

In Vivo Embryogenesis, Embryo Migration, and Embryonic Mortality in the Domestic Cat¹

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ABSTRACT

In vivo embryogenesis, embryo migration, and survival were studied in the domestic cat. Queens were naturally mated (three times daily) on the second and third days of behavioral estrus and, if ovulation occurred, ovariectomized at 64 (n = 8), 76 (n = 11), 100 (n = 8), 124 (n = 7), 148 (n = 6), and 480 h (n = 8) after first copulation. Of 52 cats mated, 48 (92.3%) ovulated (as evidenced by the presence of ovarian CL), and of these, 38 (79.2%) either produced good-quality embryos or had implantation sites. From the remaining cats, only unfertilized oocytes (n = 5), degenerating embryos (n = 4), or no oocytes/embryos (n = 1) were recovered. Embryos at 64, 76, 100, and 124 h after the first copulation typically were 1 to 4 cells (17 of 20; 85.0%), 5 to 8 cells (18 of 28; 64.3%), 9 to 16 cells (14 of 24; 58.3%), and morulae (15 of 21; 71.4%), respectively; all were within the oviducts. At 148 h, embryos primarily were compact morulae or early blastocysts (15 of 18; 83.3%), and all were within the uterus. For the preimplantation groups, the overall recovery of embryos plus oocytes per CL was 80.6%, and the mean (\pm SEM) numbers of CL and embryos were 64 h, 4.8 ± 0.3 , 3.1 ± 0.8 ; 76 h, 4.7 ± 0.3 , 3.9 ± 0.6 ; 100 h, 5.8 ± 0.5 , 3.3 ± 0.8 ; 124 h, 4.4 ± 0.5 , 4.0 ± 0.6 ; and 148 h, 6.5 ± 1.1 , 3.7 ± 0.7 , respectively. Cats in the 480-h group produced a mean of 5.6 ± 0.5 CL and 3.9 ± 0.5 implantation sites. In six of eight cats in this group, there was a disparity between CL number on a given ovary and number of implantation sites in the ipsilateral horn, supporting the concept of transuterine embryo migration. In summary, results indicated that 1) more than 90% of cats ovulated following this multiple mating regimen, but ~21% of these failed to produce any fertilized or viable embryos; 2) embryo developmental rate in vivo was biphasic, with a rapid cleavage rate to the 5- to 8-cell stage followed by a slower cleavage rate to the morula stage; 3) cat embryos entered the uterus as compact morulae or early blastocysts ~5.5 days after the first copulation; and 4) on the basis of implantation/CL ratio, ~30% of all ovulated cat oocytes underwent either fertilization failure or preimplantation embryonic mortality.

INTRODUCTION

In vitro fertilization (IVF) and embryo culture have been the focus of considerable research in the domestic cat (*Felis catus*) [1–9] and in several nondomestic felid species including the leopard cat (*Felis bengalensis*) [10], tiger (*Panthera tigris*) [11, 12], puma (*Felis concolor*) [13], and cheetah (*Acinonyx jubatus*) [14]. These studies have explored mechanisms associated with sperm capacitation, oocyte maturation, gamete interaction, fertilization, and early embryonic development in vitro and have contributed significantly to understanding the reproductive physiology of felids. However, the “normality” of IVF embryos cannot be determined without comparative baseline data on in vivo embryogenesis—knowledge that, surprisingly, is unavailable for all felid species, including the domestic cat.

The chronological events of in vivo embryo development are known for most common species, including the cow [15], horse [16], pig [17, 18], sheep [19], goat [20], rabbit [21, 22], and mouse [23]. Relatively recently, information has become available on preimplantation embryo events in vivo for a few canid species, including the dog [24–26] and sil-

ver and blue fox [26]. However, information on in vivo preimplantation embryo development in the domestic cat is scarce and is usually a by-product of studies driven by other objectives [27, 28]. Especially problematic has been 1) the lack of attention to the time of mating relative to embryo recovery (because the cat is an induced ovulator) [29–32] and 2) the confounding effects of use of exogenous gonadotropins to induce folliculogenesis and ensure ovulation after natural breeding [33].

Most of what is known about cat embryology is derived from in vitro studies [1–9] using embryos generated by IVF of in vivo-matured oocytes recovered from queens treated with exogenous gonadotropins. Although this approach provides important information about embryo developmental rates and culture requirements in vitro, questions arise that cannot be addressed without understanding in vivo events. For example, in vitro-generated embryos develop readily in vitro to morulae under a variety of media and culture conditions but then experience a partial developmental arrest at the morula-to-blastocyst transition [34]. This developmental “block” has proven resistant to alterations in culture conditions [5, 6, 35], but the early developmental competence of these IVF embryos is difficult to determine without knowledge of the developmental capacity of naturally produced embryos. Similarly, when IVF embryos are transferred into gonadotropin-synchronized recipients, embryo survival and/or pregnancy rates are low [4, 9, 36]; this is a problem that cannot be addressed adequately without comparative information on the survival of

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naturally produced embryos *in vivo*. Finally, it is likely that a comprehensive examination of *in vivo* embryo development, embryo migration, and embryo survival will yield information useful for developing and applying assisted reproductive techniques for the propagation of cats used as models for human disease [37] and propagation of endangered, nondomestic felid species [38].

This report is the first in a series that will describe *in vivo* embryogenesis in the domestic cat. These studies will systematically characterize and interrelate developmental, endocrine, and histological traits in the preimplantation and early postimplantation periods to develop a composite picture of natural reproduction in the domestic cat. Our specific objectives here were to 1) assess the ovulatory responses of cats exposed to a controlled mating regimen; 2) characterize the chronological development of preimplantation cat embryos *in vivo*; 3) examine temporal embryo transport and migration patterns within the oviduct and uterus, respectively, while investigating implantation traits; and 4) evaluate fertility and embryo survival in response to natural mating.

MATERIALS AND METHODS

Animals

Adult (mean age, 20 ± 2 mo) female domestic cats were housed in stainless steel cages (1–2 cats per cage) or in communal pens (2–8 cats per pen) and were provided a commercial feline diet (Purina Cat Chow; Ralston-Purina Co., St. Louis, MO) and water *ad libitum*. Two proven breeder males were housed singly in separate pens and fed the same diet. Cats were maintained in a controlled ambient environment under artificial fluorescent illumination (12L:12D daily) during the ~1-yr study period.

Estrus Detection and Natural Breeding

Queens were monitored daily for signs of behavioral estrus that included lordosis posturing, tail deviation, and treading of the hind feet when stroked on the perineum [39, 40]. The first day of overt estrous behavior was designated as Day 1. If observed behavioral responses were considered ambiguous, queens were allowed to interact briefly with a male to clarify sexual receptivity; however, this encounter was always terminated before coitus occurred. Beginning on Day 2, queens were allowed to mate three times daily at 3-h intervals [41] for two consecutive days (i.e., Day 2 and Day 3 of estrus). Males were used in a rotating fashion as needed. Each mating encounter was limited to a single intromission that was confirmed by the queen's demonstrating typical after-reaction behavior (i.e., rolling, vocalization, licking of the perineum) [39].

