BIOLOGY OF REPRODUCTION 60, 756–762 (1999)

Prolonged Mating in Prairie Voles (*Microtus ochrogaster*) Increases Likelihood of Ovulation and Embryo Number[1]

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ABSTRACT

Prairie voles are induced ovulators that mate frequently in brief bouts over a period of ~24 h. We examined 1) impact of mating duration on ovulation and embryo number, 2) incidence of fertilization, 3) temporal pattern of embryo development, 4) embryo progression through the reproductive tract over time, and 5) embryo development in culture. Mating was videotaped to determine first copulation, and the ovaries were examined and the reproductive tracts flushed at 6, 8, 10, 12, 16, 20, and 24 h and 2, 3, and 4 days after first copulation. The number of mature follicles and fresh corpora lutea and the number and developmental stage of embryos were quantified. One, two-, and four-cell embryos were cultured in Whitten's medium. Mature follicles were present at the earliest time examined (6 h). Thirty-eight percent of females that had been paired for < 12 h after the first copulation ovulated, whereas all females paired ≥ 12 h after the first copulation ovulated. Virtually all (> 99%) oocytes recovered from females paired for ≥ 12 h after first copulation were fertilized. Pairing time after first copulation and mean copulation-bout duration were significant ($p < 0.05$) determinants of embryo number. Embryos entered the uterine horns and implanted on Days 3 and 4, respectively, after first copulation (Day 0). Embryos cultured in vitro underwent approximately one cell division per day, a rate similar to that in vivo. We conclude that prairie voles ovulate reliably after pairing for ≥ 12 h, although some females showed exceptional sensitivity not predicted by the variables quantified. Prolonged mating for longer than 12 h increased the total embryos produced. This mechanism likely has adaptive significance for increasing offspring number.

INTRODUCTION

Successful reflex, or induced, ovulation in mammals differs from spontaneous ovulation in that copulation is needed to trigger an LH surge. Physical stimulation of the cervix and vagina during mating activates afferent neural pathways to GnRH-producing centers in the hypothalamus, culminating in an LH surge and eventually ovulation [1].

For most induced ovulators, the phases of reproductive activation (follicular development and behavioral estrus) occur spontaneously in response to appropriate environmental cues [2, 3]. However, microtine rodents (genus *Microtus*) are induced ovulators that do not consistently respond to seasonal environmental changes but rather to social factors [4, 5]. Prairie voles (*Microtus ochrogaster*) do not display any detectible spontaneous ovarian activation but are induced into estrus by pheromonal exposure from a novel, conspecific male [6]. Behavioral estrus occurs 1–2 days after introduction, and prairie voles mate repeatedly (6–39 times) in bouts of 0.4–6.4 min over a 1-day period [7, 8]. The functional relationship between prolonged mating and ovulation is poorly understood. One study indicates that ovulation occurs within 8 days in 50% of female prairie voles after only three intromissions [9], whereas another suggests that ovulation occurs ~24 h after first copulation [10].

The purpose of the present study was to examine the functional significance of prolonged mating in *M. ochrogaster* with respect to the incidence of ovulation and number of corpora lutea (CL) and embryos produced. We hypothesized that prolonged mating facilitates ovulation and increases embryo number. Although the reproductive behavior of prairie voles has been studied for more than 30 yr [11], little is known about fertilization, embryogenesis, and embryo development. Our specific objectives were to examine 1) the effect of pairing time after first copulation, copulation duration (mean copulation-bout duration and total copulation duration), and number of copulation bouts on ovulation onset and total embryos produced; 2) the incidence of fertilization; 3) the kinetics of embryo transport through the reproductive tract; and 4) embryo development in vivo and in vitro.

