BIOLOGY OF REPRODUCTION **61,** 188–194 (1999)

Circannual Variations in Intraovarian Oocyte but Not Epididymal Sperm Quality in the Domestic Cat[1]

Rebecca E. Spindler[2] and David E. Wildt

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

ABSTRACT

Ovaries and testes were collected throughout the year from domestic cats spayed and neutered at local veterinary clinics. Fresh oocytes recovered from minced ovaries were subjected to in vitro maturation and then stained to determine stage of maturation or were inseminated with conspecific sperm. The cauda and corpus regions of each epididymis were dissected into pieces and placed in medium; 30 min later, the epididymal tissue was removed, the medium centrifuged, and the sperm pellet resuspended. Samples were assessed for total sperm count and sperm motility traits, morphology, acrosomal integrity, and ability to penetrate cat oocytes in vitro. Fewer excellent (grade I) oocytes were recovered per ovarian pair during September–November (mean ± SEM, 19.2 ± 2.1%) than during January–July (36.8 ± 3.6%, $p < 0.05$), while the remaining months had intermediate percentages of grade I oocytes ($p > 0.05$). A high percentage of oocytes recovered from November–April completed nuclear maturation (64.3 ± 6.8%), which was different ($p < 0.05$) from the values for May–July (32.2 ± 3.8%) and August–October (10.4 ± 2.9%). Percentage of oocytes with bound sperm was lowest ($p < 0.01$) in September and October (32.0 ± 3.1%) compared to February and March (91.4 ± 1.7%). Percentage of oocytes with sperm within the perivitelline space was highest ($p < 0.05$) in May–August (33.8 ± 4.6%) compared to all other months. In contrast, the period of highest ($p < 0.01$) fertilization (i.e., ≥ 4-cell embryo formation) was March–April (51.7 ± 3.1%) as compared to May–July (17.2 ± 1.8%) or November–January (12.4 ± 2.6%). Negligible numbers of oocytes recovered during August–October developed beyond the 2-cell stage (1.1 ± 0.3%). Blastocyst development from cleaved embryos was highest during February–April (44.3 ± 2.3%) and lowest during August–October (0.6 ± 0.1%; $p < 0.01$). Sperm recovered from the epididymides throughout the year did not differ ($p > 0.05$) in concentration or in any of the motility, structural, or functional variables evaluated. In summary, cat oocyte nuclear maturation in vitro is depressed during August–October, and the ability to form cleaved embryos remains low even when the capacity to achieve nuclear maturation is relatively high (November–January and May–July). In contrast, male cats are capable of consistently producing viable, progressively motile sperm throughout the year.

INTRODUCTION

There are no documented studies on circannual variation in gamete quality in the female or male domestic cat. Although some free-ranging, short-haired cat breeds reproduce year-round [1], most litters are born in spring or summer [2, 3], and therefore females generally are considered reproductively seasonal. This circannual variation may be an evolutionary strategy to avoid the energy-expensive period of lactation during winter months and to increase food availability for the growing litter [4]. Accordingly, this reproductive pattern is seen most often in species that inhabit extreme climates, but persists in individuals even after translocation to milder climates, possibly under the influence of photoperiod [4]. It is well known that the laboratory-maintained cat exposed to 12 h of daily light expresses estrus cyclicity (female) or produces sperm (male) throughout the year [5, 6]. Circannual variations in ovarian and testicular activity have been studied in some wild felid species—including the tiger (*Panthera tigris*) [7–9], clouded leopard (*Neofelis nebulosa*) [10], snow leopard (*Uncia uncia*) [11] and Pallas' cat (*Otocolobus manul*) [12, 13]—but largely to examine cyclicity versus reproductive quiescence rather than variations in gamete quality.

It is possible to recover intraovarian oocytes from ovariectomized female cats [14–20] or sperm from the epididymides of castrated males [21, 22]. Some of these oocytes will mature, fertilize, and develop in vitro to morulae or early blastocysts [17, 19, 20]. Embryo transfer has proven that these embryos can produce live young [19]. However, most oocytes subjected to in vitro maturation (IVM) and fertilization (IVF) do not consistently develop to the blastocyst stage [14, 16, 18, 20]. Cat IVM/IVF embryos [23, 24] also are less developmentally competent than oocytes matured and fertilized in vivo [14, 16, 18, 20].

A recent study has revealed that the domestic cat experiences a naturally high incidence of intraovarian follicular atresia (~65%) that reduces the number of high-quality oocytes available for IVM [25]. It also is questionable whether most follicular oocytes are capable of completing cytoplasmic maturation in vitro [16], a crucial prerequisite in other mammals for oocyte activation, fertilization, and development to the blastocyst stage [26]. Additionally, reduced IVM/IVF efficiency may be related to premature zona hardening, a response to fertilization triggered by cortical granule content release to prevent polyspermy [27], which may be brought on prematurely by in vitro culture [28, 29]. Although zona hardening has never been confirmed in the cat, mechanically piercing the cat zona increases penetration in vitro by conspecific sperm [30].

One objective of this study was to examine the influence of season on nuclear and cytoplasmic maturation and zona receptivity of intraovarian cat oocytes after IVM. Another aim was to examine the total number of sperm recovered from the epididymis as well as sperm motility traits, viability, morphology, acrosomal integrity, and ability to penetrate oocytes in vitro. Because previous observations have revealed that most domestic cat litters are born in the spring [2, 3], our hypothesis was that intrinsic oocyte and sperm quality and response within a standard IVM/IVF system would vary over time and throughout the year.

