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The Effects of the Ovarian Components on the Motility and Movement of Mouse Sperms in a Capillary Tube

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卵巢 構成成分이 微細管內에서의 精子의 運動能 및 移動能에 미치는 影響에 관하여

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적 요

난소 구성성분의 일부인 여포난자와 난구세포가 배양중인 정자의 운동능과 이동능에 미치는 영향을 미세관 배양법을 개량한 배양장치를 고안하여 조사한 결과 다음과 같은 결과를 얻었다.

1. 배양중인 정자의 운동능은 배양시간이 길어짐에 따라 혹은 정자의 농도를 희석시킴에 따라 점차 감소했다. 그러나 온도의 변화(22°C~36°C)에는 거의 영향을 받지 않았다.
2. 난자는 정자의 운동능을 억제하는 경향을 보여주었다. 그러나 이 효과는 8시간 이후에는 나타나지 않았다.
3. 난구세포나 난자-난구세포의 복합체가 모세관을 통과하는 정자의 이동능을 증진시키는 것으로 보아 난구세포가 어떤 정자유인요소를 분비하는 것으로 추정할 수 있었다.

INTRODUCTION

The mechanism of sperm transport through the female reproductive tract is still obscure. It has been reported that the factors concerning the transport of mammalian sperms at the present are; (1) intrinsic motility of sperm, (2) uterotubal contraction of female reproductive tract, (3) ciliary movement of oviductal epithelium, and (4) the combination of the upper three factors (Brown, 1944; Saad *et al.*, 1974). Bishop and Tyler (1956) reported that sperm-agglutinating properties exist in the mammalian egg and Thibault and Dauzier (1960) reported that there exist sperm-immobilizing properties in the mammalian egg. It has been assumed that in hamster, the sperm can bind species-specifically

to the egg by a vitelline factor of the egg (Hartmann and Hutchison, 1974) and in mouse, certain sperm-activating factors which stimulate the sperm motility exist in cummulus cells (Pavlok and McLaren, 1972; Bavister and Morton, 1974).

The above reports can be grouped into two categories; one active and the other passive transports of sperms in the female reproductive tract. The difference of results of these two groups may be resulted from the difference of the observation. Some reports were based on the *in vivo* observation made on the transported sperms in certain parts of the female reproductive organ that was cut out at various time intervals after the coitus (Chang and Sheaffer, 1957; Thibault and Winterberger-Torres, 1967), while some were deduced from the observation made on the sperm motility *in vitro* (Pauffler and Foote, 1968; Morita and Chang, 1970; Mukherjee and Lippes, 1972). Still others come from the observation of the sperm transport in the cultured female reproductive organ (Moghissi, 1968; Blandau and Gaddum-Rosse, 1974).

The present studies were conducted in order to improve the research method and to investigate the role of ovarian components on the sperm movement *in vitro*.

MATERIALS AND METHODS

Sperms were obtained from the cauda epididymis of 2~3 month old A-strain mice bred randomly in our laboratory. The animals were sacrificed by cervical dislocation and epididymis was dissected out and placed in modified Krebs-Ringer bicarbonate solution (m-KRBS; Biggers *et al.*, 1971) to trim fats and blood clots out from the epididymal surface. The cleaned epididymis was transferred to a watch glass containing 0.3 m*l* of m-KRBS supplemented with 3.2% bovine serum albumin (BSA, Sigma Chemical Co.), covered with paraffin oil (Hopkins and Williams, light) and sperms were squeezed out. The concentration of the sperm in the suspension was adjusted to $8\sim11\times10^6$ sperms/m*l* (average, 9.2×10^6/m*l*).

The ova and cummulus cells were obtained as follows: Three to 4 week old A-strain mice were injected intraperitoneally with 5 i.u. of pregnant mare's serum gonadotropin (PMSG) (Sigma), and then after 48 hours with 5 i.u. of human chorionic gonadotropin (HCG) (Sigma). Ampulla portion of the oviduct was dissected in m-KRBS to obtain the ova and cummulus cells. The superovulated ova-cummulus cells complexes (OCC) were treated with hyaluronidase (12.5 u.s.p./m*l*, Sigma) in order to isolate the ova from cummulus cells. The ova-cummulus cells complexes, ova and cummulus cells were collected with a capillary pipette (Thomas 77 60-B26) and then used as materials for culture.