Ovulation Assessment and Ovariohysterectomy

Occurrence of ovulation in all queens ($n = 52$) was assessed laparoscopically ($n = 60$ total evaluations) by ex-

amining all aspects of each ovary for fresh CL [4, 42] at one of six times after first copulation (64 h, $n = 8$; 76 h, $n = 14$; 100 h, $n = 9$; 124 h, $n = 10$; 148 h, $n = 9$; 480 h, $n = 10$). These times were chosen on the basis of knowledge that cats exposed to this mating frequency usually ovulate ~48–64 h after the first copulation [41]. Although range in ovulation onset is variable in this species [41, 43, 44], we anticipated that the 64-h time would coincide with 1- to 2-cell embryo development whereas 480 h would correspond to the postimplantation stage. For laparoscopy, anesthesia was induced with an injection (i.m.) of a ketamine hydrochloride (Vetalar; Parke-Davis, Morris Plains, NJ) plus acepromazine maleate (Ayerst Labs., Rouses Pt., NY) mixture (10:1 ratio, 10 mg/kg BW and 0.1 mg/kg BW, respectively). A surgical plane of anesthesia was maintained with isoflurane (Aerane; Anaquest, Madison, WI) gas anesthesia (1–2%) delivered by a face mask. At laparoscopy, a Verres needle was used to manipulate the ovaries to allow complete ovarian examination. Ovulating queens ($n = 48$) were subjected immediately to a midventral laparotomy and ovariohysterectomy. The reproductive organs were excised carefully to avoid damaging ovarian structures, embryos, or implantation sites.

Embryo Recovery/Assessment and Implantation Determination

For the preimplantation embryo groups (64–148 h), the reproductive tract was processed immediately by severing the oviducts from the uterine cornua at the uterotubal junctions and the uterine cornua from the uterine body. Each oviduct and uterine cornu was flushed separately and repeatedly into plastic petri dishes using 1–5 ml of warm (37°C) tissue culture medium (Ham's F-10; Sigma Chemical Company, St. Louis, MO) supplemented with 5% fetal calf serum (FCS; Irvine Scientific, Santa Ana, CA). Dishes were searched for embryos and/or oocytes through use of a stereomicroscope at 12× magnification, and recovered embryos were assessed for quality and developmental stage. Criteria for three quality grades were as follows. Grade 1 embryos had symmetrical blastomeres that were dark brown or black in color with no fragmentation or vacuolation. Grade 2 embryos had slightly asymmetrical blastomeres, some with minimal fragmentation or vacuolation. Grade 3 (or degenerate) embryos had blastomeres of variable size and were very light or highly variable in color, most having pronounced fragmentation or vacuolation. Embryo developmental stage was based on number of distinct blastomeres at microscopic examination (i.e., 1 cell, 2 cells, 3 cells, 4 cells, 5 to 8 cells, 9 to 16 cells, morula [> 16 cells], compact morula, or blastocyst). All recovered embryos were cultured *in vitro* for a companion study on embryo development [45] and so were unavailable for fluorescent staining of nuclear structures. Because the germinal vesicle, polar bodies, and pronuclei cannot be visualized in cat embryos without fluorescent staining [4], cleavage of 1-cell embryos

TABLE 1. Ovulatory response and fertility of queens naturally mated using a controlled breeding regimen.^a

Category	Number of cats (%)
Total cats naturally mated	52
Cats failing to ovulate	8 (15.4%) ^b
Cats ovulating	44 (84.6%) ^b
Total cats ovulating	48 ^c
Ovulating cats with no oocytes or embryos	1 (2.1%)
Ovulating cats with only unfertilized oocytes	5 (10.4%)
Ovulating cats with embryos or implantations	42 (87.5%)
Cats with embryos or implantations	42
Cats with only grade 3 (degenerate) embryos	4 (9.5%)
Cats with grade 1 or 2 embryos	30 (71.4%)
Cats with implantations	8 (19.1%)

^aQueens were naturally mated three times daily at 3-h intervals on Days 2 and 3 of behavioral estrus.

^bResponse of queens to the initial series of matings.

^cIncludes four previously anovulatory queens that ovulated in response to mating at a subsequent estrus.

within 24 h of culture was used to distinguish these from nondegenerate, unfertilized oocytes. For the postimplantation-interval group (480 h), the number, size (gestational sac diameter), and distribution of implantation sites within the uterine cornua were determined, and gestational sacs were incised to assess presence or absence of a fetus. Ovaries of all ovariohysterectomized queens were evaluated for CL number (to confirm earlier laparoscopic assessments).

Statistics

Values are presented as means \pm SEM. Differences in mean number of CL, total embryos, good-quality (grade 1 or 2) embryos, degenerate (grade 3) embryos, unfertilized oocytes, and implantations were evaluated by analysis of variance using the Statistical Analysis System [46] and least significant difference (LSD) means comparison. Difference between mean number of CL and implantations was analyzed using a Student's *t*-test. Differences in implantation rate between queens with lower vs. higher ovulation number and differences in embryo/oocyte recovery rates between interval groups were compared using chi-square analysis [47].

RESULTS

Ovulatory Response and Embryo Recovery

Of the 52 queens mated three times daily for 2 days beginning on Day 2 of estrus, 84.6% ovulated (Table 1). Four cats failing to ovulate when first subjected to the mating regimen ovulated after a second, identical copulatory series during a subsequent estrus. Three of the nonovulating queens were subjected to the same serial breeding regimen on two or three occasions (two cats, twice; one cat, three times), but none ovulated. Of the 48 ovulating queens, 87.5% had embryos that were recovered from the oviducts or uterine horns or had uterine implantation sites (Table 1). On the basis of total cats mated, 80.8% ovulated with evidence of fertilization (embryos or implantations). Of the 42 cats with embryos or implantation sites, four had only degenerate (grade 3) embryos, whereas 30 queens had good-quality embryos and eight (the 480 h group) had uterine implantation sites. The overall fertility rate (defined as percentage of mated females with at least one grade 1 or 2 embryo or at least one implantation site) was 73.1% (38 of 52).