MATERIALS AND METHODS

Gonadal Collection, Storage, and Transport

Female and male domestic cat reproductive tracts were recovered after ovariohysterectomy and castration, respec-

Accepted February 23, 1999.
Received December 3, 1998.
[1]This project was supported by the Smithsonian Scholarly Studies Program and Friends of the National Zoo.
[2]Correspondence: Rebecca Spindler, Conservation & Research Center, 1500 Remount Road, Front Royal, VA 22630. FAX: 540 635 6506; e-mail: rspindler@crc.si.edu

tively, performed at local veterinary clinics throughout each week of the year. The on-duty veterinarian confirmed with the client that each cat was free-living or had daily access to fluctuations in natural photoperiod and was at least 1 yr old and/or reproductively mature. If age of the cat was unknown, experienced veterinary and technical staff estimated the age. Ovarian pairs (n = 832) were dissected from the reproductive tracts and immediately classified as follicular (at least one visible, mature follicle ≥ 2 mm in diameter on at least one ovary), luteal (one or more corpora lutea on at least one ovary), or inactive (having neither follicular or luteal activity). Epididymides (n = 231 pairs) were left attached to the respective testis for transport. During transport, ovarian and testis pairs were stored in Dulbecco's PBS (pH 7.4, containing 100 IU/ml penicillin and 100 mg/ml streptomycin; Sigma Chemical Co., St. Louis, MO) at 4°C using ice packs and paper padding. Container temperature was monitored using a thermocouple (Omega Engineering Inc., Stanford, CT). Ovaries and testes were processed for gamete collection within 6 h of gonadectomy.

Oocyte Recovery, Maturation, and Culture

Ovaries were minced with a scalpel blade to liberate oocytes into modified H-MEM (Hepes-buffered Minimum Essential Medium; Gibco Laboratories, Grand Island, NY) supplemented with 1.0 mM pyruvate, 2.0 mM glutamine, 100 IU/ml penicillin, 100 mg/ml streptomycin, and 4 mg/ml BSA (Miles Pentex, Bayer Diagnostics, Kankakee, IL) and held at 38°C. Because grade of oocyte significantly affects IVM success [14, 19, 20], only grade I oocytes were chosen (defined by presence of a uniformly dark cytoplasm and an intact cumulus cell investment) [20]. Of the 10 973 oocytes collected from 832 ovarian pairs for the year, 3155 oocytes (28.8%) met grade I criteria and were studied further. Oocytes were categorized according to ovarian type (follicular versus luteal versus inactive), and then all oocytes on a given day were pooled for experimentation. Oocytes were washed three times in H-MEM and incubated in groups of 10 in 50 μl of maturation medium (MEM) containing 1.0 mM glutamine, 1.0 mM pyruvate, 100 IU/ml penicillin, 100 mg/ml streptomycin, 4 mg/ml BSA, 1 mg/ml FSH (1.64 IU/ml; NIDDK-oFSH-17, lot 3082; National Hormone and Pituitary Program, Rockville, MD), 1 mg/ml LH (1.06 IU/ml; NIDDK-oLH-25, lot 3502; National Hormone and Pituitary Program), and 1 mg/ml estradiol (Sigma). Oocytes were cultured in this medium under mineral oil for 32 h at 38°C in a humidified environment of 5% CO_2 in air [18].

Epididymal Sperm Recovery

After arrival at the laboratory, each epididymis was dissected from the testis and subdivided into the cauda (~10-mm closest to ductus deferens), corpus (middle segment), and caput (~10- to 15-mm region immediately adjacent to the testis) regions. The cauda and corpus regions were further dissected into 2-mm pieces and placed into 1.5-ml Eppendorf tubes (Fisher Scientific, Pittsburgh, PA) containing 0.5 ml Ham's F-10 medium (Irvine Scientific, Santa Ana, CA) supplemented with 1.0 mM pyruvate, 2.0 mM glutamine, 5% (v:v) fetal calf serum (Hyclone Laboratories, Logan, UT), and 100 IU/ml penicillin and streptomycin (Ham's F-10 complete) [31]; incubation was performed at 38°C in 5% CO_2 in air to passively release sperm. After 30 min, epididymal tissue was discarded, and the tubes were centrifuged for 8 min at 300 × *g*. Supernatant was removed and the sperm pellet resuspended in 50 μl Ham's F-10 complete before assessment of sperm quality.

Oocyte Nuclear Maturation Analysis

After maturation culture, one third of the oocytes were washed free of cumulus cells using 0.2% hyaluronidase (Sigma) and gentle mechanical manipulation. These oocytes were fixed in 2% formaldehyde, 0.04% Triton X-100 and stained with Hoechst 33342 [18, 31]. Stage of the meiotic cycle was clearly visible when the oocyte was examined using a fluorescent microscope (330–380-nm excitation). Oocytes were classified as mature when chromosomes were in telophase or metaphase II [14, 17, 18].