MATERIALS AND METHODS

Chemicals and Media

PB1 medium [12] was prepared using BSA (crystallized: cat. no. 81–001–4) purchased from Bayer Corp. (Kankakee, IL) and modified Dulbecco's PBS (cat. no. 14280-036) purchased from Gibco BRL-Life Technologies (Grand Island, NY). Whitten's medium [13] was prepared by the NIH Media Unit (Bethesda, MD). Tert-amyl alcohol (cat. no. 24,048-6) and 2,2,2-tribromoethanol (cat. no. T4, 840-2) were purchased from Aldrich Chemical Co. (Milwaukee, WI). All other chemicals were obtained commercially from Sigma Chemical Co. (St. Louis, MO).

Animals

Research animals were first- and second-generation, outbred descendants of wild-caught prairie voles from populations at the Illinois Natural History Survey experimental pond sites in Urbana, Illinois. Voles were maintained in the National Zoo's Department of Zoological Research Animal Facility under a 16L:8D cycle (lights-on at 0500 h local time). Animals were housed in 20 × 25 × 45-cm polycarbonate cages containing a substrate of shredded aspen bedding (Aspen Bed #1; Harlan Teklad, Madison, WI) with

Accepted October 27, 1998.
Received April 13, 1998.
[1]Supported by a Smithsonian Institution National Board Fellowship and Smithsonian Institution Restricted Endowment Funds Award to R.L.R.
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alfalfa hay for nesting material. Diet consisted of vole chow (Purina Mills, St. Louis, MO), sunflower seeds, rolled oats, and water ad libitum, with weekly supplements of lettuce.

This research was conducted in accordance with the Guiding Principles for the Care and Use of Research Animals promulgated by the Society for the Study of Reproduction and standards set forth in the NIH Guide for the Care and Use of Laboratory Animals.

Mating Protocols

Mating was monitored by time-lapse videotaping to determine time of first copulation and number and length of subsequent mating bouts. A closed-circuit camera (Panasonic model WVBP310, Secaucus, NJ) with a wide-angle infrared lens (Panasonic model WVLA408C3) was positioned ~40 cm from each cage, and activity was recorded (Panasonic model AG6730 time-lapse VCR) on a standard S-VHS videotape at the 24-h tape-speed setting (frame speed = 4.6 frames/sec). During the dark period (2100–0500 h), the area in proximity to the mating pairs was illuminated using an infrared illuminator (Burle model TC82451RW, Lancaster, PA). Each tape was replayed at the 2-h tape-speed setting (SP; 12 times the recorded tape speed) to determine time of first copulation. About one third of the pairs mated within 4 days of introduction. The remaining pairs (those that did not mate) were separated, and the females were housed singly for at least 10 days before pairing with a different male. This process was repeated until the females mated (which almost always occurred by the third pairing).

Incidence of Ovulation

Preliminary data indicated that ovulation occurred in this species approximately 12 h after mating onset. To assess the relative contributions of pairing time (i.e., time from first copulation to time of separation of breeding pairs) and time of embryo collection to the incidence of ovulation, some pairs in the < 12 h groups were separated 2–6 h before embryo collection (Table 1). In this way, we determined whether a shorter mating period (6–10 h) followed by single housing for 2–6 h was as effective in eliciting ovulation as continued male exposure for the entire period. The variable "pairing time after first copulation" was defined as the number of hours from first copulation to separation from the male. The variable "time of collection after first copulation" was defined as the number of hours from first copulation until time of embryo collection. Time of collection after first copulation was 0–6 h longer than pairing time after first copulation for females paired < 12 h.

Impact of Prolonged Mating on CL and Embryo Number

To assess the contribution of prolonged male-female interactions on CL and embryo number (beyond the postulated 12-h ovulatory "threshold"), pairs were allowed to interact for periods of 16 h to 4 days during which copulatory interactions occurred ad libitum. The ovulatory response of females was quantified and examined statistically as a function of specific copulatory interactions (see below), as well as duration of overall male exposure.

Behavioral Assessment

Time-lapse videotaped mating sessions were analyzed for the total duration of time that copulation was engaged in (copulation duration = total min engaged in lordosis while mounted) using The Observer 2.0 behavioral event-recording software (Noldus Information Technology, Wageningen, The Netherlands). Time-lapsed durations were converted to real times using a conversion factor of 12 (24 h activity per 2 h tape).

