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Effects of various cultured cells on the survival and fertilizing ability of fowl spermatozoa

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Sperm-host glands are present in the infundibulum and uterovaginal junction of the fowl oviduct, where spermatozoa survive for long periods (Van Drimmelen, 1946; Bobr, Lorenz & Ogasawara, 1962, 1964; Fujii & Tamura, 1963; Takeda, 1964, 1965). However, there have been few investigations of the mechanisms of survival.

Ashizawa, Nishiyama & Nagae (1976) reported that spermatozoa incubated at 41°C with cultured cells from the epithelia of the oviduct were almost immotile whilst resting on the surface of the cells and their potential for motility, assessed afterwards at room temperature, was maintained for 5 to 6 days. Fertilizing ability was also retained for at least 4 days. Ashizawa *et al.* suggested that fowl spermatozoa are quiescent while stored in the sperm-host glands close to the epithelial cells, and that these conditions favoured the prolonged survival of spermatozoa within the oviduct. They further suggested that the cells of the sperm-host glands may not exert a specific influence, and that the environment provided by living cells is all that is necessary for the survival of spermatozoa *in vivo*.

In the present study to investigate this possibility, cells prepared from several body tissues were cultured and their effects on the survival and fertilizing ability of spermatozoa were compared.

Methods

White Leghorns (Shaver strain) obtained from Ohta Poultry Breeding Farm Inc., Saga, Japan, were used.

The cells to be cultured were prepared from the pullet kidney, pullet oviduct (uterovaginal junction), male chick kidney, and skeletal muscle (femoral muscle) of the 9-day chick embryo. The methods for dispersing and culturing the cells were as described previously (Ashizawa *et al.*, 1976). A quantity (2 ml) of the cell suspension (2×10^6 cells/ml) was seeded into small Petri dishes and cultured at 41°C in 5% CO_2 + 95% air by the stationary monolayer method.

On the 3rd day of culture, the culture medium (MEM used by Ashizawa *et al.*, 1976) was exchanged and about 0·05 ml germ-free semen collected from the ductus deferens of a rooster was added to give a sperm concentration of $2{\cdot}0 \times 10^8$/ml. The spermatozoa and cells were incubated as described above for the culture of cells alone. Spermatozoa incubated in cell-free culture medium were used as controls. After addition of semen, sperm motility was assessed at room temperature (20–23°C) at intervals during the incubation period at 41°C. The number of abnormal spermatozoa was determined on the 2nd and 4th day of incubation (see Ashizawa *et al.*, 1976).

Fertilizing ability was examined using the spermatozoa incubated with the cultured cells or in the cell-free medium for 4 days. Approximately 10^8 spermatozoa in about 0·5 ml culture medium were inseminated into the magnum or isthmus (see Ashizawa *et al.*, 1976). From 2 days after insemination, any eggs laid were collected, incubated and examined on the 4th day of incubation for fertility. The duration of fertility was also determined.

Results and discussion

The changes of sperm motility in the various incubations are shown in Table 1. Spermatozoa in the cell-free medium (control) lost motility rapidly, while those incubated with cells of any source maintained motility for 7 to 8 days. The numbers of abnormal spermatozoa on the 2nd and 4th days of incubation were significantly greater in the control incubations than in those with cultured cells (Table 2).

Table 1. Motility score* (at 20–23°C) of fowl spermatozoa incubated with cultured cells at 41°C in 5% CO_2 + 95% air

Cells in incubation medium	Days after addition of semen								
	0	1	2	3	4	5	6	7	8
None (control)	5·0	2·8	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Uterovaginal junction	5·0	4·8	4·1	3·8	2·5	1·0	0·5	0·1	0·0
Pullet kidney	5·0	4·6	3·9	3·4	2·9	1·5	0·6	0·1	0·0
Male chick kidney	5·0	4·8	4·0	3·0	2·5	1·1	0·4	0·1	0·0
Embryonic skeletal muscle	5·0	4·6	3·5	2·9	2·1	0·8	0·2	0·0	0·0

* Adjudged on the 0 to 5 system; 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 in various media for 2 or 4 days

Cells in incubation medium	2 days	4 days
None (control)	$85·9 \pm 2·7^a$	$86·7 \pm 1·6^b$
Uterovaginal junction	$18·2 \pm 3·4^c$	$29·7 \pm 3·9^d$
Pullet kidney	$14·5 \pm 2·9^c$	$25·0 \pm 5·9^d$
Male chick kidney	$17·1 \pm 5·5^c$	$30·0 \pm 2·8^d$
Embryonic skeletal muscle	$17·4 \pm 2·4^c$	$34·3 \pm 7·7^d$

Within columns, values with different superscripts differ significantly ($P < 0·01$) from each other.

The spermatozoa incubated for 4 days at 41°C with the cells from the various tissues described above were highly fertile, as judged by the number of fertile eggs laid during the 1st and 2nd week after insemination (Table 3).

Table 3. Fertilizing ability of fowl spermatozoa incubated with cultured cells for 4 days at 41°C in 5% CO_2 + 95% air

Cells in incubation medium	No. of hens inseminated	Fertilized eggs (%)		Mean duration of fertility (days)
		1st week	2nd week	
None (control)	5	0·0 (0/25)*	0·0 (0/27)	0·0
Uterovaginal junction	5	87·5 (28/32)	51·5 (17/33)	11·8
Pullet kidney	5	90·3 (28/31)	54·8 (17/31)	12·8
Male chick kidney	4	84·0 (21/25)	46·2 (12/26)	10·3
Embryonic skeletal muscle	5	90·0 (27/30)	59·4 (19/32)	13·6

* No. of fertilized eggs/no. of eggs laid.

These results show that the maintenance of motility and fertilizing ability of spermatozoa are prolonged by cultured cells of any of the origins tested, and support the suggestion of Ashizawa *et al.* (1976) that an environment provided by living cells is all that is necessary for prolonged sperm survival.

However, more work is required to determine whether proximity only is important or whether a substance or substances derived from the juxtaposed cells is involved.

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