCG on ovarian activity within each group were analysed using a Student's *t* test (Snedecor & Cochran, 1980). Data expressed as a proportion (pregnancy rates between cat groups) were compared by χ^2 analysis (Snedecor & Cochran, 1980).

Results

The dose of hCG (75 versus 100 iu) had no effect ($P > 0.05$) on the number of queens ovulating, or the number of follicles or the number of corpora lutea detected on the day of insemination. Likewise, dose of hCG did not influence ($P > 0.05$) ovarian activity or the number and quality of embryos recovered 6 days later. Pregnancy rates were also similar ($P > 0.05$) between the cats receiving 75 or 100 iu hCG. Data from the two hCG groups were therefore pooled.

A high proportion of females (53 of 54; 98.1%) demonstrated follicular activity after PMSG/hCG treatment. At the time of AI, both ovaries of 23 females contained only pre-ovulatory follicles. A retrospective analysis of the times of hormone injection in this pre-ovulatory group revealed that hCG had been given 25–33 h earlier. At the time of AI, 30 cats had begun ovulation based on the presence of at least one corpus luteum. In this post-ovulatory group, hCG had been administered 31–50 h earlier. At the time of AI, pre-ovulatory females produced more follicles and fewer corpora lutea ($P < 0.05$) than post-ovulatory females (Table 1). Characteristics of the inseminate were similar ($P > 0.05$) between the two groups (Table 1). Overall, the mean percentage sperm motility, progressive motility, structurally normal spermatozoa and total number of motile spermatozoa inseminated per female were 76 ± 3 , 4.1 ± 0.1 , 67.2 ± 5.0 and $6.2 \pm 0.9 \times 10^6$, respectively.

Six days after AI, the ovaries of the pre-ovulatory females still contained more follicles and fewer corpora lutea than those of post-ovulatory females ($P < 0.05$; Table 1). At this time, the gross appearance of the follicles did not differ between groups. In the post-ovulatory group, there was a 2.5-fold increase ($P < 0.05$) in the number of follicles and corpora lutea observed 6 days after AI than in those detected on the day of insemination (Table 1).

Cats inseminated after ovulation produced about 11-fold more ($P < 0.05$) embryos than those inseminated before ovulation (Table 1). Neither the percentage fertilization (overall rate, $67.5 \pm 13.0\%$) nor the mean number of unfertilized ova per female (overall mean, 0.7 ± 0.5) differed ($P > 0.05$) between the two groups. A wide range in stage of development was observed in embryos recovered at 6 days after AI (range, two-cell to blastocysts; Table 2; Fig. 3), but this variation was independent of pre-ovulatory versus post-ovulatory AI time (data not shown). Nevertheless, 57.1% of all embryos were morulae or blastocysts, and the mean quality grade of these embryos was higher ($P < 0.05$) than that of any of the earlier stage classifications (Table 2). A large proportion (96.7%) of the morulae and blastocysts were classified as QG 1 or QG 2, whereas none of the two- to 16-cell embryos were rated as QG 1, and only 9.1% of these earlier stage embryos were classified as QG 2. Of the blastocysts recovered, 80% and 20% were graded as excellent (QG 1) and good (QG 2) quality, respectively. Expanded blastocysts were observed in 80% of the cats producing blastocysts and constituted 30% of the total blastocysts recovered. Of the morulae recovered, 30%, 60% and 10% were classified as QG 1, QG 2 and QG 4, respectively.

A 3.5-fold increase in the incidence of pregnancy occurred when cats were inseminated after ovulation compared with those before ovulation (Table 1). In the pre-ovulatory group, both queens that became pregnant were given hCG 26 h before insemination. In the post-ovulatory group, the mean interval between hCG administration and AI was 37.9 ± 0.9 h (range, 33–41 h). The number of motile spermatozoa inseminated in all queens that became pregnant ($6.6 \pm 1.3 \times 10^6$; range

Table 1. Insemination of domestic cats before or after ovulation and either ovari hysterectomized 6 days after AI or allowed to complete gestation^a