TABLE 1. Proportion of females in each group that ovulated as a function of the pairing time and time of embryo collection after first copulation.[a]

Pairing time after first copulation (h)	Time of embryo collection after first copulation (h)	No. females ovulating/ No. collected (percent ovulating)
6 h	6 h	0/4 (0)
6 h	8 h	1/3 (33)
6 h	10 h	0/2 (0)
6 h	12 h	2/3 (67)
8 h	8 h	1/3 (33)
8 h	10 h	3/3 (100)
8 h	12 h	0/2 (0)
10 h	10 h	2/3 (67)
10 h	12 h	1/3 (33)
12 h	12 h	3/3 (100)
16 h	16 h	7/7 (100)
20 h	20 h	7/7 (100)
24 h	24 h	10/10 (100)
Day 2	Day 2	14/14 (100)
Day 3	Day 3	23/23 (100)
Day 4	Day 4	10/10 (100)

[a] Females in groups with pairing times ≤ 12 h were assigned to collection times equal to or greater than their pairing time in order to determine whether prolonged mating or a prolonged period of time was necessary for ovulation to occur; females in groups with pairing times > 12 h remained paired with the male until the time of embryo collection.

Ovarian Morphology and Embryo Collection

Embryos were collected at 6, 8, 10, 12, 16, 20, and 24 h or 2, 3, or 4 days after first copulation (see Table 1 for number of females per time period). Initially, only ova/embryos were collected from the mated females, and the ovaries were not examined (n = 26). Subsequently, ova/embryos were recovered from mated females (n = 74), and their ovaries were examined for mature follicles and fresh CL. Briefly, females were anesthetized with a 1.5% tribromoethyl alcohol solution (tert-amyl-alcohol 3%; tribromoethanol 3%; 0.9% saline) and, when necessary, supplemented with gaseous Metofane (methoxyflurane; Pittman-Moore, Mundelein, IL) delivered via a nose cone. Once a surgical plane of anesthesia was reached, each female was ovariohysterectomized, and the uterine horns and ovaries were aseptically excised. The laparotomy site was sutured and females were wrapped in a towel and allowed to recover in a clean cage under an incandescent light for warmth.

Ovaries were assessed using a stereo microscope (×10) for number of mature follicles and fresh CL. Each oviduct and uterine horn was flushed using a 33-gauge blunt-tipped needle attached to a 1-ml syringe filled with PB1 medium. The needle was inserted into the infundibulum of the oviduct, and the entire tract was flushed with ~0.3 ml of medium. The contents of each uterine horn were discharged separately into a Petri dish and examined by stereomicroscopy for number, condition, and developmental stage of embryos.

One-cell ova were considered to be fertilized if two polar bodies were present (Fig. 1, top left). Developmental stages beyond the one-cell stage were determined by counting blastomere number.