For the purpose of the present studies, the ovarian components and sperms were

cultured in a microtube as shown in Fig. 1. We designed the culture system by modifying the microtube culture system (Cho, 1974). The whole length of the present culture system was 90 mm and the middle part of it (capillary part) was 20±1 mm in length and 360±25 μ in diameter. The capillary part was made by stretching out the microtube by flame. When medium is inserted into the middle part of the tube, the medium is divided into two parts (A and B, see Fig. 1) and the capillary vessel become an internal channel between media of both sides.

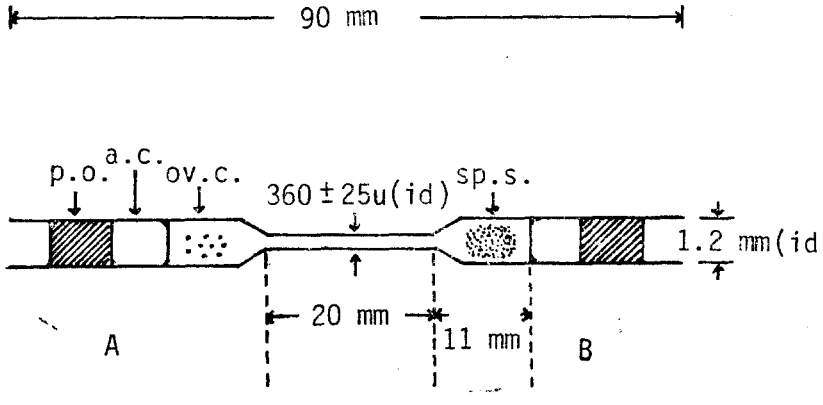

Fig. 1 Basic scheme of the culture system for mouse epididymal sperms.
a.c.: air compartment p.o.: paraffin oil
id: internal diameter sp.s.: sperm suspension
ov.c.: ovarian components

The basic culture medium used in this study was the m-KRBS supplemented with 3.2% BSA. The culture system was set up by inserting 10 μl of the medium to the middle part of the tube and plugged at one end with paraffin oil. Before introducing ovarian components and sperms, the tube with medium was incubated with 5% CO_2 in fully moistened air at 37°C for up to 10 hours to obtain an equilibrium. In experimental groups, 30~50 OCC, 30~50 ova, or cummulus cells obtained from 30~50 OCC were introduced into the medium of one side (part A) and 5 μl of sperm suspension was inserted into the medium of the other side (part B). In the control group, ovarian components were replaced by 2~3 μl of Dulbecco's phosphate buffered saline (PBS). During culture period, both ends of the tube were sealed with 5 μl of paraffin oil and air compartments between medium and oil were filled with 5% CO_2 in moistened air. The culture system was placed in an incubator at 37°C supplied with 5% CO_2 in fully moistened air. At the end of culture period, the tube was cut out at mid-point and the total number of sperms, number of motile sperms, and number of sperms moved

into the part of the ovarian components (part A) through the capillary channel were counted with a hemocytometer (Fisher) under a phase-contrast microscope (Wild M20). All glasswares and equipments for the culture were sterilized in a hot air sterilizer and the media were filtered with Millipore filter (0.45 μ) before use.

RESULTS

In order to examine the effects of culture period and temperature on the sperm motility, the sperms were cultured in the incubator at 37°C or at room temperature (22°C). The results are shown in Table 1. Motility was rated in terms of percentage of sperm moving. The initial motility of sperms obtained from cauda epididymis was in the range of 60~70% (average, 64%). When sperms were cultured for 24 hours, the motility was reduced to 8.8% in the room temperature (22°C) and 6.0% in the incubator (37°C). The percentage of sperms moving decreased gradually as the culture period was prolonged. The pH of the media in the culture system showed 7.0~7.1 in both temperature through the culure period. The above results showed that the culture period is one of the important factors which influence the motility of sperms *in vitro*.