In Vitro Insemination and Development Assessment

Remaining oocytes were placed in 40-μl droplets (10 oocytes per droplet) of Ham's F-10 complete. Frozen-thawed sperm were used for assessing oocyte zona receptivity and cytoplasmic maturation (by fertilization competence). Semen was recovered by electroejaculation from two normospermic male cats housed under a controlled light cycle (12L:12D) [32]. Sperm from these males were pooled, diluted in an egg yolk-lactose-4% glycerol extender, cooled to 4°C, and frozen in 30-μl pellets on dry ice (−40°C/min for 2 min) before being plunged into liquid nitrogen for storage [33]. On the day of insemination, pellets from this pool were removed from liquid nitrogen, thawed in air for 10 sec, and placed in 100 μl of Ham's F-10 complete at 38°C for 30 sec. Sperm samples with ratings of ≥ 80% sperm motility and good forward progression [34] were swim-up processed [31, 35]. In brief, this involved diluting the thawed semen with an equal volume of Ham's F-10 complete in a 1.5-ml Eppendorf tube followed by centrifugation (8 min, 300 × *g*). Supernatant was aspirated and discarded, and 100 μl of Ham's F-10 complete was layered slowly onto the resulting pellet and incubated to allow sperm to swim up into the medium for 30 min. Sperm concentration was determined using a hemacytometer [34], and motile sperm (6×10^5 motile cells/ml) were added to all (but one) medium drops containing oocytes (3×10^4 motile sperm per drop). Oocytes in the remaining drop with no sperm served as parthenogenetic controls. All drops were maintained under mineral oil at 38°C in 5% CO_2 in air. After 12–16 h, oocytes were rinsed in medium and placed in a fresh 30-μl droplet of Ham's F-10 complete. Oocytes were transferred to fresh medium every 48 h, and after 7 days of culture were stained with Hoechst 33342, to determine the extent of sperm penetration in unfertilized oocytes and the number of cells per embryo [18, 31]. Oocytes were classified as having bound sperm when sperm were attached to the outer or inner zonal layer [36]. Oocytes were considered to have allowed sperm penetration when sperm were visible in the perivitelline space or when fertilization had been successful [37]. The precise stage of embryo development was determined by counting fluorescing nuclei. Embryos with four or more nuclei were considered to be fertilized. Parthenogenetic controls in this study generally arrested at the 2-cell stage of development.

Epididymal Sperm Quality Assessment

Processed sperm from the combined cauda and corpus regions of each epididymis were assessed for sperm concentration (as above) and for percentage motility and progressive motility status (graded scale: 0 = no forward

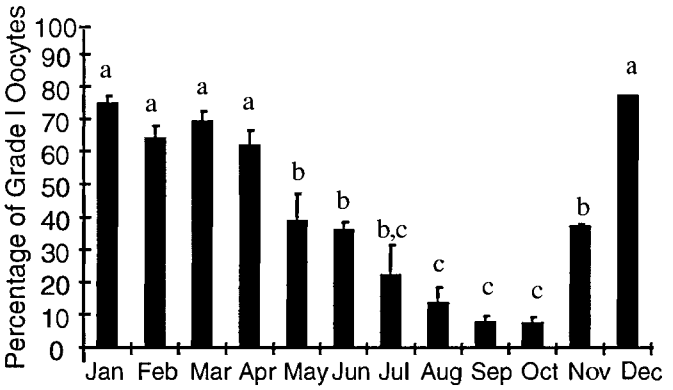

FIG. 1. Percentage of grade I oocytes achieving nuclear maturation on the basis of month of intraovarian harvest. Columns with different superscripts differ ($p < 0.01$).

movement to 5 = rapid, linear, forward movement) [34]. Sperm viability was assessed using the SYBR-14/propidium iodide, Live/Dead Sperm Viability Kit (Molecular Probes, Eugene, OR). Dead sperm were recognized as red under fluorescent light (400–485-nm broadband excitation filter), whereas live sperm were green-colored. Acrosomal integrity was examined using fluorescein isothiocyanate-conjugated *Arachis hypogaea* (peanut) agglutin stain (FITC-PNA, Sigma) whereby all cells with intact acrosomes fluoresced brightly and evenly when excited by UV light (400–485-nm broadband filter) [35]. Acrosome-reacted sperm had only faint or patchy fluorescence over the head region, a single band at the equatorial region, a ballooning acrosome, or an acrosome lifted away from the head region [35]. An aliquot of sperm was fixed in 0.3% glutaraldehyde in PBS (pH 7.1), and morphology was assessed by light microscopy to detect gross defects, including a bent or coiled flagellum, a bent midpiece, or the presence of a proximal or distal cytoplasmic droplet [34, 38]. The ability of epididymal sperm to penetrate the zona pellucida was assessed using a salt-stored oocyte penetration assay [36, 37, 39]. In brief, intraovarian oocytes collected during April, May, and June were matured, denuded of cumulus cells, and stored in a hypertonic solution at 4°C. At the time of sperm analysis, oocytes (n = 10–20 per analysis) were equilibrated in Ham's F-10 complete for ~4 h, then cocultured with 2×10^5 sperm/ml. The degree of oocyte penetration was determined using differential interference contrast (DIC) microscopy (×320) in combination with fluorescence after staining the oocyte with Hoechst 33342. Sperm often were more easily located using fluorescence, and then the position was determined using DIC. Percentage of oocytes with sperm 1) bound to the outer zona or within the outer zona, 2) bound to the inner layer of the zona, or 3) penetrating into the perivitelline space [37] was determined.

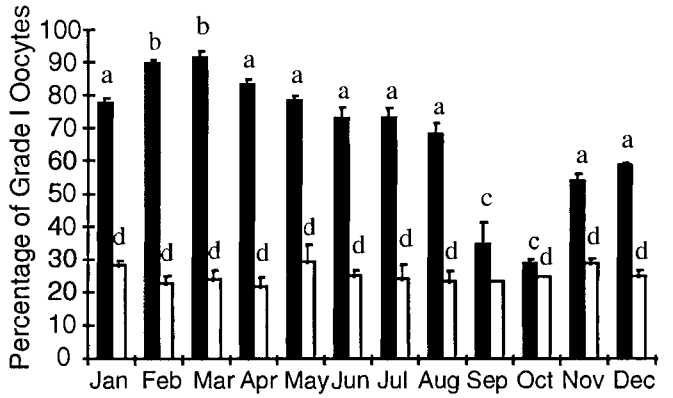

FIG. 2. Percentage of grade I oocytes with sperm bound to the outer (solid bars) or inner (open bars) zonal layers on the basis of month of intraovarian harvest. Solid columns with different superscripts differ (a–b, $p < 0.05$; a–c, b–c, $p < 0.01$).