	Pre-ovulation insemination	Post-ovulation insemination
Day of Insemination		
Number of females	23	30
Number of follicles per female	10.5 ± 1.1 ^b (5–18)	1.9 ± 0.5 ^c (0–11)
Number of corpora lutea per female	0.0 ^b (0)	7.5 ± 0.9 ^c (2–23)
Sperm motility of inseminate (%)	75 ± 4	76 ± 2
Sperm progressive motility of inseminate (scale 0–5)	4.1 ± 0.1	4.1 ± 0.1
Structurally normal spermatozoa per inseminate (%)	70.2 ± 5.4	65.2 ± 4.8
Number of motile sperm inseminated per female (× 10 ⁶)	5.5 ± 1.3	6.8 ± 0.7
Ovari hysterectomy – 6 days after AI		
Number of females	9	12
Number of follicles per female	8.8 ± 3.3 ^b (4–22)	5.3 ± 1.6 ^c (0–20)
Number of corpora lutea per female	2.8 ± 1.5 ^b (0–8)	18.9 ± 3.3 ^c (2–34)
Number of embryos per female	0.4 ± 0.3 ^b (0–2)	4.6 ± 1.2 ^c (0–11)
Term pregnancy		
Number of females	14	18
Number of pregnant females	2 ^b	9 ^c
Pregnancy rate	14.3% ^b	50.0% ^c

^aValues are means ± SEM. Values in parentheses represent ranges.^{b,c}Within rows, means with different superscripts are significantly different ($P < 0.05$).**Table 2.** Mean (±SEM) number, developmental stage and quality grade of embryos recovered 6 days after AI in domestic cats^a

	Number of embryos per female	Quality grade
Total embryos	6.3 ± 1.2	2.3 ± 0.2
Two- or four-cell embryos	1.2 ± 0.4	3.4 ± 0.2 ^b
Eight- or 16-cell embryos	1.5 ± 0.4	3.4 ± 0.2 ^b
Morulae	1.3 ± 0.6	1.9 ± 0.3 ^c
Blastocysts	2.3 ± 1.2	1.2 ± 0.1 ^c

^a $n = 9$ females.^{b,c}Within columns, means with different superscripts are significantly different ($P < 0.05$).

$(2.4\text{--}19.2) \times 10^6$) was not different ($P > 0.05$) from that used in the nonpregnant cats (mean $(6.3 \pm 0.8) \times 10^6$; range $(1.3\text{--}12.3) \times 10^6$). The overall mean duration of gestation and litter size for all cats was 67.6 ± 0.5 days (range 66–70) and 1.8 ± 0.3 kittens (range 1–4), respectively. These values did not differ ($P > 0.05$) between the pre-ovulatory and post-ovulatory groups.

Discussion

A single injection of PMSG followed by hCG effectively stimulated ovarian activity in domestic cats to allow successful AI. However, efficiency of AI was based on the time of the anaesthesia required for laparoscopy and deposition of spermatozoa *in utero*. If ovulation had already commenced by the time of AI, a relatively high pregnancy rate could be expected. However, if anaesthesia/laparoscopy was performed with only pre-ovulatory follicles on the ovaries, then ovulation was usually inhibited and pregnancy rate was low.

The dose of PMSG used here may have been excessive. In the post-ovulatory group, the PMSG treatment produced an expected (normal) number of corpora lutea by the day of laparoscopic AI; but 6 days later, the ovaries were hyperstimulated as evidenced by almost 19 corpora lutea per female. These ancillary corpora lutea probably originate from a secondary wave of follicular activity provoked by the long biological half-life of PMSG that causes continual recruitment of antral follicles (Moor *et al.*, 1984). The adverse effects of this secondary wave are probably more related to altered endocrine profiles rather than to 'extra' oocytes. Goodrowe *et al.* (1988a) compared embryo recovery and endocrine profiles among mated, naturally oestrous and follicle-stimulating hormone pituitary FSH-treated cats and found that the gonadotrophin-treated females produce altered oestradiol and progesterone profiles and fewer embryos of overall lower quality. Tsutsui *et al.* (1989) confirmed this observation and further demonstrated that pituitary FSH-treated queens occasionally abort during the first 4 weeks of pregnancy owing to decreasing progesterone concentrations indicating inadequate luteal function. In this study, flushing the reproductive tract and examining the embryos for developmental stage and quality demonstrated that these PMSG-treated queens were experiencing considerable ovum and embryo loss. Although about 57% of the recovered embryos were classified as morulae or blastocysts of good to excellent quality at 6 days after AI, most of the remaining embryos were retarded in development and of poor/degenerative quality. On the basis of our *in vitro* data (Johnston *et al.*, 1991), cat embryos can be expected to be at the morula- or blastocyst-stage of development 5–6 days after mature oocytes are exposed to spermatozoa. The present study therefore suggests that as many as 43% of the fertilized oocytes did not survive during the early stages of development. The pregnancy interval in the naturally oestrous/naturally mated cat averages 63 days (Schmidt *et al.*, 1983), which is 3–7 days shorter than the duration of gestation measured in this study. A similar finding has been noted in cats receiving repeated PMSG injections to stimulate oestrus (Colby, 1970). Because the latter cats were not anaesthetized or artificially inseminated, it is possible that these prolonged gestations also resulted from perturbed endocrine profiles caused by suboptimal gonadotrophin therapy. We have studies in progress to determine whether a lower gonadotrophin dose could stimulate oestrus in cats without causing ovarian hyperstimulation.