Table 1. The effects of culture period and temperature on the motility of the mouse epididymal sperms in a microtube (unit:$\times 10^6$/m*l*).

Culture period (hours)	22°C			37°C		
	No. of motile sperms	No. of total sperms cultured	%	No. of motile sperms	No. of total sperms cultured	%
0	5.86	9.16	64.00	5.86	9.16	64.00
4	3.27	7.94	41.20	3.15	9.08	34.67
8	2.13	8.30	25.70	1.99	7.18	27.69
16	1.66	8.50	19.60	1.28	7.00	18.24
24	1.51	17.25	8.75	0.83	13.71	6.02

To observe the sperm dilution effect, sperm concentrations were adjusted with basic culture medium 6×10^6/m*l* (group A), 2×10^6/m*l* (group B) and 0.4×10^6/m*l* (group C). The results are shown in Table 2. As shown in the table, the number of motile sperms decreased significantly as the concentration of sperms was lowered as well as the culture period was prolonged. Even in the most concentrated group (group A), the ratio of motile sperm decreased markedly after 8 hours of culture. But agglutinated sperms maintaining the motility were found only in this group.

To examine the effects of ovarian components on the sperm motility, ovarian components were introduced into the medium of one side and 5 μl of the sperm

Table 2. The effects of culture period and sperm concentration on the motility of the mouse epididymal sperms in a microtube (unit:$\times 10^4$/m*l*).

Culture hours		Group A	Group B	Group C
2		126.8/664.8*	4.0/230.0	0.4/80.5
	%	19.07	1.74	0.50
4		92.8/629.6	6.4/294.8	0.1/39.6
	%	14.74	2.17	0.25
8		46.4/535.0	1.7/158.2	0.05/27.8
	%	8.69	1.08	0.18
16		2.5/1031.2	0.1/114.4	0.0/30.2
	%	0.24	0.09	0
24		2.0/644.0	0.0/66.5	0.0/46.0
	%	0.31	0	0

Group A: 0.1 m*l* sperm suspension+0.2 m*l* basic medium.
Group B: 0.1 m*l* of group A+0.1 m*l* basic medium.
Group C: 0.1 m*l* of group B+0.4 m*l* basic medium.
* No. of motile sperms after culture/No. of sperms cultured.

suspension containing 64% motile sperms was inserted into the other side of the capillary part. The results are shown in Table 3. In the group introduced with OCC, the motility of sperms (34.67%) was similar to that of the control group (34.33%) at 4 hours of culture, but at 8 hours the motility was reduced significantly (8.45%). On the other hand, in the group introduced with only the ova, the motility was reduced to 15.7% at 4 hours of culture but at 8 hours of culture the motility was increased to 34.6% which was higher than that of the control group (27.69%). In the group introduced with cummulus cells, the motility has increased from 4 hours to 8 hours of culture. In brief, OCC reduced the sperm motility markedly only after 8 hours of culture, but the ova which

Table 3. The effects of ovarian components on the motility of the mouse epididymal sperms in a microtube (unit:$\times 10^4$/m*l*).

Incubation hours		Control (PBS)	Ova-cummulus cell complex	Ova	Cummulus cell mass
4		314.7/907.9*	353.5/1029.6	112.6/742.8	194.5/714.8
	%	34.67	34.33	15.67	27.21
8		198.7/717.6	95.3/1127.5	242.0/699.5	488.1/1398.7
	%	27.69	8.45	34.60	34.90

* No. of motile sperms/No. of sperms inserted.
PBS: Dulbecco's phosphate buffered saline.
Ova-cummulus cell complex: superovulated ova with intact cummulus cells.
Ova: ova isolated from cummulus cells by treatment of hyaluronidase.

once suppressed the motility at 4 hours (15.7%) rather stimulated the motility at 8 hours (34.60%).