Statistical Analysis

Correlation coefficients were calculated between the incidence of an ovarian type (percentage of follicular, luteal, or inactive ovaries) and initial oocyte quality (percentage grade I oocytes recovered) using percentage monthly values. For oocyte quality assessment, the percentage of oocytes that completed nuclear maturation and allowed sperm binding, sperm penetration, and fertilization/continued embryo development (≥ 4-cell) was expressed as a percentage of the total number of oocytes undergoing treatment (one third maturational assessment, two thirds IVF), unless otherwise stated. For epididymal sperm assessment, penetration was expressed as the percentage of oocytes with sperm bound, penetrated into the inner layer, or penetrated into the perivitelline space. Percentage data were transformed using arc sine transformation before analysis. Differences within assessments among months of the year were analyzed by ANOVA and Dunnett's multiple comparison testing [40]. All data were expressed as mean ± SEM.

RESULTS

Oocyte Quality Over Time

Compared to the values for other months, the percentage of follicular ovaries was elevated ($p < 0.05$) during December–February (mean range, 26.7–34.4%), and was highest during March (45.2%; $p < 0.01$) and lowest in August–November (mean range 13.3–15.2%; $p < 0.05$). The highest ($p < 0.01$) percentage of luteal ovaries occurred in June (31.2%) and July (41.7%), with the lowest ($p < 0.05$) in October–January (4.7%). For the entire year, the percentage of grade I oocytes recovered from follicular (22.5 ± 1.6%), luteal (22.8 ± 1.5%), or inactive (21.4 ± 1.4%) ovaries did not differ ($p > 0.05$), and the incidence of grade I oocytes was not correlated with any particular ovarian type (follicular $R^2 = 0.35$; luteal $R^2 = 0.17$; inactive $R^2 = 0.41$; $p > 0.05$).

The average percentage of oocytes meeting grade I criteria was consistent during January–July (mean range, 31.7 ± 8.0% to 40.4 ± 4.2%) and was lowest ($p < 0.05$) in September–November (mean range, 18.6 ± 2.8% to 20.2 ± 2.7%). Values in August and December were intermediate (26.8 ± 4.0, 29.3 ± 3.1, respectively; $p > 0.05$).

Few grade I oocytes recovered during August–October completed nuclear maturation (mean range, 7.2 ± 1.9% to 13.6 ± 4.9%; n = 238 oocytes; Fig. 1), with most arresting in the germinal vesicle or germinal vesicle breakdown stages. The incidence of nuclear maturation in grade I oocytes was highest ($p < 0.05$) during December–April (mean range, 61.8 ± 4.7% to 77.2 ± 0.1%; n = 488). Oocytes recovered in May–July (mean range, 22.2 ± 9.2% to 38.6 ± 8.5%; n = 217) and November (37.1 ± 0.7%; n = 86) were intermediate in nuclear maturation capacity (Fig. 1).

Percentage of oocytes inseminated that had sperm bound to the outer layer of the zona pellucida was higher ($p <$

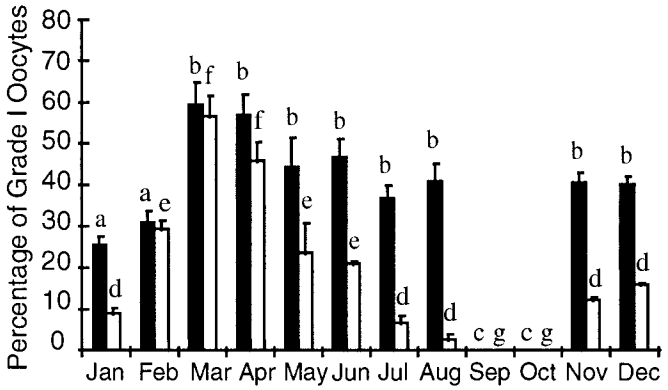

FIG. 3. Percentage of grade I oocytes that allowed sperm penetration past the zona (solid bars) and that fertilized and formed embryos of 4 cells or more (open bars) on the basis of month of intraovarian harvest. Within each variable, columns with different superscripts differ (c vs. a and b; g vs. d, e, and f: $p < 0.01$; all others, $p < 0.05$).

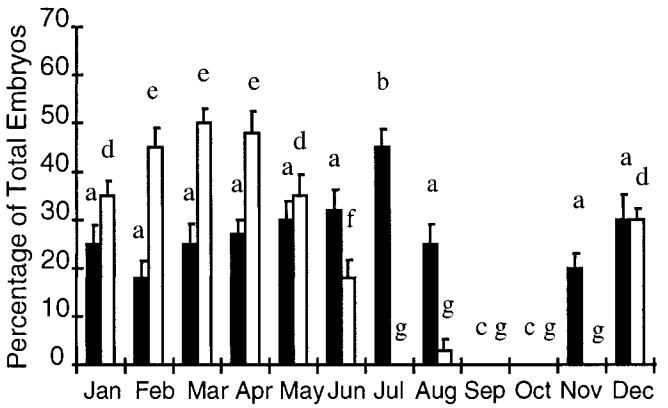

FIG. 4. Percentage of total embryos that developed into morulae (solid bars) or blastocysts (open bars) after 7 days of culture on the basis of month of intraovarian harvest. Within each variable, columns with different superscripts differ (c vs. a and b; g vs. d, e, and f: $p < 0.01$; e–f: $p < 0.01$; all others: $p < 0.05$).