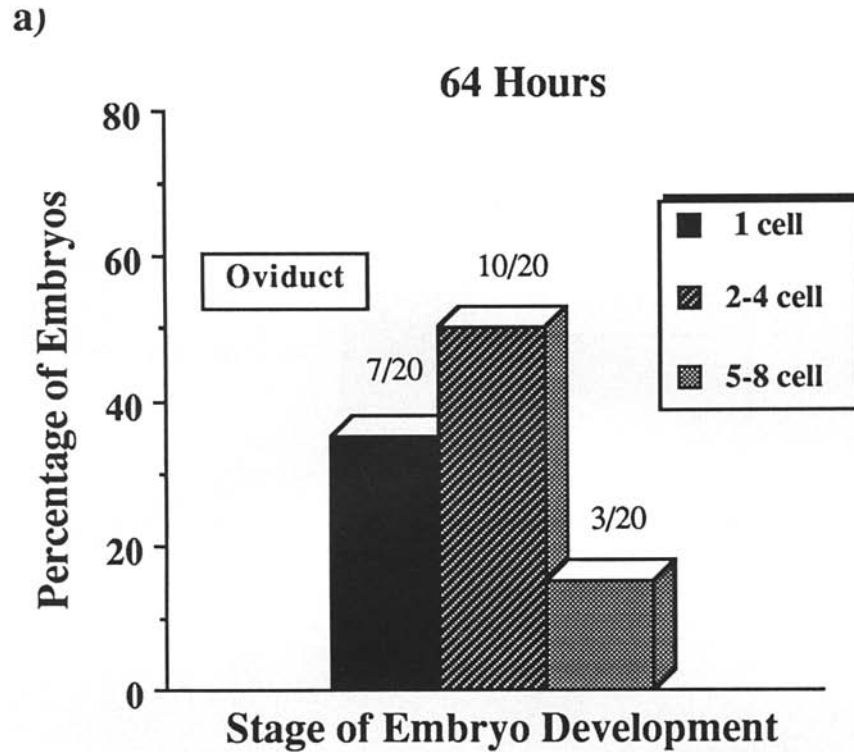
Mean CL number did not differ ($p > 0.05$) among most interval groups (64–480 h); however, CL number for the 148-h group was greater ($p < 0.05$) than that for the 64-, 76-, and 124-h groups (Table 2). Mean number of total (grade 1, 2, or 3) embryos, good-quality (grade 1 or 2) embryos, degenerate (grade 3) embryos, or unfertilized oocytes did not differ ($p > 0.05$) among cats in the preimplantation-interval groups (64–148 h) (Table 2). Mean number of implantations in the 480-h group (3.9) did not differ ($p > 0.05$) from the mean number of total embryos (range, 3.1–4.0) or good-quality embryos (range, 2.5–3.0) in the preimplantation groups (Table 2). Similarly, for the 40 queens in the preimplantation embryo groups, the disparity between CL ($n = 206$) and embryo ($n = 144$) number was 30.1%.

For the preimplantation groups, flushing the oviducts and uterine cornua resulted in an overall recovery rate of embryos plus oocytes per CL of 80.6% (166 of 206): 64 h, 86.8% (33 of 38); 76 h, 90.4% (47 of 52); 100 h, 76.1% (35 of 46); 124 h, 93.5% (29 of 31); 148 h, 56.4% (22 of 39). Recovery

TABLE 2. Mean number (\pm SEM) of CL, embryos/unfertilized oocytes, or implantations for ovulating queens at specific time intervals after first copulation.

Interval group (h post-first copulation)	Number of ovulating females per group	CL number per female	Number of embryos and oocytes recovered				Implantation number per female
			Total embryos (Grade 1, 2, or 3)	Grade 1 or 2 embryos	Grade 3 embryos	Unfertilized oocytes	
64	8	4.8 \pm 0.3 ^a	3.1 \pm 0.8	2.5 \pm 0.8	0.6 \pm 0.6	1.0 \pm 0.7	—
76	11	4.7 \pm 0.3 ^a	3.9 \pm 0.6	2.5 \pm 0.7	1.4 \pm 0.7	0.4 \pm 0.4	—
100	8	5.8 \pm 0.5 ^{ab}	3.3 \pm 0.8	3.0 \pm 0.8	0.3 \pm 0.3	1.1 \pm 0.6	—
124	7	4.4 \pm 0.5 ^a	4.0 \pm 0.6	3.0 \pm 0.5	1.0 \pm 0.8	0.1 \pm 0.1	—
148	6	6.5 \pm 1.1 ^b	3.7 \pm 0.7	3.0 \pm 0.9	0.7 \pm 0.7	0.0	—
480	8	5.6 \pm 0.5 ^{ab}	—	—	—	—	3.9 \pm 0.5

^{a,b}Within columns, different letters indicate that means were significantly different ($p < 0.05$).



b)

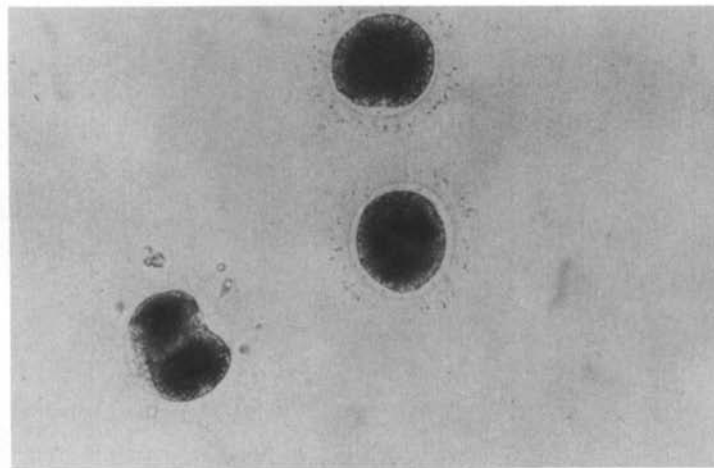
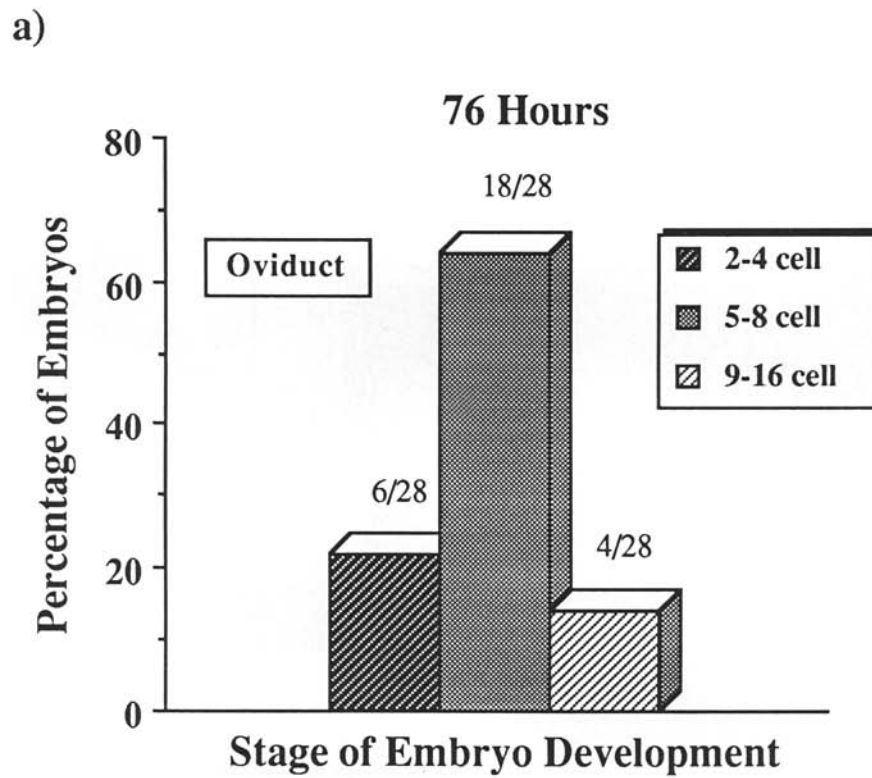