To observe the effects of ovarian components on the movement of sperms, the ovarian components and sperm suspensions were introduced into the capillary tube as described above and cultured for 4 or 8 hours *in vitro*. The results are shown in Table 4. In the control group, the movement ratio of the total inserted motile sperms into the opposite part of the culture tube was 0.35% at 4 hours of culture and 2.76% at 8 hours of culture. In the group introduced with OCC, the ratio was 0.66% (about 2 folds to that of the control group) at 4 hours of culture but at 8 hours the ratio was 0.4% (about 1/7 fold to that of the control group). In the ova group, the movement ratio was 0.06% at 4 hours of culture but at 8 hours it was increased to 1.5%. In the group introduced with cummulus cells the ratio was 1.38% (about 4 fold to control group) at 4 hours but at 8 hours the ratio was reduced to 0.38%.

Table 4. The effects of ovarian components on the movement of the mouse epididymal sperms through a capillary tube (unit:$\times 10^4$/m*l*).

Incubation hours		Control (PBS)	Ova-cummulus cell complex	Ova	Cummulus cell mass
4		2.50/707.20*	4.63/699.00	0.28/495.60	6.90/500.10
	%	0.35	0.66	0.06	1.38
8		14.62/530.40	3.13/780.75	7.25/484.90	3.75/960.75
	%	2.76	0.40	1.50	0.38

* No. of sperms moved toward the opposite side/No. of total sperms inserted.

DISCUSSION

There have been many investigations on the mechanism of mammalian sperm motility and transport but their results are still conflicting. These different results might be due to the different methods they used. Some investigators have observed the transported sperms at time intervals in certain parts of cultured female reproductive tract that had been cut out immediately after coitus (Moghissi, 1958; Blandau and Gaddum-Rosse, 1974). With this method, it is impossible to find out the proper mechanism of motility and transport of sperm because the number of sperms cannot be counted exactly and there could be various other factors which influence the sperm transport *in vivo*. There were attempts to observe the sperm activity cultured *in vitro* by other investigators (Mukherjee and Lippes, 1972; Bavister, 1974). The sperm suspension was cultured in a drop of medium under paraffin oil. But in this method, oil soluble substance in sperm, ova or cummulus cells may be dissolved out into the oil. So, it is

probable that some important factors which are essential for sperm activity could not be detected. The present culture system has several advantages. Culture with oil-soluble substances is now feasible because the medium is isolated from oil. Changes in pH, humidity and gas tension are minimized with this system. The handling and observation of the culture system is very simple and easy. Therefore, we are sure that the present culture system is adequate to study the reaction of sperms to the ova and to the cummulus cells.

Bavister (1974) reported an increase of motility of cauda epididymal sperms at 25~30°C rather than 37°C in the hamster. In the present experiment the motility of mouse sperm showed no change between 22°C and 37°C. Therefore, it is assumed that temperature is not a significant factor for motility of mouse sperm. Bavister (1974) also reported that there are sperm motility stimulating substances in the sperm extracts. If it is correct, the dilution of sperm suspension might cause the dilution of these suspension and would cause a decrease of sperm motility. The decrease of sperm motility by sperm dilution was showed in the present experiment and the same phenomena were also observed in the hamster (Nevo and Mohan, 1969; Morton and Chang, 1973).

Merton(1939) reported that the sperm motility maintained for 13 hours in the female reproductive tract in the mouse. However, Morton and Chang (1973) reported that the sperm motility was decreased as the culture period was prolonged in the hamster *in vitro*. In the present study, it was found that mouse sperms are very sensitive to the culture period. After 24 hours of culture, it was found that there were few sperms which maintained motility. This loss of activity might be due to decomposition of the sperm motility stimulating substances or inactivation and/or dilution of these substances during the culture period.