0.05) in February and March (89.7 ± 2.3%, 91.3 ± 2.0%; n = 467 oocytes; Fig. 2) than in April–August (mean range, 73.1 ± 2.2 to 83.2 ± 2.1%; n = 493) and November–January (mean range, 53.8 ± 3.4 to 77.6 ± 2.6%; n = 825), and the latter intervals were higher ($p < 0.01$) than in September and October (34.8 ± 6.6%, 28.7 ± 2.5%, respectively; n = 384 oocytes). Percentage of oocytes with sperm bound to the inner zona layer did not change ($p > 0.05$) throughout the year (mean range, 23.5 ± 1.5 to 29.4 ± 3.1%; Fig. 2).

Fertilization was assumed to have been successful when oocytes developed to the 4-cell stage or beyond (embryos blocked at the 2-cell stage were assumed to be parthenogenetic). Parthenogenetic development did not vary ($p > 0.05$) during November–July (mean range, 3.1 ± 1.0 to 5.6 ± 1.3%), and did not occur August–October. Total sperm penetration past the zona (sperm in perivitelline space plus embryo formation) was high during March–August and November–December (mean range, 59.5 ± 5.2 to 36.7 ± 3.7%; Fig. 3), but satisfactory (> 25%) embryo development was restricted to February–June (Fig. 3). The percentage of oocytes fertilizing and developing in vitro was lower ($p < 0.05$) in July–October (mean range, 0.0 ± 0.0 to 6.7 ± 1.7%) compared to the rest of the year, and was highest ($p < 0.05$) in March and April (56.6 ± 4.8%, 45.8 ± 4.7%, respectively; Fig. 3). Of these embryos, the percentage reaching the morula stage (after 7 days culture) was greatest ($p < 0.01$) in July (45.0 ± 3.8%; 3.0 ± 0.1% of total grade I oocytes), coincident with decreasing blastocyst development (Fig. 4). The highest ($p < 0.05$) success at producing blastocysts occurred during February–April (mean range, 45.1 ± 3.9 to 50.3 ± 2.6% of embryos; 8.1 ± 1.0 to 13.0 ± 1.0% of total grade I oocytes; Fig. 4). In contrast, the lowest ($p < 0.01$) success at blastocyst production occurred in July–November oocytes (mean range, 0.0 ± 0.0 to 3.1 ± 1.9% of embryos; 0.0 ± 0.0 to 0.8 ± 0.0% of total grade I oocytes; Fig. 4).

Sperm Quality

There were no differences ($p > 0.05$) among months in epididymal sperm number or quality variables (sperm motility, progressive motility status, viability, acrosomal integrity, morphology) or in binding and penetrating capacity (Table 1). Sperm morphology data revealed a high number of sperm with retained cytoplasmic droplets (proximal 18.4 ± 3.1%; distal 27.8 ± 7.1%) in contrast to that previously published for ejaculated samples [32].

DISCUSSION

Results demonstrate that time of year has a differential impact on the quality of domestic cat oocytes. Particularly interesting was the finding that the intervals of successful nuclear maturation and sperm penetration were longer than that for cytoplasmic maturation, as indicated by fertilization and embryo development. Therefore, acquisition of oocyte cytoplasmic (or developmental) competence was not nec-

TABLE 1. Epididymal sperm quality traits (mean ± SEM) over time.

	Period*			
Parameter	Feb–Apr (n = 34)	May–Jul (n = 22)	Aug–Oct (n = 31)	Nov–Jan (n = 25)
Million sperm/epididymal pair	5.4 ± 1.2	3.7 ± 0.9	3.1 ± 1.4	3.8 ± 0.8
Motile sperm (%)	57.3 ± 5.7	48.3 ± 5.3	52.4 ± 3.8	47.6 ± 4.9
Progressive motility status	2.8 ± 1.6	3.4 ± 1.8	3.2 ± 2.7	2.7 ± 2.4
Viable sperm (%)	77.4 ± 3.5	81.6 ± 2.9	75.5 ± 4.1	77.9 ± 4.7
Intact acrosome (%)	92.5 ± 2.5	94.8 ± 3.7	90.5 ± 1.9	89.7 ± 2.4
Normal morphology (%)	25.4 ± 3.4	22.7 ± 1.8	21.8 ± 2.4	22.4 ± 2.5
Binding to outer zona (% of oocytes)	30.5 ± 2.5	31.8 ± 3.4	32.5 ± 4.1	30.1 ± 2.8
Binding to inner zona (% of oocytes)	36.5 ± 2.5	34.8 ± 3.1	32.8 ± 2.4	34.8 ± 1.7
Penetration into perivitelline space (% of oocytes)	33.4 ± 1.5	33.2 ± 2.4	32.9 ± 3.9	34.3 ± 2.7

* There were no monthly differences ($p > 0.05$) for any variables; thus, to facilitate presentation, data were pooled into quarterly intervals.

essarily coupled to nuclear maturation and zona receptivity, suggesting that the popular method of visualizing the meiotic plate may not always accurately reflect oocyte cytoplasmic maturation in this species. In contrast, this study revealed that there was no effect of season on epididymal sperm quality or quantity. We have established that spermatogenesis occurred consistently throughout the year in free-living and outdoor companion cats.

