The Period of Ovulation and Presence of the First Polar Body of Ova Ovulated in the House Musk Shrew (*Suncus murinus*)

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Abstract: The period of ovulation in mature house musk shrews was examined in a natural mating group and a superovulation group treated with gonadotropin. In the natural mating group, ovulation started 14 hr after mating in 3 of the 7 house musk shrews (42.8%), and occurred in all 5 house musk shrews by 15 hr after mating. In the superovulation group, ovulation started 13 hr after the administration of hCG in 3 of the 5 house musk shrews, and was observed in all 5 shrews by 16 hr after the administration. In the natural mating group, ovulated ova were collected from the ovarian bursa of 14 house musk shrews 14–20 hr after mating (mean, 2.2 ± 1.0 ova) and from the oviduct of 42 animals 14–24 hr after mating (mean, 3.6 ± 1.8 ova). Among the ova ovulated 14–16 hr after mating, both mature ova with the first polar body and immature ova without the first polar body were observed. In the superovulation group, ovulated ova were collected from the ovarian bursa of 31 house musk shrews 13–22 hr after the administration of hCG (mean, 9.7 ± 6.8 ova), and from the oviduct of 28 animals 13–24 hr after the administration of hCG (mean, 20.0 ± 11.7 ova). There were also mature and immature ova in the ova ovulated 13–16 hr after the administration of hCG. The time when ova ceased to be recovered from the ovarian bursa roughly coincided with the time when new corpora lutea ceased to be found in the ovaries. These findings suggested that the period of ovulation of house musk shrews was 14–20 hr after mating in the natural mating group and 13–22 hr after the administration of hCG in the superovulation group. Both the natural mating group and superovulation group ovulated both mature ova with the first polar body and immature ova without the first polar body.

Key words: first polar body, natural mating, ovarian bursa, ovulation, superovulation

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Introduction

There are two kinds of temporal regulation of ovulation in mammals, one occurring periodically as in mice [22, 29] and other animal species [17, 23, 24, 27, 28] and the other which is induced by mating as seen in rabbits [1, 2]. In the latter kind of animals, the ovarian follicles show repeated cycles of growth, closure, and regression during the breeding season, and ovulation is induced by mating or other similar stimuli. There are two types of expression of estrus in this group of animals. One is the rabbit-type in which there is always a small number of mature ovarian follicles in the ovary during the reproductive period, and the other is the cat-type in which growth and regression of the ovarian follicles periodically repeats and estrus occurs coincidentally with this cycle [26]. The house musk shrew (*Suncus murinus*) shows the former type of estrus, and mating is therefore always possible in the species [3, 4, 8, 11, 20].

In the house musk shrew, the roles of ovarian estrogen in the growth and maintenance of the accessory genital organs differ from those in other mammals. The removal of the ovary does not significantly affect the tissue structures of the uterus and vagina, and the weights of the uterus and vagina are independent of ovarian hormones [6, 25]. Ovulation can be induced by the administration of a single gonadotropin (PMSG, hCG, FSH or LH) in the house musk shrew [5]. In the mouse [7, 33], hamster [10], and rabbit [16], superovulation can be induced, and we have developed a superovulation induction method for the house musk shrew by a combined administration of PMSG and hCG (manuscript submitted).

In the present study, we examined the time of ovulation in house musk shrews after natural mating and after inducing superovulation by the administration of hCG.

Materials and Methods

Three to four-month-old male and female house musk shrews (Jic: SUN Strain) [18, 19] bred in the Central Institute for Experimental Animals were used. The house musk shrews were kept in a room at $22 \pm 2^\circ\text{C}$, with humidity at $55.5 \pm 5\%$ and ventilation 10–15 times/hr with fresh air. The lighting condition was a 12-hr

light period (8.00 a.m.–8.00 p.m.) and a 12-hr dark period. The animals were fed solid feed (CIEA-305) [32] and allowed to drink tap water *ad libitum*.