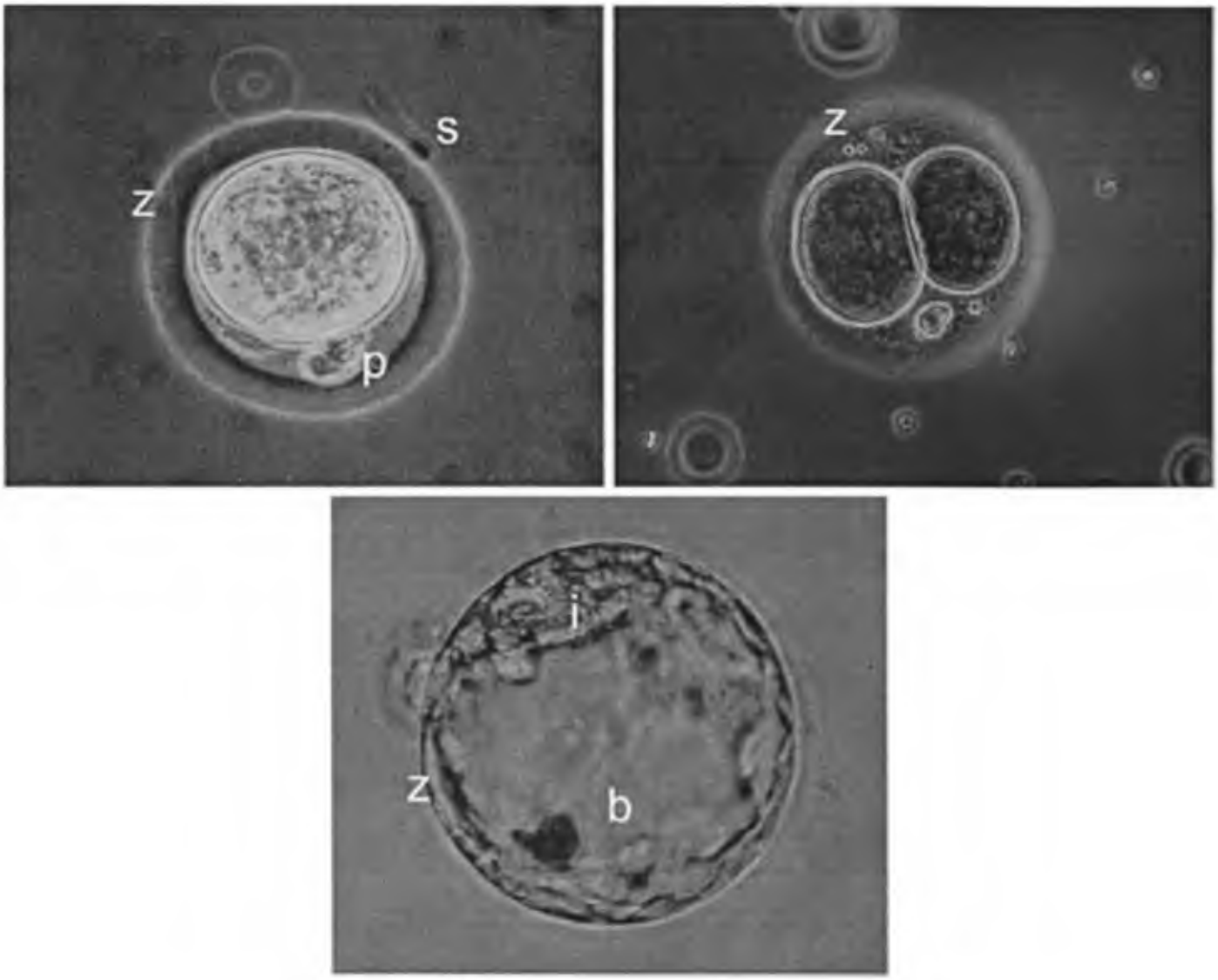

FIG. 1. Representative 1-cell and 2-cell embryos (top left and right, respectively) cultured in Whitten's medium to the expanded blastocyst stage (bottom). S, Sperm; P, polar bodies; B, blastocoele; I, inner cell mass; Z, zona pellucida. Embryo diameter is ~70 μm.

Embryo Distribution

To determine location and distribution of embryos between the oviduct and uterine horn as a function of time, reproductive tracts also were processed 2–4 days after first copulation. The oviduct and uterine horn were separated at the uterotubal junction. Each uterine horn was flushed in the normograde direction from the opening of the uterotubal junction with PB1 medium. Each oviduct was similarly flushed into a separate dish. The uterine horns of females collected beyond 85 h after first copulation were examined microscopically for evidence of implantation sites.

