—Brief Note—

Chronological Changes in Fertilized Eggs of the Mongolian Gerbil (Meriones unguiculatus)

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Abstract: *There have been very few reports about fertilized eggs of the Mongolian gerbil. This study was therefore designed to determine the chronological changes in fertilized eggs of the Mongolian gerbil. Superovulated females were monogamously paired with males and pregnant females were killed at 16, 19 and 22 hours after hCG injection. After slaughter, the presence of female and male pronuclei and sperm tail was examined. Rates of female and male pronuclei increased with the collection time for fertilized eggs (24.5, 58.9 and 67.9% at 16, 19 and 22 hr, respectively). Rates for a fertilizing sperm tail out of the total number of fertilization eggs decreased with the collection time for fertilized eggs (85.0, 58.0 and 28.0% at 16, 19 and 22 hr, respectively).*
Key words: *Mongolian gerbil, Fertilized egg, Female and male pronuclei, Sperm tail*

In most mammals, the presence of female and male pronuclei, second polar bodies and fertilizing sperm tails are indices of fertilized eggs in *in vitro* fertilization. As an exception, in the fertilization of Chinese hamster gametes, the middle piece and tail of the sperm are not almost incorporated into oocytes [10, 12]. Mongolian gerbils (*Meriones unguiculatus*) are used as laboratory animals, but there have been few reports regarding biological phenomena. The chronological change in fertilized eggs is conducted in most mammals [1–9, 11]. But there has been almost no report on fertilized eggs of the Mongolian gerbil. Marston and Chang [9] investigated the time of ovulation, fertilization and clevage in the naturally mated Mongolian gerbil. They examined the pronuclei at collection time, but they did not examine the relationship between pronuclei and the fertilizing sperm tail. In this study, we examined chronological changes in Mongolian gerbil *in vivo* fertilized eggs, particularly chronological changes in the pronuclei and fertilizing sperm tail.

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Mongolian gerbils were self-breeding in our laboratory. The animals were housed in polycarbonate cages (Seobit Co. Ltd., Tokyo, Japan) bedded with soft tip (Japan SLC Co. Ltd., Shizuoka, Japan) under conventional conditions. Lights were on from 6:00 to 18:00 (L: 12, D: 12). The diet was standard laboratory diet (FI: Funabashi Farm. Co. Ltd., Chiba, Japan) and fresh water was provided ad libitum, and formula feeds (Tsubue: Nippon Nosan Kogyo Co. Ltd., Kanagawa. Japan) were given to them every other day.

Females (2 to 3 months old) were injected with 10 IU each of pregnant mare serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG) (1 p.m. on day 0) at an interval of 48 hours. After the hCG injection, the females were monogamously paired with males (3 to 4 months old). Day 0 of pregnancy was defined as the day when spermatozoa were found in the vagina at 6 p.m. and the female was still being vigorously pursued by the male. At 4 a.m. the next day, a smear test was conducted.

Pregnant females were killed by cervical dislocation, and the oviducts were excised. Oocytes were collected from the excised oviducts by irrigation with PBI at 16, 19 and 22 hours after hCG injection. Oocytes were washed 3 times, mounted on slides, and examined under a phase-contrast microscope. Fertilization was assessed according to the formation of female and male pronuclei, a fertilizing sperm tail, and second polar body.

Figures 1, 2 and 3 show fertilized eggs obtained at 19 and 22 hours after hCG injection. In Figs. 1 and 2, pronuclei (male pronuclei or female and male pronuclei) and a fertilizing sperm tail in the egg can be seen, but in Fig. 3, only female and male pronuclei can be seen; the fertilizing sperm tail has disappeared.

Therefore, pronuclei and a fertilizing sperm tail in the egg can be seen in fertilized eggs of most mammals.

Table 1 and Fig. 4 indicate chronological changes in pronuclei and a fertilizing sperm tail. Rates of female and male pronuclei increased with collection time of

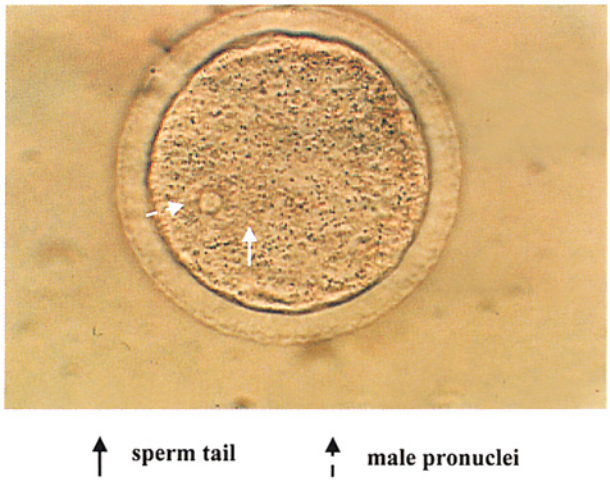

Fig. 1. Fertilized egg obtained at 19 hours after hCG injection.