In the natural mating group, each female house musk shrew was mated with a male, and after the mating was confirmed (the male licks his penis after showing mounting behavior), the female and male of each pair were separated after being housed a cage for 2 hr. In the superovulation group, the females were then superovulated by receiving an intraperitoneal injection of 5.0 iu pregnant mare's serum gonadotropin (PMSG) followed by an intraperitoneal injection of 5.0 iu human chorionic gonadotropin (hCG) 72 hr later.

The PMSG (5.0 iu) and hCG (5.0 iu) were each dissolved in 0.2 ml physiological saline before injection. Ova were collected 12, 13, 14, 15, 16, 17, 18, 20, 22 and 24 hr after the first mating in the natural mating group and at the same time points after the administration of hCG in the superovulation group. To collect the ova, the female house musk shrews were killed by cervical dislocation under anesthesia with ether, the ovaries and uterus were dissected out and placed on a filter paper, and the fat was removed from the uterus and ovaries. The ovaries and oviduct were then transferred to a dish with a culture medium (TYH), and the ova were collected from the ovarian bursa and oviduct under a dissecting microscope. In some of the ova collected with the cumulus cells, the cumulus cells were removed by repeated pipetting with a glass capillary, and live ova were observed under an inverted microscope. Ovulation was confirmed by the presence of the corpora lutea on the ovary surface.

Results

1. Period of ovulation in the natural mating group

Table 1 shows the period of ovulation in the natural mating group. Large ovarian follicles were observed in the ovary of the female house musk shrew 13 hr after mating, and 14 hr after mating, ova were collected from 3 of the 7 shrews mated. Of these 3 shrews, 2 had large ovarian follicles and corpora lutea in the ovary, and one had fungiform corpora lutea but no ovarian follicles in the ovary. In each ovary of the other 4 animals without ovulation, several large ovarian follicles were observed. Fifteen hr after mating, ovulation was observed in all five of the house musk shrews

Table 1. The ovulation period of the house musk shrews after copulation

Time (hr) after copulation	No. of animals examined	No. of (%) animals ovulated	No. of (%) ovum recovered and situation of recovery						
			Total (Means $\pm$ SD)	With cumulus cells	Without cumulus cells	No. of animals	Ovarian bursa	No. of animals	Oviduct
12	3	0	0						
13	6	0	0						
14	7	3 (42.8)	9 (3.0 $\pm$ 2.7)	8 (88.9)	1 (11.1)	3/3	6 (66.7)	2/3	3 (33.3)
15	5	5 (100)	22 (4.4 $\pm$ 1.2)	20 (90.9)	2 (9.1)	5/5	13 (59.1)	4/5	9 (40.9)
16	8	8 (100)	34 (4.3 $\pm$ 1.3)	33 (97.1)	1 (2.9)	3/8	7 (20.6)	8/8	27 (79.4)
17	6	6 (100)	28 (4.7 $\pm$ 2.6)	28 (100)	0	1/6	2 (7.1)	6/6	26 (92.9)
18	5	5 (100)	14 (2.8 $\pm$ 0.8)	14 (100)	0	1/5	1 (7.1)	5/5	13 (92.9)
20	5	5 (100)	21 (4.2 $\pm$ 1.1)	20 (95.2)	1 (4.8)	1/5	2 (9.5)	5/5	19 (95.2)
22	6	6 (100)	24 (4.0 $\pm$ 0.9)	12 (50.0)	12 (50.0)	0/6	0	6/6	24 (100)
24	6	6 (100)	29 (4.8 $\pm$ 1.5)	23 (79.3)	6 (20.7)	0/6	0	6/6	29 (100)

examined (mean, 4.4 ova).

Ovulated ova were collected from the ovarian bursa of 14 animals 14–20 hr after mating. In particular, the beginning of ovulation at 14 hr after mating produced a high collection rate (66.7%). From the oviduct, ovulated ova were collected from the upper oviduct 14–16 hr after mating, and 17–24 hr after mating ovulated ova were collected from the ampulla of the oviduct and the lower oviduct. No new corpus luteum was found in the ovary 22 hr after mating.