FIG. 1. In vivo cat embryo development at 64 h after the first copulation: (a) percentage of recovered grade 1 or 2 embryos at each developmental stage; (b) photomicrograph ($\times 150$) of 1-cell and 2-cell embryos typical of this time interval. All embryos were recovered from the oviducts.

success did not differ ($p > 0.05$) among most of the interval groups. However, embryo recovery for the 100-h group was less ($p < 0.05$) than that for the 124-h group and similar ($p > 0.05$) to that for the 148-h group, which in turn

was less ($p < 0.05$) than for all other groups. Of the 144 embryos recovered, 77.1% were quality grade 1 ($n = 83$) or 2 ($n = 28$) and the remaining ($n = 33$) were classified as degenerate (grade 3).



b)

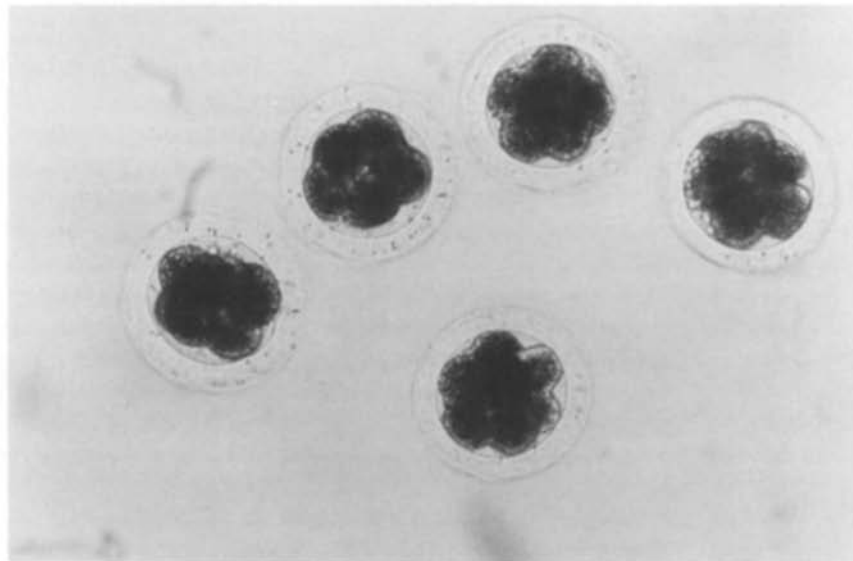
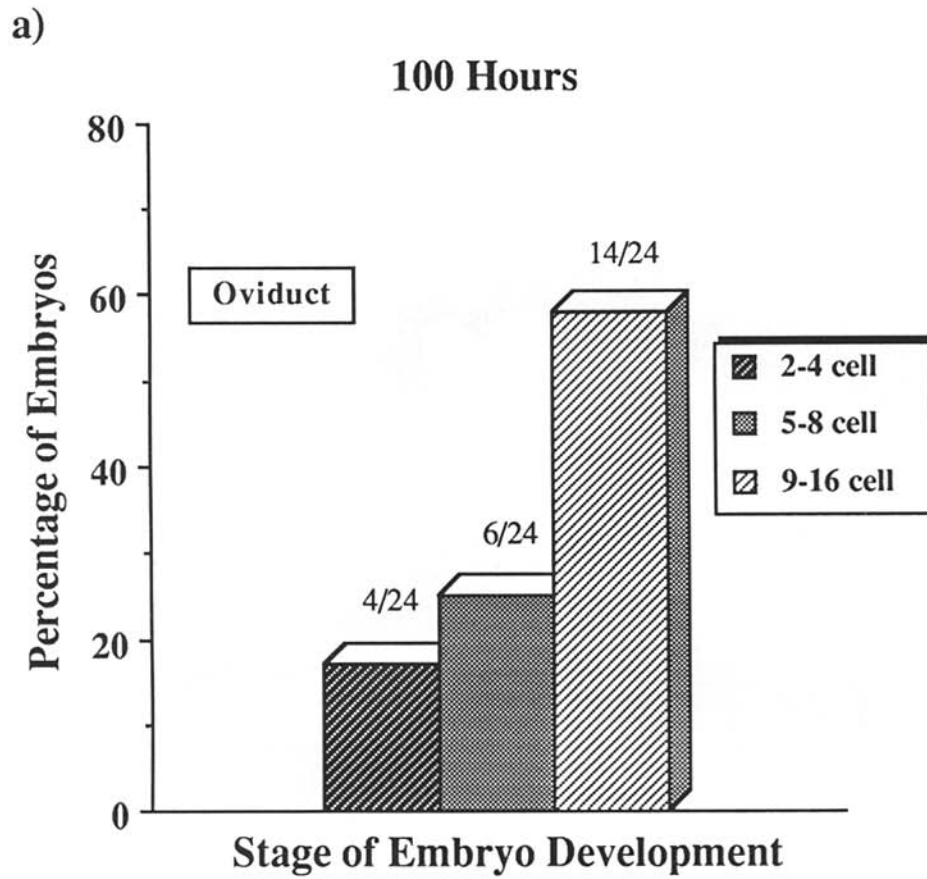


FIG. 2. In vivo cat embryo development at 76 h after first copulation: (a) percentage of recovered grade 1 or 2 embryos at each developmental stage; (b) photomicrograph ($\times 200$) of 5- to 8-cell embryos typical of this time interval. All embryos were recovered from the oviducts.



b)