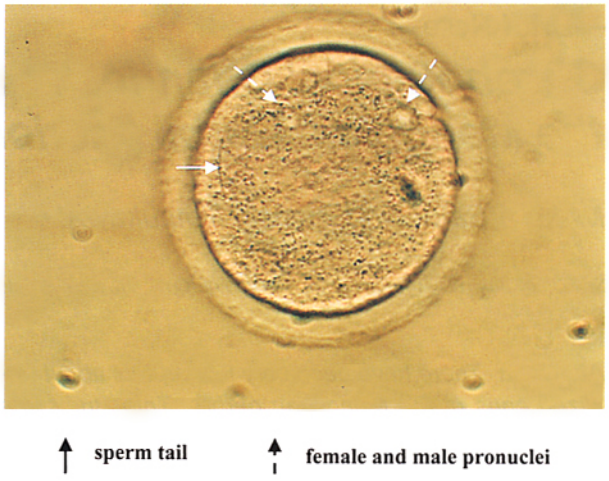

Fig. 2. Fertilized egg obtained at 19 hours after hCG injection.

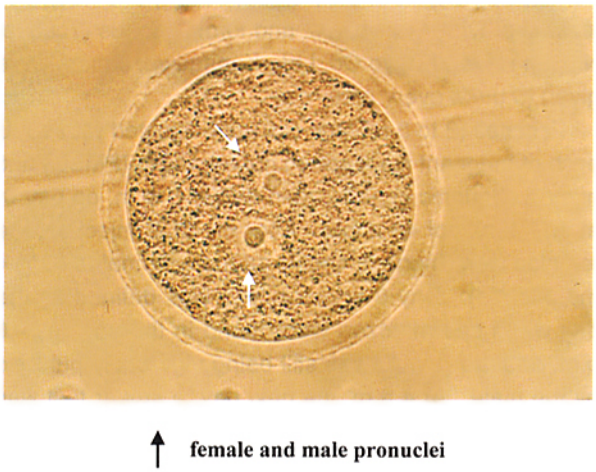

Fig. 3. Fertilized egg obtained at 22 hours after hCG injection.

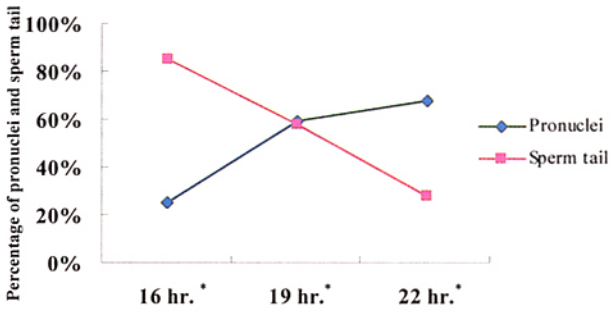

Fig. 4. Chronological changes in pronuclei and sperm tail. *: Time when oocytes were collected after hCG injection.

Table 1. Chronological changes in fertilized eggs

Time of collection	No. of animals	No. of oocytes	Proportion of pronuclei oocytes (%)	No. of pronuclei oocytes with tail (%)
16 hr.	4	53	13 (24.5%)	11 (85.0%)
19 hr.	4	56	33 (58.9%)	19 (58.0%)
22 hr	5	53	36 (67.9%)	10 (28.0%)

fertilized eggs (24.5, 58.9 and 67.9% at 16, 19 and 22 hr, respectively) (Table 1 and Fig. 4). Rates for a fertilizing sperm tail out of the total number of fertilized eggs decreased with collection time of the fertilized eggs (85.0, 58.0 and 28.0% at 16, 19 hr and 22 hr, respectively) (Table 1 and Fig. 4). Numbers of sperm tails in the total numbers of collected oocytes were very low.

Because the results of this study show the data on fertilized eggs obtained *in vivo*, chronological changes in pronuclei and the fertilizing sperm tail should be examined *in vitro* in future. And because the numbers of sperm tails in the total number of collected oocytes were

very low, ablation changes in the sperm tail in oocytes and cytoplasm (velocimetry of sperm tail ablation etc.) should be examined. Therefore, this paper will contribute to knowledge of the mechanism of fertilization of the Mongolian gerbil.

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