The ova collected with the cumulus cells were covered by the thick cumulus cells, and some of the cells were removed by repeated pipetting with a glass capillary. Of the 7 viable ova from 2 house musk shrews sacrificed at 15 hr after mating, 4 had the first polar body but 3 were immature ova without the first polar body. Similarly, of the 8 ova from 2 of the house musk shrews examined 16 hr after mating, 2 mature ova had the first polar body while 6 were immature ova without the first polar body. Of the ova of animals collected 18 hr after mating, the ova of one animal were all mature ova with the first polar body, while the ova of another animal were all immature ova without the first polar body. Sperm were observed in the perivitelline cavity of one mature ovum. The ova of a house musk shrew collected 21 hr after mating were both with and without the first polar body. Penetration of sperm was observed in the pellucid zone of all of these ova (Table 3).

2. Period of ovulation in the superovulation group

Table 2 shows the period of ovulation in the group in

which superovulation was induced by the administration of PMSG and hCG. Ovulation was not observed at 12 hr after the administration of hCG (0/4 animals), but in the ovary of these 4 animals, large ovarian follicles were observed forming a continuous array. Thirteen hr after the administration of hCG, ovulation was observed in 3 of the 5 animals examined, and large ovarian follicles were observed in the ovary of the 2 shrews without ovulation. Sixteen hr after the administration of hCG, ova were collected from all five animals examined, but large ovarian follicles were also observed in the ovary.

One to twenty-six (mean, 9.7 ± 6.8) ovulated ova were collected from the ovarian bursa in 31 of the 34 house musk shrews observed 13–22 hr after the administration of hCG. During the early ovulation period, almost all ova were collected from the ovarian bursa, and the ovarian bursa remained a site of ovum collection, though at a low rate, at 22 hr after the administration of hCG. The number of ova collected from the ovarian bursa per house musk shrew was the largest at 16 hr after the administration of hCG (12–26 ova). From 17 hr after the hCG administration, the number of the ova collected from the ampulla of the oviduct and the lower part of the oviduct increased with time. There were no new corpus lutea in the ovaries at 24 hr after the administration of hCG.

In some of the ova collected with the cumulus cells, the cumulus cells were removed, and the ova inside were observed. Fourteen hr after the administration of hCG, the first polar body was observed in 7 of the 15

Table 2. The ovulation period of the house musk shrews in which superovulation was induced with injections of PMSG and hCG

Time (hr) after hCG injection	No. of animals examined	No. of (%) animals ovulated	No. of (%) ovum recovered and situation of recovery						
			Total (Means $\pm$ SD)	With cumulus cells	Without cumulus cells	No. of animals	Ovarian bursa	No. of animals	Oviduct
12	4	0	0						
13	5	3 (60.0)	31 (10.3 $\pm$ 8.0) ^a	30 (96.8)	1 (3.2)	3/3	30 (96.8)	1/3	1 (3.2)
14	5	4 (80.0)	27 (6.8 $\pm$ 5.9) ^a	27 (100)	0	4/4	27 (100)	0/4	0
15	5	4 (80.0)	38 (9.5 $\pm$ 6.2) ^a	37 (97.4)	1 (2.6)	4/4	29 (76.3)	2/4	9 (23.7)
16	5	5 (100)	129 (25.8 $\pm$ 8.4) ^b	124 (96.1)	5 (3.9)	5/5	89 (69.0)	4/5	40 (31.0)
17	5	5 (100)	146 (29.2 $\pm$ 10.8) ^b	136 (93.2)	10 (6.8)	5/5	51 (34.9)	5/5	95 (65.1)
18	5	5 (100)	156 (31.2 $\pm$ 4.6) ^b	152 (97.4)	4 (2.6)	2/5	29 (18.6)	5/5	127 (81.4)
20	5	5 (100)	174 (34.8 $\pm$ 18.3) ^b	169 (97.1)	5 (2.9)	5/5	37 (21.3)	5/5	137 (78.7)
22	3	3 (100)	76 (25.3 $\pm$ 7.8) ^b	71 (93.4)	5 (6.6)	3/3	8 (10.5)	3/3	68 (89.5)
24	3	3 (100)	83 (27.7 $\pm$ 7.2) ^b	80 (96.4)	3 (3.6)	0/3	0	3/3	83 (100)