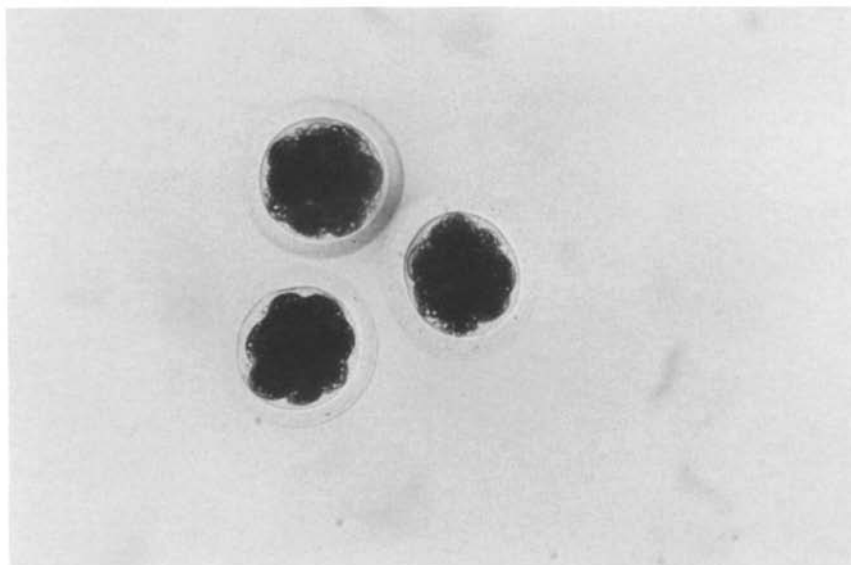
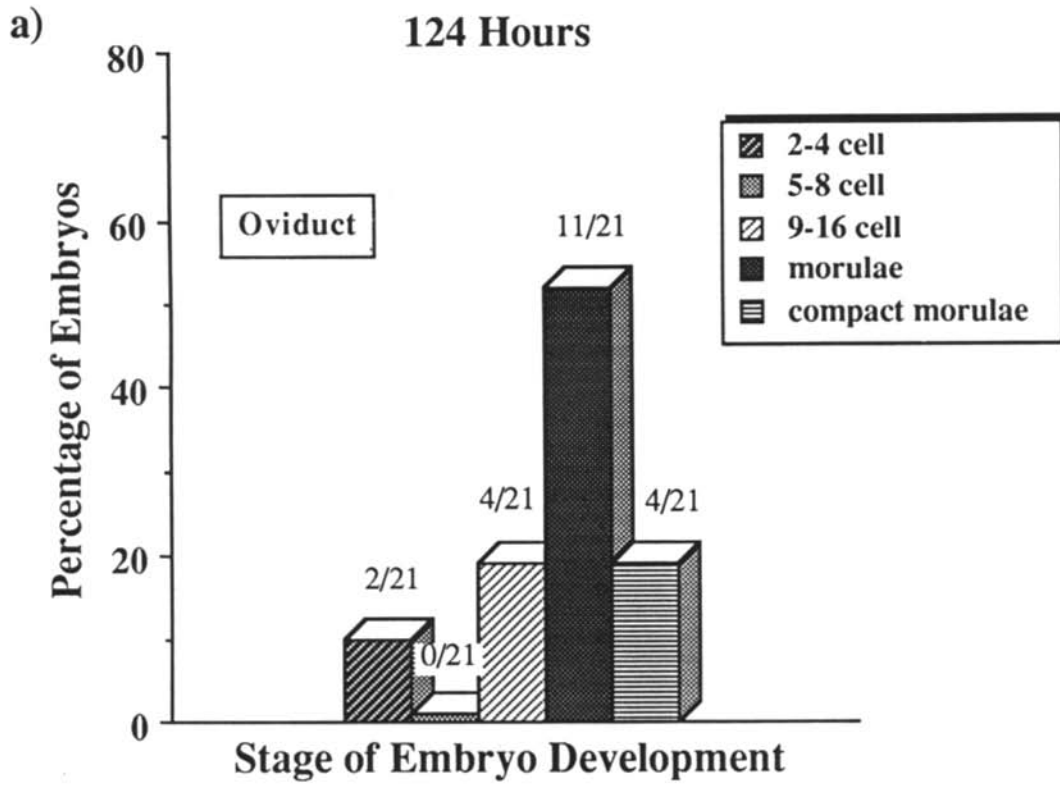


FIG. 3. In vivo cat embryo development at 100 h after first copulation: (a) percentage of recovered grade 1 or 2 embryos at each developmental stage; (b) photomicrograph ($\times 200$) of 9- to 16-cell embryos typical of this time interval. All embryos were recovered from the oviducts.



b)

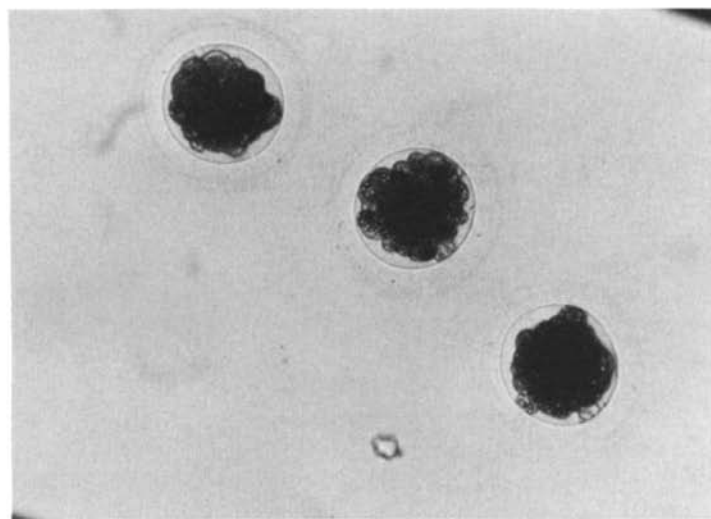
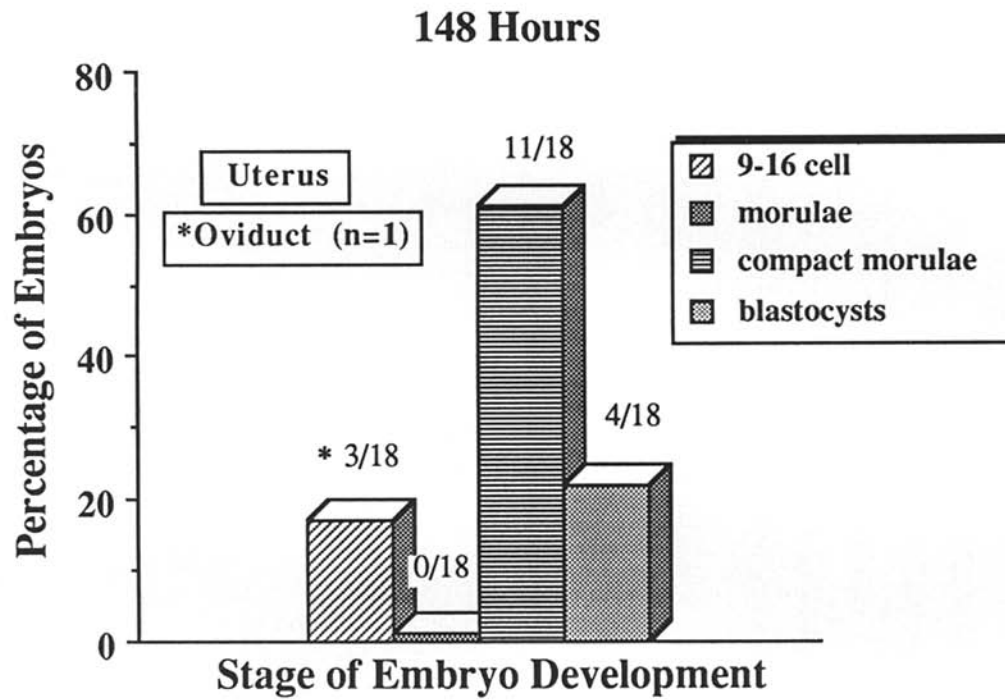