Embryo Culture

One- (Fig. 1, top left), two- (Fig. 1, top right), and four-cell embryos were cultured to the expanded blastocyst stage (Fig. 1, bottom) in Whitten's medium in a humidified atmosphere of 5% CO_2 at 38.5°C. Embryos were inspected daily at ~1400 h for developmental progress and condition. The incidence of parthenogenetic oocyte cleavage in culture and in vivo was determined by recovering ova from three donor females, each paired with a vasectomized, proven sterile male for 1–2 days after first copulation. The number of polar bodies present in oocytes and 1-cell embryos was counted for post hoc evaluation of polar body number as a reliable method for assessing fertilization.

Data Analysis

The incidence of ovulation as a function of time paired after first copulation was analyzed using Fisher's exact test. Forward stepwise multiple regression (Procedure REG; SAS 6.02; Statistical Analysis Systems Institute, Cary, NC) was used to analyze the relative importance of both the linear and quadratic components of pairing time after first copulation, copulation-bout number, mean duration of copulation bouts, total copulation duration, time of embryo collection after first copulation, latency to mating after initial introduction, and duration of side-by-side behavior (including allogrooming) on the numbers of CL and embryos. Nonsignificant factors ($p > 0.05$), including all interaction effects, were eliminated from the model. The regression ANOVA was calculated on the basis of the simplified model.

Embryo development in culture was compared to that in vivo by comparing the slopes of their linear regression models using a Student's *t*-test. Proportion of embryos recovered from each donor was calculated as total number of embryos recovered divided by fresh CL number. Fertilization success was calculated as the proportion of embryos recovered that were at the 2-cell or greater stage plus the proportion of 1-cell embryos that contained two polar bodies.

RESULTS

The proportion of females with at least one ovulation varied depending on the time animals were paired after first copulation. As evidenced by ovarian morphology, all females initiated ovulation by 12 h after first copulation, and few mature follicles remained by 24 h after first copulation (Table 2). However, only 38% (10 of 26) of females that had been paired for 6–10 h after first copulation ovulated, and these females had more ($p < 0.001$) mature ovarian follicles remaining than females in the 16-h and later groups (Table 2). None of the specific characteristics of copulatory behavior measured in this study (copulation frequency, total copulation duration, mean bout duration), or courtship (latency from pairing to first copulation), or affiliative behavior (duration of side-by-side behavior) had an impact ($p > 0.05$) on the likelihood of ovulation occurring or number of CL produced (Table 3).

Regression analysis revealed that CL number increased steadily for pairing times up to Day 2, with the greatest increase from 16 h to Day 2, and that it did not change at longer pairing times (Table 2; $r^2 = 0.61$, F = 27.6; $p < 0.001$). The linear (partial $r^2 = 0.48$; $p < 0.001$) and quadratic (partial $r^2 = 0.13$; $p < 0.002$) components of pairing time after first copulation significantly affected total CL number. Similar trends were identified for the regression of copulation duration and pairing time on embryo number (Table 2; $r^2 = 0.70$, F = 27.1; $p < 0.001$). The linear (partial $r^2 = 0.50$; $p < 0.001$) and quadratic (partial $r^2 = 0.16$; $p < 0.001$) components of pairing time after first copulation accounted for most of the variability associated with embryo number. The mean duration of copulation bouts also made a small but significant ($p < 0.05$) contribution to the regression model for embryo number (partial $r^2 = 0.04$).

The significant effect of extended pairing times after first copulation on the number of CL and embryos was attributable to additional mature follicles found at ~24 h after first copulation and additional 1-cell embryos in females collected at pairing times beyond 24 h (Table 2). In 11% (5 of 47) of females collected on Days 2–3 after first copulation, at least one embryo was recovered at a developmental stage two or more cell divisions earlier than that of the remaining embryos. These early-stage embryos collected from females on Days 2–3 after first copulation were viable in culture. All embryos collected beyond Day 3 after first copulation were at an advanced developmental stage (> 8-cell).

Embryos were located only in the oviducts or in the uterine horns within 77 h of, or 83 h after, first copulation, respectively (Table 4). Embryos were located in both the oviduct and uterine horns of two of three voles examined 77–83 h after first copulation. Implantation sites were located in the uterine horns of two of three voles examined beyond 93 h after first copulation.