Values with different alphabetical superscripts within each column are significantly different from each other ($p < 0.01$). The values at 18 hr, 20 hr and 22 hr, 24 hr are not significantly different from each other, Student's *t*-test.

Table 3. Presence of the first polar body of ovulated ova in the house musk shrews

Ovulation	Time (hr) after copulation or hCG injection	No. of animals examined	No. of ova ovulated	Development of ovum	
				With 1st polar body	Without 1st polar body
Copulation	15	2	7	4	3
	16	2	8	2 ¹⁾	6
	18	2	7	4 ²⁾	3
	21	1	4	2 ³⁾	2
Super-ovulation	14	1	15	7	8
	17	1	19 (8) ⁴⁾	2	6
	18	1	23 (10)	3	7

1) Sperm adhered to cumulus cells of 1 ovum. 2) Sperm were confirmed in the perivitelline space of 1 ovum. 3) Sperm penetrated all of the ova. 4) Parentheses indicate numbers of ova examined.

ova, and the remaining 8 ova were immature ova without the first polar body (Table 3). Seventeen to eighteen hr after the administration of hCG, there were ova with and without the first polar body, showing the same result as in the natural mating group.

Discussion

To clarify the time of ovulation in the house musk shrew, ovulation after natural mating and after hormone treatments was examined. The ovaries of the house musk shrew are very small, measuring about 1.5 mm, which is much smaller than the ovaries of the mouse,

and the short oviduct goes around the ovary once and adheres to the ovarian bursa [9, 13].

The time of ovulation in the house musk shrew has been reported to be 13 hr [21] or 15 hr [12] after mating in ovulation induced by natural mating. In the present study, it was 14 hr (3/7; 42.9%) after mating in the natural mating group and 13 hr (3/5; 60.0%) after the administration of hCG in the superovulation group. In both groups, ova were collected from the ovarian bursa. It has been shown that ovulated ova induced by mating remain in the ovarian bursa for a very short time and reach the ampulla of the oviduct even in an animal in which ovulation is in progress [21], but Hasler

[12] was able to collect ova from the ovarian bursa of two house musk shrews among 16 animals in which mating was confirmed. In our study, 1 to 4 ova per animal were collected from the ovarian bursae of 14 females after mating, and 1 to 26 ova per animal were collected from the ovarian bursae of 29 females in the superovulation group. These results indicate that ovulated ova do not move to the fimbria immediately but rather stay temporarily in the ovarian bursa.

The cessation of ovulation was estimated to be 20 hr after mating in the natural mating group and 22 hr after the administration of hCG in the superovulation group, since new corpora lutea were observed only during the period when ova could be collected from the ovarian bursa. The reason for the longer ovulation period in the superovulation group compared to the natural mating group was probably because the growth of many ovarian follicles and the maturation of the ova induced by the administration of PMSG were not simultaneous.

In most cases, ovulation in mammals occurs after the formation of the first polar body (metaphase 2) [14, 15]. In the house musk shrew, ovulation occurs either after the discharge of the first polar body following the resumption of cleavage of mature oocytes, which is 13 to 16 hr after mating, or before the formation of the first polar body [12]. In the present study, more than half (15/26) of the ova collected 13 to 14 hr after mating were immature and thus lacking the first polar body (metaphase 1). This finding suggests that completion of the first meiosis and formation of the first polar body occur within the oviduct, as is the case with dogs.

Much remains to be clarified regarding the process of maturation of ovulated ova as well as fertilization in the house musk shrew. A detailed examination of the characteristics of ovulated ova induced by the administration of hormones is also necessary.

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