FIG. 4. In vivo cat embryo development at 124 h after first copulation: (a) percentage of recovered grade 1 or 2 embryos at each developmental stage; (b) photomicrograph ($\times 200$) of morulae and compacting morulae typical of this time interval. All embryos were recovered from the oviducts.

a)



b)

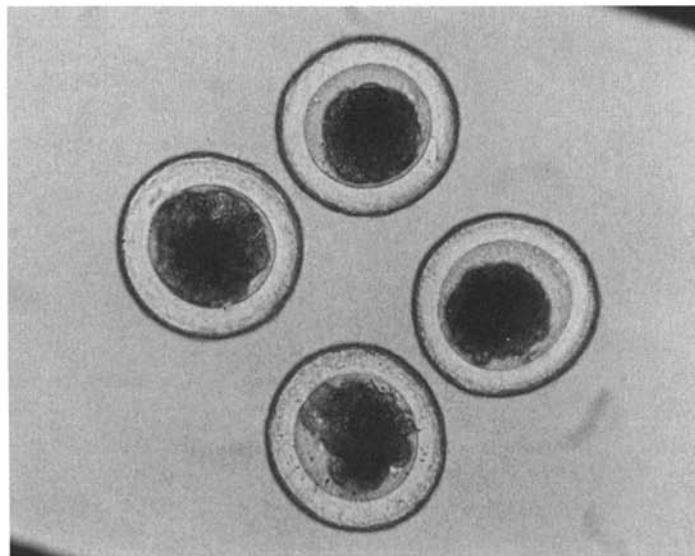


FIG. 5. In vivo cat embryo development at 148 h after first copulation: (a) percentage of recovered grade 1 or 2 embryos at each developmental stage; (b) photomicrograph ($\times 200$) of compact morulae typical of this time interval. All embryos (except one 9-16-cell embryo within the oviduct) were recovered from the uterine horns.

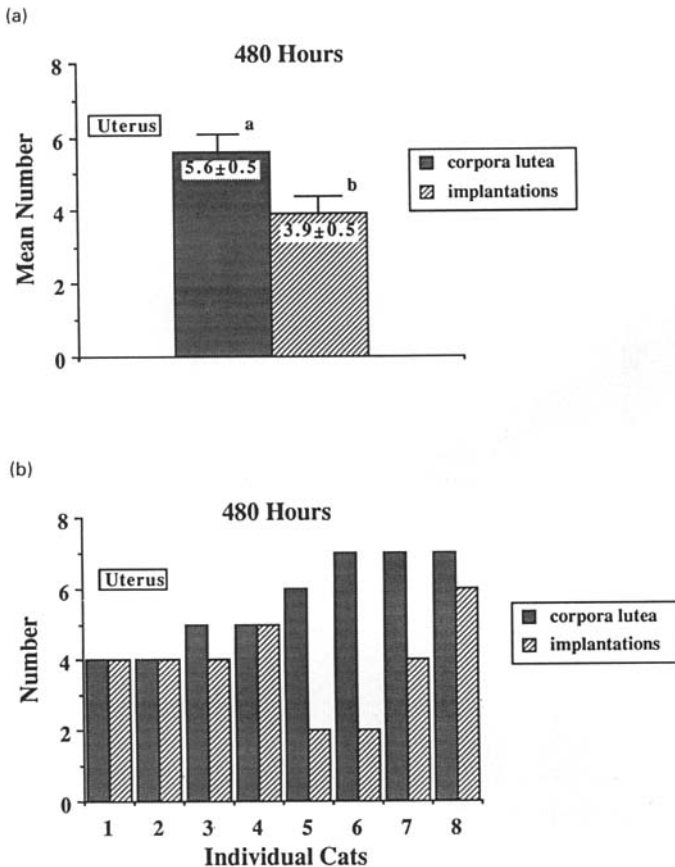


FIG. 6. CL/implantation disparity in cats at 480 h after the first copulation: (a) mean (\pm SEM) number of CL and implantation sites. Columns with different superscripts differ ($p < 0.05$); (b) number of CL and implantations for individual queens within the 480-h-interval group.

Embryo Developmental Stages

Developmental stages for grade 1 or grade 2 embryos were characterized for each preimplantation time interval. At 64 h after the first copulation, 65% (13 of 20) of embryos had completed at least one cleavage division with the remaining embryos still at the 1-cell stage (Fig. 1). At 76 h, most embryos (64.3%) were 5 to 8 cells (Fig. 2); at 100 h, most (58.3%) were at the 9- to 16-cell developmental stage (Fig. 3). At 124 h, 71.4% (15 of 21) of embryos had developed to the morula or compact morula stage (Fig. 4). All

embryos in the 64–124-h-interval groups were recovered from the oviducts. At 148 h after the first copulation, 61.1% of embryos were compact morulae and 22.2% were blastocysts (Fig. 5), and all but one were recovered from the uterine horns. A single 9- to 16-cell, grade 1 embryo was flushed from the oviduct of one queen; no other embryos were recovered from this cat.

Implantation and Transuterine Migration

For queens in the postimplantation group, there was a disparity of 31.1% ($p < 0.05$) between number of implantation sites ($n = 31$) and CL ($n = 45$) (Fig. 6a). Analysis of results for individual queens (Fig. 6b) revealed that the implantation/CL disparity for queens ($n = 4$) with 5 CL or fewer was 5.5% (1 of 18) but for cats ($n = 4$) with more than 5 CL was 48.1% (13 of 27) ($p < 0.01$).

In six of the eight cats in the postimplantation group, there were more implantation sites in one uterine horn than CL on the ipsilateral ovary, indicating transuterine embryo migration from the contralateral horn (Table 3). Implantation sites averaged 2.0 ± 0.1 cm in diameter and (for queens with multiple implantation sites in one uterine horn) were spaced an average of 2.5 ± 0.3 cm apart. When incised, all gestational sacs except a single site in cat #5 contained a developing fetus. This vacant gestational sac was 1.1 cm in diameter and was positioned immediately adjacent to the only other implantation site in the left uterine horn.

The chronological sequence of *in vivo* embryo development after natural mating in the domestic cat is summarized in Figure 7.