The percentage of high-quality (grade I) oocytes was unaffected by ovarian type (follicular, luteal, or inactive ovary), confirming previous observations [14]. Number of grade I oocytes, however, was influenced by time of ovarian harvest, with the fewest recovered in the autumn months (September–November). Intraovarian grade I oocytes had a marked decrease in nuclear maturational capacity and the ability to form embryos in July–October. Because most of these oocytes had no sperm bound or penetrated completely through the zona, it could be speculated that reduced zona receptivity was the primary barrier to fertilization during this time. However, a preliminary study in our laboratory has demonstrated that bypassing the zona of these oocytes using intracytoplasmic sperm injection fails to enhance fertilization [41], suggesting that an unreceptive zona is not the only fertilization barrier. More importantly, when oocyte maturation as well as sperm binding and penetration increased during November–January and May–July, fertilization and embryo development remained low. Although the zona appeared more receptive to sperm during these periods, the cytoplasm apparently was deficient in some factor(s). Therefore, there appear to be intervals of transition between the natural breeding season (February–April) and nonbreeding season (August–October), whereby reduced fertilization and embryo development in vitro may be due largely to cytoplasmic deficiency.

It remains unclear what mechanism(s) might be acting at the nuclear, cytoplasmic, or zonal level, or why there is seasonal rhythm in oocyte quality and receptivity to IVM. Seasonality in cats is most likely induced by altered hypothalamic-pituitary activity due to changing photoperiod. Changes in GnRH secretion affect FSH concentrations, which in turn regulate follicle development and viability [42]. Circannual fluctuations in reproductive hormone patterns or receptor concentrations is one factor that may affect oocyte quality and developmental competence. For example, FSH is considered essential, not only for dominant follicle development, but also for general follicle viability and control of atresia [42]. We have recently established that a relatively high incidence of follicular atresia occurs in the domestic cat ovary, affecting the availability of excellent-grade oocytes [25]. Even a normal-appearing oocyte may have difficult-to-distinguish traits that may reduce its capacity to respond to in vitro maturation. Studies are under way to examine the role of FSH receptors and follicular atresia in the fluctuations in cat oocyte quality.

Regardless of the factors modulating cat oocyte quality over time, it is possible that markers for cytoplasmic maturation are likely to be more accurate determinants of developmental capacity than visualization of the meiotic plate. There are assays for some of the processes involved in cytoplasmic maturation, including glutathione content and sperm decondensation [43], intracellular calcium concentrations [44], cortical granule distribution [45], and male pronucleus formation [46]. However, it is possible that the deficiency of the cytoplasm and, therefore, fertilization and developmental competence may not influence all of these parameters. One of the best indicators of general embryo health and activity is its metabolism [47–49]. We have determined in preliminary studies that glucose, glutamine, and palmitate metabolism increases as oocytes progress through the stages of meiotic maturation [50]. More importantly, oocytes of high developmental competence after maturation in vivo metabolize more glucose than oocytes that have reached the same stage of nuclear maturation in vitro [50]. We anticipate that metabolic markers may be an accurate means of selecting felid oocytes that have the best chance of maturing and fertilizing in vitro.

Sperm can be recovered from epididymides of free-living male domestic cats and are known to be motile, viable, and capable of penetrating oocytes [21, 22]. However, epididymal sperm naturally have more cytoplasmic droplets that normally are lost during transport through this duct [51]. In our study, more than 40% of sperm could be classified as morphologically abnormal because of the presence of proximal or distal cytoplasmic droplets. Spermatogenesis occurred consistently throughout the year, and the lack of discernible variations was likely related to the reduced energy cost of male reproduction [4]. The energy invested by a male domestic cat ends with copulation and is minimal compared to that of the female, whose costs continue throughout pregnancy and lactation. Therefore, it is a significant genetic advantage for domestic male cats to be reproductively active throughout the year, or at least to remain active well outside of the usual female breeding season.

This strategy is apparent in wild felids. For example, the Siberian tiger female exhibits more estrual activity in January–June than at other times of the year [7], but sperm quality is fairly consistent throughout the year [9]. Although the reproductive cost to the male is minimal compared to that of the female, breeding still incurs a cost. Quantity and quality of some felids' sperm can vary when distinctive female reproductive seasonality is known, as in the snow leopard [11] and Pallas' cat [13], for example; but the males remain reproductively active for longer periods than the females of the same species.

Present findings have application to parallel studies involving the rescue of gametes from endangered species. Most nondomestic felids maintained in accredited zoos are genetically valuable and may have never reproduced. It is logical to use reproductive technologies, including intraovarian oocyte and epididymal sperm recovery, to capture this valuable genetic material. This domestic cat study has demonstrated the profound impact of the time of year on oocyte quality. Thus, it would be useful to clearly understand seasonality in wild felid species, so that when medically necessary, elective ovariectomies can be scheduled appropriately. We know surprisingly little about seasonal fluctuations in ovarian activity in the 37 wild Felidae species [52], although noninvasive fecal hormone monitoring is allowing rapid expansion of this database [53].

In summary, oocyte quality in cats has a circannual rhythm. Even after excision and removal from maternal influences during seasonal reproductive quiescence, oocytes remain resistant to maturation and fertilization and have reduced developmental competence in vitro. It appears that compromised nuclear and cytoplasmic maturation and zona receptivity may all play a role in the seasonal decrease in oocyte developmental competence in vitro. In contrast, the male domestic cat is under little or no circannual control. It is likely that energy expended by the male to continue

reproduction is negligible, thereby allowing spermatogenesis to occur throughout the year.