Our findings of a higher pregnancy rate in domestic cats inseminated after onset of ovulation (50.0%) compared with before ovulation (14.3%) appears related to an inhibition and/or delay of ovulation caused by the pre-ovulatory administration of anaesthesia. After ketamine HCl anaesthesia, circulating cortisol is increased significantly for at least 60 min in male cats (Carter *et al.*, 1984). It is therefore possible that we provoked an acute stress response resulting in high cortisol concentrations which compromised ovulation. Restraint, high temperature or administration of adrenocorticotrophin releasing hormone (ACTH) reduce conception and embryo survival in mice, rats, sheep and pigs (Euker & Riegle, 1973; Doney *et al.*, 1976; Kittinger *et al.*, 1980; Hemsworth *et al.*, 1986). Administration of ACTH to pigs interferes with ovulation by changing the timing or suppressing the luteinizing hormone (LH) peak (Hennessy & Williamson, 1983). The endogenous secretion of LH and subsequent ovulation in cats also appear sensitive to anaesthetics. Johnson & Gay (1981) reported that using ketamine HCl, halothane or pentobarbital in conscious, gonadectomized cats promptly reduces both the frequency and amplitude of pulsatile LH peaks and mean circulating LH concentrations. When anaesthesia was induced in another study with ketamine HCl 22 h after mating, only 7 of 15 females (46%) ovulated (Shille *et al.*, 1983).

When anaesthesia was delayed and performed 28–40 h after mating, 10 of 14 cats (71%) ovulated. In retrospect and in the context of the present results, it appears that the use of ketamine HCl in mated or gonadotrophin-primed, pre-ovulatory cats suppresses ovulation in the late follicular stage of the cycle. This conclusion is also supported by earlier AI studies. Sojka *et al.* (1970) reported a 50% conception rate in non-anaesthetized, naturally oestrous queens induced to ovulate with 50 iu hCG. In contrast, Platz *et al.* (1978) achieved only a 10.6% conception rate in queens anaesthetized with ketamine HCl using frozen–thawed spermatozoa. Although this relatively low rate of efficiency was originally attributed to low viability of spermatozoa after cryopreservation, it is also likely that ovulation was suppressed by the anaesthetic in some of these queens.

The mechanism by which anaesthesia compromises ovulation after hCG injection is unknown. Since hCG has been demonstrated to compete with LH for the same receptors in ovarian tissue, the site of dysfunction may be the ovary with specific uncoupling of the LH–receptor complex on the granulosa cell surface, thus reducing cyclic AMP production needed to activate protein kinases and protein phosphorylation events essential for ovulation (Koch *et al.*, 1974). The mechanism by which these anaesthetics specifically influence this sequence remains to be studied.