DISCUSSION

Studies of natural reproductive processes in domestic cats historically have focused on the physiological mechanisms and temporal relationships associated with behavior, ovulation induction, and endocrine patterns throughout the various phases of the estrous cycle, luteal phase, and pregnancy (for reviews see [34, 48, 49]). The present data represent the first substantive information on *in vivo* embryogenesis in the domestic cat encompassing embryo developmental rate, survival, and intrauterine migration.

TABLE 3. Distribution of CL and implantation sites in naturally mated queens at 480 h after the first copulation and evidence of transuterine embryo migration.

Cat number	Right ovary (CL number)	Right uterine horn (Implantation number)	Left ovary (CL number)	Left uterine horn (Implantation number)	Embryo migration?
1	0	1	4	3	yes
2	2	2	2	2	unknown
3	0	2	5	2	yes
4	2	3	3	2	yes
5	5	0	1	2	yes
6	7	1	0	1	yes
7	3	4	4	0	yes
8	5	4	2	2	unknown

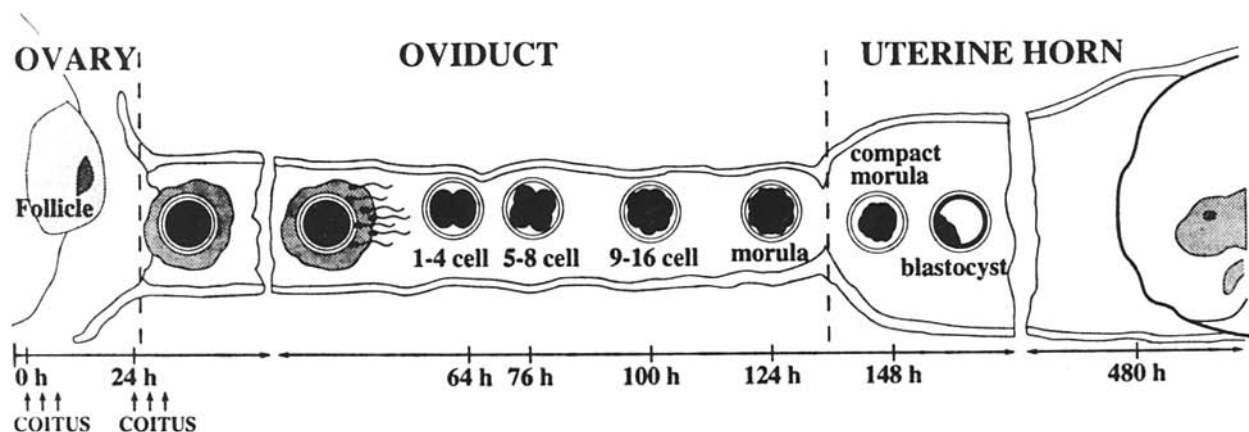


FIG. 7. Summary diagram of in vivo embryo development in the domestic cat after mating three times per day on Days 2 and 3 of natural estrus.

More than 85% of the cats in the present study ovulated in response to multiple matings on the second and third day of estrus; these results were comparable to those reported by others [31, 32, 41]. Concannon et al. [31] demonstrated that ad libitum copulations on Day 3 of estrus resulted in 100% (36/36) of cats ovulating. We have shown earlier that 83% (10 of 12) of cats ovulated when mated three times on either Day 1, 2, or 3 of estrus, whereas all cats (12 of 12) ovulated when mated three times daily for the first 3 days of estrus [32, 41]. As induced ovulators, cats depend on copulatory activity to cause the hypothalamic LHRH secretion that subsequently elicits ovulatory LH surges [31, 32, 41, 44]. Mating frequency has a profound effect on LH surge peak/duration and ovulatory response [31, 32]. Although LH was not measured in the present experiments, previous studies have shown that anovulatory queens generally do not produce an LH surge [31, 32, 41, 44]. In the present investigation, half of the eight queens failing to ovulate in response to the initial series of matings ovulated during a later estrus, indirectly suggesting that different mating experiences within the same female provoked variations in hypothalamo-pituitary response. However, three queens were consistently anovulatory (despite being mated during two or three estrous periods) although their age, parity, and sexual behavior were similar to those for the rest of the study population. All mated queens exhibited typical estrous behavior and postcoital reactions regardless of whether ovulation resulted, perhaps because sexual behavior and LHRH release in the cat are uncoupled and are controlled by separate hypothalamic centers [50].

Results also indicated that it was unnecessary to mate queens on the first day of estrus to achieve ovulation and to produce viable embryos. Day 2, and not Day 1, was chosen for first mating on the basis of recent data suggesting that follicular oocytes on the first day of estrus are immature and frequently of poorer quality than during later estrus [8]. In that study, oocytes were laparoscopically aspirated from naturally estrual queens receiving hCG (to induce final follicular maturation) on either Day 1, 2, or 3 of estrus.

Oocytes recovered from Day 1 follicles were markedly compromised in fertilizability in vitro compared to oocytes aspirated from Day 2 or 3 follicles. Such results may mean that matings occurring during the very early stages of estrus either have little functional relevance or serve to promote gonadotropin release that in turn is more important for follicle/oocyte maturation than ovulation induction.

A true assessment of embryo developmental rates requires relating findings to estimates of time of ovulation, a difficult task in the case of this induced ovulator because oocyte release is sensitive to the specifics of the mating regimen [34]. Ovulation has been observed to occur from 24 to 32 h [43, 44] and from 48 to 64 h [41] after first copulation, the difference being attributable largely to the number of copulations and the interval between them. Queens mated beginning on Day 3 or 4 of estrus and permitted one to four copulations within one 1/2-h period ovulate 24–32 h after the first copulation (44). In contrast, the earliest ovulation detected in queens mated beginning on Day 1 of estrus (three copulations at 3-h intervals during each of the first three days of estrus) is 48 h later [41]. Delaying coitus until the later days of estrus may make the ovarian follicles more responsive to copulatory stimuli, accelerating both the final stage of follicular/oocyte maturation and ovulation onset. Our developmental data here supported this assertion. With in vivo-matured, in vitro-fertilized oocytes, first cleavage division occurs 24–30 h post-insemination [2, 4]. In the present study, most (65%) in vivo-generated embryos had cleaved at least once by 64 h after the first breeding. Because a parallel study indicated that early developmental rates of in vivo-generated and IVF embryos were similar [45], it can be assumed that fertilization in vivo occurred ~30 h before recovery of the embryo at 64 h. Within this time frame, ovulation was estimated to have occurred ~30–36 h after the first mating.