Embryos underwent approximately one cell division per day in culture. This developmental rate was not different

TABLE 2. Ovarian activity and embryo number in female prairie voles as a function of pairing time after first copulation.

Pairing time after first copulation[a] (pooled across collection times)	Total no. ovulating females	Mature follicles[b] (mean ± SEM)	Fresh CL (mean ± SEM)	CL/ovulating female (mean ± SEM)	1-Cell	2-Cell	4-Cell	8-Cell	>8-Cell	Total no. embryos	Embryos/female (mean no. ± SEM)
6 h	3	3.8 ± 0.7	0.8 ± 0.4	3.0 ± 0	6	0	0	0	0	6	2.0 ± 0.6
8 h	4	2.8 ± 0.7	1.5 ± 0.7	3.0 ± 0.7	4	0	0	0	0	4	1.0 ± 0.7
10 h	3	2.0 ± 0.7	1.8 ± 1.1	3.7 ± 1.8	4	0	0	0	0	4	1.3 ± 1.3
12 h	3	2.0 ± 0	3.5 ± 0.5	3.5 ± 0.5	10	0	0	0	0	10	3.3 ± 0.9
16 h	7	0.8 ± 0.8	4.2 ± 0.6	4.2 ± 0.6	25	0	0	0	0	25	3.6 ± 0.7
20 h	7	0.7 ± 0.3	3.8 ± 0.3	3.8 ± 0.3	17	5	0	0	0	22	3.1 ± 0.5
24 h	10	1.0 ± 0.6	4.4 ± 0.6	4.4 ± 0.6	21	18	1	0	0	40	4.0 ± 0.5
Day 2	14	0 ± 0	4.9 ± 0.5	4.9 ± 0.5	4	26	17	6	0	53	3.8 ± 0.4
Day 3	23	0.8 ± 0.6	5.5 ± 0.5	5.5 ± 0.5	7	3	6	55	12	83	3.6 ± 0.4
Day 4	10	0 ± 0	4.8 ± 0.4	4.8 ± 0.4	0	0	0	0	36	36	3.6 ± 0.4

[a] Pairing time after first copulation is defined as the period from time of first copulation until ovariohysterectomy and embryo collection.
[b] Of the 100 total animals in this study, 74 were examined for ovarian activity.

TABLE 3. Nonrelationship between copulatory variables and likelihood of ovulation from each of the three pairing times in which some females failed to ovulate.[a]

Female ID	Pairing time after first copulation (h)	No. CL	Copulation bout frequency	Total copulation bout duration (min)	Mean copulation bout duration (min)
1056	6	0	16	36.0	1.3
1122	6	0	3	9.3	3.1
1113	8	0	7	11.6	1.7
1123	8	0	11	66.2	6.0
1184	10	0	25	45.4	1.8
1121	10	0	20	45.8	2.3
1132	10	1	31	40.9	1.3
1204	8	2	9	18.1	2.0
1183	6	3	10	8.8	0.9
1193	6	3	14	25.4	1.8
1167	8	3	9	47.4	5.3
1207	10	7	12	33.8	2.8

[a] There was no relationship ($p > 0.05$) between copulatory frequency, total copulation duration, or mean copulatory-bout duration and likelihood of ovulation or CL number.

($p > 0.05$) from that observed for embryos developing in vivo. Cultured embryos developed to the expanded blastocyst stage and degenerated without hatching. All of the oocytes (n = 16 ova) collected from females that had been paired with a vasectomized male contained a single polar body, and none cleaved during in vitro culture. Only one unfertilized oocyte was collected from a female that had been paired with a fertile male, as determined by the presence of a single polar body at the time of collection and failure to divide in culture within 24 h of collection. On the basis of the number of fresh CL compared to the number of embryos recovered, 84% of the oocytes and embryos were collected successfully from females paired with males at least 12 h after first copulation. The overall mean (± SEM) number of embryos recovered was 3.2 ± 0.2 per female.