ACKNOWLEDGMENT

The authors thank Brandon Sitzmann for assistance with ovary collection, oocyte processing, and sperm evaluation.

REFERENCES

1. Jemmett JE, Evans JM. A survey of sexual behaviour and reproduction of female cats. J Small Anim Pract 1977; 18:31–71.
2. Robinson BL, Cox HW. Reproductive performance in a cat colony over a ten-year period. Lab Anim 1970; 4:99–112.
3. Johnston I. Reproductive patterns of pedigree cats. Aust Vet J 1987; 64:197–200.
4. Bronson FH. Mammalian reproduction: an ecological perspective. Biol Reprod 1985; 32:1–26.
5. Beaver BV. Mating behavior in the cat. Vet Clin North Am 1977; 16: 729–733.
6. Wildt DE, Guthrie SC, Seager SWJ. Ovarian and behavioral cyclicity of the laboratory maintained cat. Horm Behav 1978; 10:251–257.
7. Seal US, Plotka ED, Smith JD, Wright FH, Reindl NJ, Taylor RS, Seal MF. Immunoreactive luteinizing hormone, estradiol, progesterone, and androstenedione levels during the breeding season and anestrus in Siberian tigers. Biol Reprod 1985; 32:361–368.
8. Seal US, Tilson RL, Plotka ED, Reindl NJ, Seal MF. Behavioral indicators and endocrine correlates of estrus and anestrus in Siberian Tigers. In: Tilson RL, Seal US (eds.), Tigers of the World. Park Ridge: Noyes Publications; 1987: 244–254.
9. Byers AP, Hunter AG, Seal ES, Graham EF, Tilson RL. Effect of season on seminal traits and serum hormone concentrations in male Siberian tigers (*Panthera tigris*). J Reprod Fertil 1990; 90:119–125.
10. Wildt DE, Howard JG, Hall LL, Bush M. Reproductive physiology of the clouded leopard: I. Electroejaculates contain high proportions of pleiomorphic spermatozoa throughout the year. Biol Reprod 1986; 34:937–947.
11. Johnston LA, Armstrong DL, Brown JL. Seasonal effects on seminal and endocrine traits in the captive snow leopard (*Panthera uncia*). J Reprod Fertil 1994; 102:229–236.
12. Brown JL, Swanson WF, Graham LH. Reproductive patterns in male and female Pallas' cats determined by fecal steroid metabolite analysis. Biol Reprod 1997; 56(suppl 1):88.
13. Swanson WF, Brown JL, Wildt DE. Influence of season on reproductive traits of the male Pallas' cat (*Felis manul*) and implications for captive management. J Zoo Wildl Med 1996; 27:234–240.
14. Johnston LA, O'Brien SJ, Wildt DE. In vitro maturation and fertilization of domestic cat follicular oocytes. Gamete Res 1989; 24:343–356.
15. Johnston LA, Donoghue AM, O'Brien SJ, Wildt DE. Influence of culture media and protein supplementation on in vitro oocyte maturation and fertilization in the domestic cat. Theriogenology 1993; 40: 829–839.
16. Schramm RD, Bavister BD. Effects of gonadotrophins, growth hormone, and prolactin on developmental competence of domestic cat oocytes matured in vitro. Reprod Fertil Dev 1995; 7:1061–1066.
17. Wood TC, Byers AP, Wildt DE. Influence of protein and hormone supplementation on in vitro maturation and fertilization of domestic cat eggs. J Reprod Fertil 1995; 104:315–323.
18. Wolfe BA, Wildt DE. Development to blastocysts of domestic cat oocytes matured and fertilized in vitro after prolonged cold storage. J Reprod Fertil 1996; 106:135–141.
19. Pope CE, McRae MA, Plair BL, Keller GL, Dresser BL. In vitro and in vivo development of embryos produced by in vitro maturation and in vitro fertilization of cat oocytes. J Reprod Fertil Suppl 1997; 51: 69–72.
20. Wood TC, Wildt DE. Effect of the quality of the cumulus-oocyte complex in the domestic cat on the ability of oocytes to mature, fertilize, and develop into blastocysts in vitro. J Reprod Fertil 1997; 110: 355–360.
21. Goodrowe KL, Hay M. Characteristics and zona binding ability of fresh and cooled domestic cat epididymal spermatozoa. Theriogenology 1993; 40:967–976.
22. Hay M, Goodrowe KL. Comparative cryopreservation and capacitation of spermatozoa from epididymides and vasa deferentia of the domestic cat. J Reprod Fertil Suppl 1993; 47:297–305.
23. Roth TL, Swanson WF, Wildt DE. Developmental competence of domestic cat (*Felis catus*) embryos fertilized in vivo versus in vitro. Biol Reprod 1994; 51:441–451.
24. Swanson WF, Roth TL, Godke RA. Persistence of the developmental block of in vitro fertilized domestic cat embryos to temporal variations in culture conditions. Mol Reprod Dev 1996; 43:298–305.
25. Wood TC, Montali RJ, Wildt DE. Follicle-oocyte atresia and temporal taphonomy in cold-stored domestic cat ovaries. Mol Reprod Dev 1997; 46:190–200.
26. Eppig JJ. Coordination of nuclear and cytoplasmic oocyte maturation in eutherian mammals. Reprod Fertil Dev 1996; 8:485–489.
27. George MA, Johnson MH, Vincent C. Use of fetal bovine serum to protect against zona hardening during preparation of mouse oocytes for cryopreservation. Hum Reprod 1992; 7:408–412.
