《Research Note》

Effects of Proximity of Spermatozoa to Cultured Cells on the Survival of Fowl Spermatozoa *in vitro*

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Sperm-host glands are present in the infundibulum and uterovaginal junction of the fowl oviduct, in which many spermatozoa survive for a period of days or weeks¹⁻⁵⁾. However, little informations have been available about the mechanisms of long survival of spermatozoa in these glands.

ASHIZAWA, NISHIYAMA and NAGAE⁶⁾ reported that spermatozoa incubated at 41°C with cultured cells from the epithelia of the oviduct were almost immotile remaining on the surface of the cells and their motility, assessed at room temperature, was maintained for 5 days. Fertilizing ability was also retained for at least 4 days. The favourable effects of the oviducal cells on the prolonged maintenance of motility and fertilizing ability of spermatozoa were similarly observed in the cells from the tissues with sperm-host glands (infundibulum and uterovaginal junction) and those from the tissue lacking host glands (shell gland)⁶⁾. Similar effects were also observed in the cells from the pullet kidney, chick kidney and skeletal muscle of the 9-day chick embryo⁷⁾. Thus they suggest that the cells of the sperm-host glands may not exert a specific influence, and that the favourable environment provided by living cells is all that is necessary for the survival of spermatozoa.

From these evidences, it is assumed that the proximity of spermatozoa to the cultured cells during incubation is important for prolonged maintenance of motility and fertilizing ability of spermatozoa. In the present study, this assumption was tested by changing the distance between the incubated cells and spermatozoa.

Materials and Methods

White Leghorn roosters and pullets were used throughout the study.

The cells to be cultured were prepared from the epithelia from the shell gland of the oviduct. The methods for dispersing and culturing the cells were as described previously⁶⁾. A quantity (2ml) of the cell suspension (2.0×10^8 cells/ml) was seeded into small Petri dishes (39mm in diameter, and two pieces of glass rods, 1 or 3 mm in

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thickness, were attached on the bottom of the dish) and cultured at 41°C in 5% CO₂+95% air by the stationary monolayer method.

On the 3rd day of culture, the culture medium (5 ml, MEM used by ASHIZAWA *et al.*⁶⁾) was exchanged and a polyvinyl chloride ring (14 mm in outside diameter, 10 mm in inside diameter, 5 mm in thickness) attached a membrane-filter to the bottom of the ring was laid on the glass rods (Fig. 1). We chose such a filter (0.45 µm pore size, HA type, Millipore Corp.) that fowl spermatozoa could not pass through it (the diameter of the head of fowl spermatozoon is about 0.5 µm⁸⁾), but a substance or substances derived from the cultured cells, if provided, were able to pass through the filter. Soon after setting of the ring, the medium flowed into the ring through the filter. Then an appropriate volume of germ-free semen collected from the ductus deferens of a rooster to give a sperm concentration of 2.0×10^8 /ml was added to the medium in the ring. The distances between cells and filter were 1 mm and 3 mm (1 mm, 3 mm-lot). Semen added to the outside of the ring was used as the proximitylot (sperm concentration, 2.0×10^3 /ml) and semen added in the ring set in the cell-free medium were used as the control-lot (Fig. 1). After addition of semen, the spermatozoa and cells or spermatozoa alone were incubated as described above for the culture of cells alone. The spermatozoa incubated at 41°C were almost immotile^{6,9)} remaining on the surface of the cells or the filter, so that the distance between incubated spermatozoa and cells were kept at 0, 1 or 3 mm in each lot.

During the incubation period at 41°C, sperm motility was examined at room temperature (20-25°C) everyday by the hanging drop method and the motility was scored on the 0 to 5 system¹⁰⁾.

The number of abnormal spermatozoa was determined on the 2nd day of incubation (see ASHIZAWA *et al.*⁶⁾).

Fig. 1. Procedure of experiment. C, cultured cells; F, membrane-filter; G, glass rod; R, polyvinyl chloride ring; S, spermatozoa.

Results and Discussion

The changes of motility assessed at 20-25°C during incubation at 41°C are shown

Table 1. Motility score* (at room temperature, 20-25°C) and duration of survival of fowl spermatozoa incubated with cultured cells at 41°C in 5% CO₂+95% air.

Lot	Days after addition of semen							Duration of survival (days)
	0	1	2	3	4	5	6	
Proximity	5.0	4.8	4.2	2.8	1.6	0.4	0.0	5.4 ^{a**}
1 mm	5.0	4.2	1.6	0.4	0.0	0.0	0.0	3.4 ^b
3 mm	5.0	4.0	0.6	0.0	0.0	0.0	0.0	2.6 ^{b c}
Control (cell-free medium)	5.0	1.2	0.0	0.0	0.0	0.0	0.0	1.6 ^c

* Sperm motility assessed on the 0 to 5 system.

** Values with different superscripts differ significantly ($P<0.01$) from each other. Each figure represents an average of 5 trials.

Table 2. The mean±S.E.M. (of 5 trials) percentage of abnormal fowl spermatozoa after incubation for 2 days.

Lot	Percentage of abnormal spermatozoa
Proximity	17.8± 3.1 ^{a*}
1 mm	53.4±12.7 ^b
3 mm	77.6± 4.3 ^{b c}
Control (cell-free medium)	85.1± 2.9 ^c

* Values with different superscripts differ significantly ($P<0.01$) from each other.

in Table 1. Spermatozoa in the cell-free medium (control-lot) lost motility rapidly, while those incubated in close proximity to the cultured cells (proximity-lot) maintained motility for 5 days. These results were similar to those of ASHIZAWA *et al.*⁶⁾ and ASHIZAWA and NISHIYAMA^{7,11)}. Comparing the three lots of spermatozoa incubated with the cultured cells, the survival period was longest in the proximity-lot, then 1 mm-lot, and 3 mm-lot was shortest which however slightly longer than that of the control-lot. The number of abnormal spermatozoa on the 2nd day of incubation increased with the distance between the cells and spermatozoa, and the control-lot was highest in the number (Table 2).

These results show that living cells provide a favourable environment on the survival of spermatozoa and that the effectiveness is inversely proportional to the distance between the cells and spermatozoa.

This gradual decrease of the effectiveness appears to suggest the presence of diffusible substance as an effective factor. Therefore, there is a possibility that substance or substances which are provided by living cells and reach the spermatozoa by diffusion may be effective for prolonged survival of spermatozoa.

Spermatozoa are stored in close proximity to the epithelial cells of the sperm-host glands^{3,12-14)}, and LAKE¹⁵⁾ assumes that substrates for sustenance, if provided, most probably reach the spermatozoa by diffusion from secretions of the cells of sperm-host glands. The results of this experiment appear to present a presumptive evidence to support this assumption, though direct evidence is not yet obtained.

Summary

Fowl spermatozoa and oviducal cell were incubated together at 41°C. The life-span of spermatozoa decreased proportionately with the increase of distance between the cells and spermatozoa, and abnormal spermatozoa increased with the distance.

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