The developmental rate of cat embryos in vivo appeared biphasic, with an initial fast cleavage period followed by a period during which developmental rate within the oviducts was slower. Between the time of ovulation and 64 h

after first copulation, most embryos (65%) had undergone at least one cleavage division. By 76 h, most embryos (58%) had completed a third cleavage (5 to 8 cells). Given our estimate of ovulation occurring 30–36 h after the first mating, cat blastomeres cleaved about once every 12 h up to the 5- to 8-cell stage. Assuming that the first cleavage occurs 24–30 h after fertilization (as evidenced by *in vitro* data) [2, 4], the second and third cleavage divisions both occurred within the next 12–22 h. Subsequent cleavages then occurred about once every 24 h from the 5- to 8-cell to the morula and compact morula stages. Embryo developmental rates for other species are known to be multiphasic with variable cleavage intervals, especially during early periods of development [51]. In cattle, initial cleavage rate to the 4- to 8-cell stage is related to maternal control of protein synthesis, and slowed growth is associated with the maternal-zygotic transition [51]. The timing of the maternal-zygotic transition is unknown for the cat, but observed *in vivo* developmental kinetics indicates that the shift from maternal to embryonic control may well occur at about the 5- to 8-cell stage when the cleavage rate slows substantially.

For 124 h after the first copulation, cat embryos remained within the oviducts, growing into morulae. From 124 to 148 h, embryos underwent compaction, transversed the uterotubal junctions, and entered the uterine cornua as compact morulae or early blastocysts. Therefore, compared to embryos of many domestic species, cat embryos were sustained within the oviducts for a longer period, making the oviductal-to-uterine transition at a relatively advanced stage. For example, cattle, sheep, rabbit, and pig embryos are typically transported through the oviducts within 2–4 days of ovulation, entering the uterus as 4 to 16 cells [15, 17–19, 21, 22]. The cat appears most like the horse and the dog, because embryos from the latter two species enter the uterus as late-stage morulae to early-stage blastocysts about 148 h and 216 h post-ovulation, respectively [16, 24–26]. The relatively prolonged oviductal transit time in the cat may be associated with the slow onset of luteal function after copulation, because circulating progesterone does not rise above basal concentration until 48–72 h after mating onset [41, 52, 53]. Delayed embryo transport in the cat also may be affected by circulating estradiol-17 β , which can remain elevated for 2–5 days after initial matings [41, 53, 54]. Exogenous estrogen retards embryo transport through the cat [27] and rabbit [21] oviduct, whereas progesterone accelerates transport in the rabbit [21]. Therefore, it is likely that the kinetics of embryo location within the cat reproductive tract is hormone mediated and driven.

Cat morulae were similar to horse [16] and dog [26] embryos in undergoing compaction at the transition period, before or coincident with entering the uterine cornua. Compaction and the formation of tight intracellular junctions appear essential for subsequent blastocoel formation [55]. It is presently unknown whether or not the transition between the oviductal and uterine environments has any

influence on the phenomenon of compaction and cat blastocyst formation. However, it was interesting that cat IVF morulae did not undergo discernible compaction *in vitro* [45]. We now speculate that perhaps this first visible evidence of a difference between *in vitro*- and *in vivo*-generated cat embryos holds the clue for addressing the problem of the pronounced *in vitro* developmental block to blastocyst formation.

As with embryos of the litter-bearing pig [56] and dog [57], cat embryos reportedly undergo transuterine migration before implantation [29, 58]. This was confirmed in the present study with six of eight cats manifesting definitive evidence of transuterine migration so that embryos tended to become equally spaced throughout the reproductive tract, perhaps to help promote embryo survival. There also was a tendency for cats having more than 5 CL to have fewer implantation sites. We have no explanation for this except that perhaps there was a wider range in ovulation time in the more prolific females, making these queens more susceptible to fertilization failure or embryo loss; alternatively, perhaps some of the oocytes originating from the additional follicles were of poorer fertilizable quality. Nonetheless, the data clearly suggested that optimal embryo survival was associated with the formation of 4 or 5 CL.

The domestic cat as observed in the present study experienced substantial fertilization failure and embryonic mortality from the time of ovulation through early implantation. Of 48 ovulating queens, 12.5% experienced a definitive infertile estrus (no embryos recovered or implantation sites identified). An additional 8.3% were tentatively classified as experiencing an infertile cycle because only degenerate embryos were recovered. Therefore, the overall "infertility rate" for ovulating queens was ~20%. Embryo/oocyte recovery was < 100% in all preimplantation-interval groups (mean recovery, 80.6%), possibly as a consequence of physiological factors (i.e., failure of oocyte retrieval by the fimbriae or anatomical obstructions) [25] or technical error in flushing the reproductive tracts. For example, the relatively low recovery success (56.4%) in the 148-h group was potentially due to embryo/oocyte loss associated with severing the oviducts at the uterotubal junction during the transition of embryos to the uterus. However, we have no evidence to suggest that recovery rates differed for unfertilized oocytes, degenerate embryos, or viable embryos, although this is a possibility. Ovulatory queens in the pre- and post-implantation groups had comparable numbers of ovulations and total embryos or implantations, with an overall disparity of ~30% between CL and embryo or implantation numbers. This disparity was higher than the implantation failure rate noted by Tsutsui et al. [58] (~16%). Tsutsui et al. examined a larger group ($n = 169$) of cats from random sources and with unknown mating and fertility histories. It may well be that more of these queens were older, multiparous females in contrast to our predominantly young (mean age, 20 mo), nulliparous (85%) group.

An overall pregnancy failure rate of ~30% from ovulation through early implantation was comparable to values

reported for other domestic species. In pigs, embryonic mortality before Day 25 of pregnancy ranges from 20 to 30% [17, 59], whereas 25–30% of cattle pregnancies are lost before Day 35 after breeding [60, 61]. There are multiple causes of early embryonic mortality, including genetic (oocyte/embryo chromosomal abnormalities), physiological (age, suboptimal semen quality, endocrine dysfunction), and environmental (nutrition, climate) factors [60]. The incidence of genetic abnormalities in *in vivo*-generated cat embryos has not yet been studied, but heteroploidy is a significant cause of early embryonic death in humans [62]. In our study, most of the physiological and environmental factors (age, health, parity, semen quality, nutrition, climate) were strictly controlled and generally optimized for reproductive success. The one uncontrollable variable was the endocrine milieu in mated queens, but previous studies [31, 41, 53, 54] have extensively characterized endocrine patterns in naturally copulating cats and have demonstrated a general uniformity among ovulating queens. Therefore, although it deserves more systematic attention, the etiology of embryonic mortality in the cat may well be related primarily to the gametes, especially their structural, functional and genetic competence.

In summary, most queens ovulated in response to multiple matings beginning on the second day of estrus, and this copulatory regimen was adequate to produce embryos *in vivo*. After fertilization, the resulting embryos exhibited an initial rapid phase of cleavage followed by a slower phase after three cell cycles. Embryos developed to morulae over a 4-day period within the oviduct, underwent compaction, and entered the uterus 5.5 days after the first copulation. Blastocysts within the uterus migrated between uterine horns before implantation. Lastly, 88% of ovulating females conceived (produced fertilized oocytes/embryos); but as many as 30% of ovulated oocytes failed to fertilize or implant, indicating considerable opportunity for reproductive failure in the naturally estrual and mated domestic cat.

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