28. Zhang X, Rutledge J, Armstrong DT. Studies on zona hardening in rat oocytes that are matured in vitro in a serum free medium. Mol Reprod Dev 1991; 28:292–296.
29. Schiewe MC, Araujo EJ, Ash RH, Balmaceda JP. Enzymatic characterization of zona pellucida hardening in human eggs and embryos. J Assist Reprod Genet 1995; 12:2–7.
30. Roth TL, Howard JG, Wildt DE. Zona pellucida-piercing enhances zona penetration by spermatozoa from normospermic and teratospermic domestic cats. J Androl 1994; 15:165–173.
31. Goodrowe KL, Wall RJ, O'Brien SJ, Schmidt PM, Wildt DE. Developmental competence of domestic cat follicular oocytes after fertilization in vitro. Biol Reprod 1988; 39:355–372.
32. Wildt DE, Bush M, Howard JG, O'Brien SJ, Meltzer D, van Dyk A, Ebedes H, Brand DJ. Unique seminal quality in the South African cheetah and a comparative evaluation in the domestic cat. Biol Reprod 1983; 29:1019–1025.
33. Swanson WF, Howard JG, Roth TL, Brown JL, Alvarado T, Burton M, Starnes D, Wildt DE. Responsiveness of ovaries to exogenous gonadotropins and laparoscopic artificial insemination with frozen-thawed spermatozoa in ocelots (*Felis pardalis*). J Reprod Fertil 1996; 106:87–94.
34. Howard JG, Bush M, Wildt DE. Semen collection, analysis, and cryopreservation in nondomestic animals. In: Fowler ME (ed.), Zoo and Wildlife Medicine II. Philadelphia: WB Saunders Co.; 1986: 390–399.
35. Long JA, Wildt DE, Wolfe BA, Critser JK, DeRossi RV, Howard JG. Sperm capacitation and the acrosome reaction are compromised in teratospermic cats. Biol Reprod 1996; 54:638–646.
36. Andrews JC, Howard JG, Bavister BD, Wildt DE. Sperm capacitation in the domestic cat (*Felis catus*) and leopard cat (*Felis bengalensis*) as studied with a salt-stored zona pellucida penetration assay. Mol Reprod Dev 1992; 31:200–207.
37. Howard JG, Donoghue AM, Johnston LA, Wildt DE. Zona pellucida filtration of structurally abnormal spermatozoa and reduced fertilization in teratospermic cats. Biol Reprod 1993; 49:131–139.
38. Howard JG, Brown JL, Bush M, Wildt DE. Teratospermic and normospermic domestic cats: ejaculate traits, pituitary-gonadal hormones, and improvement of spermatozoal motility and morphology after swim-up processing. J Androl 1990; 11:204–215.
39. Boatman DE, Andrews JC, Bavister BD. A quantitative assay for capacitation: evaluation of multiple sperm penetration through the zona pellucida of salt-stored hamster eggs. Gamete Res 1988; 19:19–29.
40. Miller RG. Simultaneous Statistical Inference. New York: Springer-Verlag; 1981.
41. Penfold LM, Wildt DE. Morphologically normal sperm from teratospermic cats remain compromised in fertilization ability even after intracytoplasmic sperm injection (ICSI). Biol Reprod 1997; 56(suppl 1):(abstract 197).
42. Erickson GF, Danforth DR. Ovarian control of follicle development. Am J Obstet Gynecol 1995; 172:736–747.
43. Perreault SD, Barbee RR, Slott VI. Importance of glutathione in the acquisition and maintenance of sperm nuclear decondensing activity in maturing hamster oocytes. Dev Biol 1988; 125:181–186.
44. Miyakazi S. Repetitive calcium transients in hamster oocytes. Cell Calcium 1991; 12:205–216.
45. Raz T, Ben-Yosef D, Shalgi R. Segregation of the pathways leading to cortical reaction and cell cycle activation in the rat egg. Biol Reprod 1998; 58:94–102.
46. Yoshida M, Ishigaki K, Pursel VG. Effect of maturation media on male pronucleus formation in pig oocytes matured in vitro and in vivo. Mol Reprod Dev 1992; 31:68–71.
47. Barnett DK, Bavister BD. What is the relationship between the metabolism of preimplantation embryos and their developmental competence. Mol Reprod Dev 1996; 43:105–133.

48. Gardner DK, Leese HJ. Assessment of embryo viability prior to transfer by the non-invasive measurement of glucose uptake. J Exp Zool 1987; 242:103–105.
49. Gardner DK, Pawelczynski M, Trounson AO. Nutrient uptake and utilization can be used to select viable day 7 bovine blastocysts after cryopreservation. Mol Reprod Dev 1996; 44:472–475.
50. Spindler RE, Wildt DE. Relationship of substrate metabolism to cat oocyte maturation. Theriogenology 1999; 295.
51. Briz MD, Bonet S, Pinart B, Egozcue J, Camps R. Comparative study of boar sperm coming from the caput, corpus, and cauda regions of the epididymis. J Androl 1995; 16:175–188.
52. Wildt DE, Brown JL, Swanson WF. Cats (*Felidae*). In: Knobil E, Neill J (eds.), Encyclopedia of Reproduction. New York: Academic Press; 1998: 497–510.
53. Brown JL, Wildt DE. Assessing reproductive status in wild felids by non-invasive faecal steroid monitoring. Int Zoo Yearb 1997; 35:173–191.