The laparoscopic intrauterine insemination technique was effective for artificial breeding in domestic cats. Although vaginal deposition of spermatozoa was not assessed in this study, AI trials in other carnivore and livestock species have demonstrated the advantages of depositing spermatozoa directly into the uterus. Vaginal AI with fresh or frozen semen fails to result in pregnancies in the blue fox (*Alopex lagopus*), despite using specialized equipment for preventing seminal backflow from the vagina (Aamdal *et al.*, 1972). In contrast, intrauterine deposition of spermatozoa via the cervix in the blue and silverfox (*Vulpes vulpes*) results in pregnancy rates comparable to those obtained after natural mating (Fougner, 1989). Our studies in domestic ferrets have confirmed the ineffectiveness of vaginal AI, whereas laparoscopic insemination *in utero* consistently results in pregnancy rates of 70% or greater using fresh or thawed spermatozoa (Wildt *et al.*, 1989; Howard *et al.*, 1991a). In sheep, abnormal transport of spermatozoa through the cervix has been associated with gonadotrophin stimulation, oestrous synchronization treatments and reduced viability of frozen–thawed spermatozoa (Evans & Armstrong, 1984; Hawk *et al.*, 1987). However, intrauterine insemination via laparotomy (Trounson & Moore, 1974) or laparoscopy (Killeen & Caffery, 1982; Armstrong & Evans, 1984; Schiewe *et al.*, 1984) has circumvented the problem of poor transport of spermatozoa. The cervix and its secretions have also been shown to play a ‘filtering’ role in preventing certain types of morphologically abnormal spermatozoa from reaching the site of fertilization (Freundl *et al.*, 1988). If this is the case in felids, then perhaps the cervix and cervical mucus also act as a partial barrier to some normal spermatozoa as well. Because many felid species ejaculate relatively low concentrations of spermatozoa and a relatively high incidence of abnormal forms with compromised sperm function (Wildt *et al.*, 1983; Howard *et al.*, 1984, 1990, 1991b, 1991c; Wildt, 1991), it is understandable that vaginal insemination may be inefficient. Bypassing the cervical barrier undoubtedly places more spermatozoa near the site of fertilization.

Although the minimum number of spermatozoa necessary to achieve a pregnancy in domestic cats is unknown, we showed that conceptions occurred when as few as 2.4×10^6 motile spermatozoa were deposited *in utero*. Domestic cats produce low volume ejaculates (about 100 μ l), and the number of motile spermatozoa per ejaculate ranges from $(17 \text{ to } 24) \times 10^6$ (Howard *et al.*, 1990). The ejaculate from a single male can therefore be divided and used to inseminate several females. A similar strategy was effective in domestic ferrets, a species producing approximately 28×10^6 motile sperm per ejaculate (Wildt *et al.*, 1989; Howard *et al.*, 1991a). Ferret pregnancies result after intrauterine AI using as few as 1.4×10^6 motile spermatozoa.

Because pregnancies resulted in cats when AI was conducted as late as 41 h after hCG injection, and, assuming that ovulation usually occurred 25–27 h after hCG injection (Hamner *et al.*, 1970; Sojka *et al.*, 1970), ova of domestic cats must retain their fertilizing capacity *in vivo* for at least 14 h

after ovulation. We have demonstrated from *in vitro* studies that unovulated oocytes of cats remain viable within the follicle for extended periods before collection for subsequent *in vitro* fertilization (Goodrowe *et al.*, 1988b; Donoghue *et al.*, 1992). Adult cats were treated with PMSG followed by hCG 72, 80, 84, 88, 92 and 96 h later. Follicular oocytes were aspirated laparoscopically 25–29 h after hCG and co-cultured *in vitro* with ejaculated spermatozoa. Oocytes in each PMSG–hCG interval were fertilized *in vitro*. Cat ova are therefore sustained for long periods both within the follicle (before ovulation) and the oviduct (after ovulation).

In summary, AI in anaesthetized, gonadotrophin-treated cats was efficient when freshly ejaculated, washed spermatozoa were deposited laparoscopically, but only after the onset of ovulation. We have used this procedure to help propagate laboratory cat models with lysosomal storage diseases including mucopolysaccharidosis (Howard *et al.*, 1992). There is also no reason why laparoscopic AI could not be used to infuse genetic diversity into genetically stagnant populations or to assist in the captive breeding of rare species. In this context, we have extended the laparoscopic AI technique to the endangered leopard cat (*Felis bengalensis*), a small (2–5 kg body weight) felid whose natural habitat extends through much of Asia (Howard & Wildt, 1990). One pregnancy has resulted after PMSG–hCG administration and intrauterine AI performed 38 h after hCG injection (Howard, 1991a). The leopard cat was post-ovulatory at the time of AI. The laparoscopic AI technique has also been successful in the highly endangered cheetah (*Acinonyx jubatus*), a species exhibiting extreme lack of genetic variation and a high incidence of teratospermia (approximately 70% abnormal spermatozoa per ejaculate) (Wildt, 1991; Wildt *et al.*, 1983; Howard *et al.*, 1984, 1991c). A single cheetah cub was produced after a modified PMSG–hCG stimulation regimen and intrauterine insemination in a post-ovulatory female (Howard, 1991b). If this technique is to be extrapolated to other wild felids in which limited gamete information is available, knowing that AI can be performed up to 41 h after hCG injection provides a relatively wide window of opportunity to ensure successful gamete interaction *in vivo*.

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