DISCUSSION

Prairie voles are remarkable among induced ovulators in the extent to which stimulation from a male is required for successful reproduction. We have determined that prairie voles ovulate reliably only after 12 h or more of pairing after mating onset. For females in which pairing time was varied independently from time of collection (Table 1), the pairing time, and not the collection time, was the factor that contributed most to the likelihood that females would ovulate and to the number of CL and embryos produced. Females allowed to mate beyond an apparent threshold of 12 h also exhibited a graded response in terms of the numbers of CL (Table 2) and embryos produced.

TABLE 4. Progression of embryos in the reproductive tract of prairie voles as a function of time.[a]

Pairing time after first copulation (h)	Position in reproductive tract	Developmental stage of embryos	No. embryos
22	Oviduct	1-cell	3
24	Oviduct	1-cell	3
48	Oviduct	2-cell	4
53	Oviduct	2-cell	5
72	Oviduct	8-cell	4
73.5	Oviduct	8-cell	1
75	Oviduct	8-cell	4
77	Oviduct	8-cell	3
78.5	Oviduct	8-cell	2
	Uterine horns	8-cell	3
80	Oviduct	8-cell	1
	Uterine horns	8-cell	2
83	Uterine horns	8-cell	5
85	Uterine horns	Morula	1
		Early blastocyst	3
93	Uterine horns	Implanted	4
96	Uterine horns	Blastocyst	4
110	Uterine horns	Implanted	5

[a] For these 15 females, embryos were collected from oviducts and uterine horns separately in order to determine embryo distribution (see *Materials and Methods* for detailed description of procedures).

Most of the female voles mating for 10 h or less failed to ovulate (62%). The females that did ovulate after pairing for 6–10 h after first copulation did not display any remarkable features in copulatory frequency or duration that might explain their heightened ovulatory responsiveness (Table 3). Our data are not consistent with the previous report of Gray et al. [9], which indicated that females ovulated after only one or two ejaculatory series. However, in those studies, females were primed for 3 days with male-soiled bedding prior to introduction of the male for mating, and ovarian responses were evaluated 8 days after removal of the male from the test cage. During the interim (from removal of the male to ovarian examination), the female was housed in the test cage where the male had been housed before and during pairing. Therefore, the female was continuously exposed to the pheromones of her mate even after his removal. Gray et al. [9] also varied the mating stimulation provided to female prairie voles within a window of no more than two ejaculatory series and found that the number of intromissions and the number of thrusts per intromission positively affected the incidence of ovulation. In another study, Richmond and Stehn [14] found that a single mating was sufficient to induce ovulation, but CL were not retained without continued cohabitation with the male. However, results from the present study clearly indicated that ovulation did not occur in most females until ~10–12 h after first copulation.

In addition to an abrupt increase in the incidence of ovulation after 12 h of mating, the number of CL and embryos Iproduced also increased as a function of pairing time after first copulation (Table 2) irrespective of the total copulatory duration. The increased embryo number as a function of pairing time after first copulation was accounted for by the appearance of additional fresh CL (Table 2), and the recovery of 1- and 2-cell embryos on the second and third day after first copulation (Table 4), when most embryos were

at a more advanced developmental stage. By Day 4 after first copulation, all collected embryos were at the morula or blastocyst stage, suggesting that embryos from ovulations occurring 2–3 days after first copulation might be able to attain the developmental stage of their older counterparts coincident with entering the uterine horns. An alternative explanation is that the late-ovulated embryos were lost on Day 4. Our regression analysis did not reveal a significant third-order effect indicating embryo loss on later days, and these embryos divided normally in culture. Furthermore, mean litter size for this species (3.58 pups; calculated from Keller [15]) was not significantly smaller than the peak embryo number observed in the present study (4.0 ± 0.5). Therefore, we cannot conclude that the early-stage embryos that apparently ovulated on Days 2 and 3 were lost. It is possible that prolonged mating interactions and subsequent oxytocin release facilitated embryo transport [16], so that embryos appearing late were moved through the oviduct more quickly than those produced earlier.