In some vertebrates and invertebrates, sperms approach the egg by an active agent, fertilizin, which are emanated from the egg. But in mammals sperms are transported by other mechanisms suggested by Chang(1951), Austin(1951), Thibault and Winterberger-Torres (1967), Blandau and Gaddum-Rosse (1974). The existence in mammals of active egg substances possessing sperm-agglutinating (Bishop and Tyler, 1956) or sperm-immobilizing properties (Thibault and Dauzier, 1960) has been proposed. There may be a fertilizin like substance emanating from the egg which initiates the events of the acrosome reaction in rabbit (Austin *et al.*, 1973; Overstreet and Bedford, 1975). Bavister(1975) reported that human serum sperm motility-stimulating component which had a molecular weight between 100~200 stimulated the sperm motility of hamster *in -vitro*.

In the present experiment (Table 3), in the group introduced with only the ova, the sperm motility was reduced to 15.7% at 4 hours of culture but at 8 hours the motility was increased to 34.6% again. The above results seem to

imply that sperm-immobilizing factors were decomposed or inactivated during the culture period. In the group introduced with cummulus cells, the motility showed no significant decrease within 4 hours. This result seems to indicate that cummulus cells have no capacity to produce the sperm immobilizing factors. To some extent, sperm motility rather increased after 8 hours in this group. This result seems to confirm that cummulus cells produce a substance capable of inducing capacitation suggested by Pavlok and McLaren (1972). In the group introduced with OCC, the sperm motility was decreased significantly at 8 hours of culture. It is assumed that when the culture period was prolonged, the ova and cummulus cells which were bound firmly in OCC at first were dissociated and then the sperm immobilizing factor of the ova was secreted. As shown in Table 3, in the group introduced with cummulus cells, the sperm motility was low (27.21%) than that of the control group (34.67%) at 4 hours of culture; nonetheless, more sperms moved to the counter part (1.38%) than those of the control group (0.35%) at 4 hours culture (Table 4). From this result, we can assume that cummulus cells may produce the sperm attracting factors. But these factors seem to be very unstable because there was no such an increase of movement after 8 hours in this group (Table 4). In the group introduced with the ova only, the movement of sperms to the counter part was suppressed within 4 hours of culture. This result might be due to the restrained sperm motility by the sperm immobilizing factor of ova. But as the culture period was prolonged, the number of sperms transferred has significantly increased. This result might be due to the attracting factor which was secreted by the ova, or due to the significant increase of sperm motility after 4 hours of culture (Table 3). Hartman and Hutchison (1974) reported that there may be vitelline factors in an egg which make sperms bind species-specifically to the egg. Overstreet and Bedford (1975) reported that the egg has a factor inducing sperm acrosomal reaction. Considering these reports, it is assumable that there might be sperm attraction factors in the egg. In the group introduced with OCC in the present experiments, the movement of sperms showed the mid-range between the ova and cummulus cells group. The results may be due to the antagonistic reaction between inhibitory substances secreted by the ova and the stimulatory factor produced by cummulus cells.

On the basis of these results, it would be assumed that motility and movement of the sperms are deeply associated with certain kinds of substances secreted by the ova and cummulus cells. In order to understand the real mechanism of motility and movement of the sperms in the female reproductive tract, further investigations should be conducted on the nature of those inhibitory or stimulatory factors of the egg and of the mode of their action on

sperm movement *in vitro*.

SUMMARY

The present experiments were undertaken to find out the effects of ova and cummulus cells on the motiliy and movement of mouse sperms in a capillary tube modified from the microtube culture system (Cho, 1974).

The results obtained were as follows:

1. The motility of the mouse sperms cultured *in vitro* was decreased gradually as the culture period was prolonged or the concentration of sperms was diluted with the culture medium.
2. The ova whose cummulus cells were removed have some effects of reducing the sperm motility, but this effect seems to disappear at 8 hours of culture, whereas ova-cummulus cells complex showed a motility suppression effect only after 8 hours of culture.
3. Cummulus cells or ova-cummulus complex stimulated the movement of the sperm through a capillary tube by some degree. It is, therefore, assumed that cummulus cells secrete some factors which induce the movement of the sperm toward them.

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