Although copulatory duration and frequency accounted for a negligible portion of the variability in cumulative ovulatory response, time paired after first copulation accounted for a total of 61% and 66% of the variability in CL and embryo number, respectively. This suggested that even a minimal amount of copulatory stimulation, combined with prolonged exposure to other components of courtship and mating (e.g., pheromonal and/or tactile stimulation, not quantified in this study), might contribute to ovulation induction in this species. This interpretation may explain differences between the present study and that of Gray et al. [9].

Among mammals that exhibit reflex ovulation, there is substantial variability in the amount of stimulation required in order for ovulation to occur. In one study, rabbits were observed to ovulate after 200 msec of copulatory stimulation [17]. In the ferret (*Mustela furo*), intromissions of a duration as short as 1 min are sufficient to stimulate full ovulatory response [18], and minks (*Mustela vison*) have been observed to ovulate in response to pairing without copulation [19]. In the domestic cat, reliable ovulation (83%) was achieved after three copulation bouts at 4-h intervals, but only 21% of queens experiencing a single copulation bout ovulated [20]. Of the queens that ovulated in that study, there was no difference in the number of CL produced as a function of the number of copulation bouts.

There also is variability within the genus *Microtus* with regard to ovarian cyclicity and latency to ovulation. Some species, such as *Microtus pennsylvanicus* and *Microtus agrestis,* exhibit irregular patterns of vaginal cornification and ovarian cyclicity, and ovulate after brief exposure to males or male odors without copulation [4, 21–23]. The prairie vole exhibits no indication of ovarian activation until paired with an unfamiliar male [4, 11], and, in the present study, most females required a minimum of 12 h of mating in order for ovulation to occur. The variability in amount of social stimulation required for behavioral estrus and ovulation in the genus *Microtus* may be related to mating system, with promiscuous species requiring less male stimulation than monogamous species [4, 24].

Females that underwent embryo collection at the earliest collection times (6–12 h) had copious amounts of sperm in the uterine horns, and almost all embryos recovered at all collection times were fertilized. Females from the earliest collection times also had well-formed, invasive copulatory plugs that extended into the cervical region, consistent with previous observations from this species [11]. Therefore, it is doubtful that prolonged mating increases the rate of fertilization or contributes to sperm competition in this species, although we have not addressed the latter issue in the present study. It is more likely that prolonged mating and subsequent cervical stimulation confers reproductive benefits by maximizing ovarian activity and sustaining the motility of the oviducts and uterus for gamete transport.

Microtus embryos developed normally to the blastocyst stage in Whitten's medium in standard embryo culture conditions at a rate similar to that found in vivo. Thus, these conditions should be appropriate for future studies designed to examine vole embryo development in vitro. There also was nothing remarkable about the appearance of unfertilized or fertilized oocytes. A single polar body was present in unfertilized oocytes, whereas all 1-cell embryos that divided in culture contained two polar bodies. Finally, no parthenogenic cleavage was observed, indicating that vole oocytes apparently have a high threshold for spontaneous activation.

In summary, this study has uncovered a graded ovulatory response to prolonged social stimulation in a reflex ovulator, the prairie vole. On the basis of an absence of similar findings in studies of other *Microtus* species and other reflex ovulators, we propose that this characteristic might be unique to prairie voles and may represent an adaptive mechanism to promote prolonged association between males and females, ensuring sperm-oocyte interaction by maximizing male contributions to reproductive success.

ACKNOWLEDGMENTS

We thank Michael Deal for outstanding animal care and record keeping and Peter Miller, Kathy Morrison, and James Carleton for assistance. We are grateful to Jan Norcross, Donald Dewsbury, and three anonymous reviewers for comments on earlier drafts of the manuscript.

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