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Indirect Assessment of Sperm Capacitation Using Zona-free Hamster Eggs in the Goat

II. Penetration into Zona-free Hamster Eggs by Goat Spermatozoa Preincubated in a Chemically Defined Medium

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Summary

Ejaculated and epidiymal goat spermatozoa were preserved for 0, 6, 12 and 18 h, and 0 and 18 h in a semi-aerobic condition at 20-25°C, and preincubated for 5-6 h in a CO_2 incubator in m-KRB solution. Then they were preincubated at different concentrations (3-5, 25-48 and 105-190 x 10^7/ml), and ability of penetration into zona-free hamster eggs *in vitro* was examined.

When ejaculated spermatozoa were preincubated in m-KRB solution after preservation for 12 and 18 h, 12 and 29% of zona-free eggs were penetrated, and only 4% of eggs were penetrated by epididymal spermatozoa which were preincubated after preservation for 18 h.

When spermatozoa were preincubated at a low concentration, the penetration rates were very low. But when the sperm concentration during preincubation was 25-48 and 105-190 x 10^7/ml, the penetration rates increased to about 30%.

Introduction

In domestic animals, studies on sperm penetration into zona-free hamster eggs have been conducted to examine capacitation of bull (Lorton and First, 1970; Bousquet and Brackett, 1982; Brackett et al., 1982; Imai, 1983), stallion (Brackett et al., 1982) and boar (Pavlok, 1981; Pavlok et al., 1982; Smith et al., 1983) spermatozoa preincubated in a chemically defined medium. Capacitation of goat spermatozoa using a hamster test has been performed using psermatozoa preincubated in the uterus of heterologous species (Kim et al., 1980; Kim, 1981; Kato et al; 1984; Song et al., 1985), but has not been performed on spermatozoa preincubated in a chemically defined medium. Recently Pao and Hanada (1983) reported that zona-free hamster eggs were penetrated by spermatozoa treated with Ionophore A-23187.

The present study was conducted to investigate the possibility of capacitation of goat spermatozoa in a chemically defined medium, and to examine the possibility of using zona-free hamster eggs for indirect assessment of the fertilizing capacity of goat spermatozoa.

Materials and Methods

The medium used for the manipulation of gametes was a modified Krebs-Ringer bicarbonate (m-KRB) solution (Toyoda and Chang, 1974). The composition of m-KRB solution is shown in previous paper (Song and Iritani, 1985).

Ejaculated semen was collected from two goats of the Saanen breed using an artificial vagina. For collec-

tion of epidiymal spermatozoa, male Saanen goats were anesthetized with an intravenous injection of pentobarbital sodium and one side of the testis-epidymis-vas deferens unit was removed and the other side was used after killing the goat within two weeks of the first surgery. The epididymal spermatozoa were collected by flushing the epididymal duct through the vas deferens by an air jet.

In the first experiment, the ejaculated semen was kept under a semi-aerobic condition in a 10 ml test tube with stopper at 20-25°C for 6, 12 and 18 h after semen collection. The epididymal spermatozoa were treated under the same conditions for 18 h. Both ejaculated and epididymal spermatozoa were washed once with m-KRB solution, resuspended to give a concentration of 15-20 x 10^8/ml and then preincubated in a 0.4 ml medium (3-5 x 10^8/ml) in a CO_2 incubator (5% CO_2 in air at 37°C) for 5-6 h. Freshly collected spermatozoa was also treated by the same procedures as mentioned in previous paper (Song and Iritani, 1985).

In the second experiment, a portion of the ejaculated semen kept under a semi-aerobic condition for 18 h was introduced into a 0.4 ml medium after washing once or twice, or without washing. Sperm concentration during preincubation was about 3-5 x 10^7, 25-48 x 10^7 and 105-190 x 10^7/ml, respectively. After preincubation, inseminations were made at a concentration of 0.02-2.1 x 10^6 spermatozoa/ml in the fertilization medium.

The methods for preparing the zona-free hamster eggs, staining, and the criteria of sperm penetration and egg activation were the same as described in previous paper (Song and Iritani, 1985).

Results and Discussion

When ejaculated and epididymal spermatozoa were preincubated in m-KRB solution after preservation for 12-18 h under a semi-aerobic condition. The percentage of motile spermatozoa (50-60%) was lower than fresh semen (more than 80%). However, the percentage of motile spermatozoa after additional preincubation for 5-6 h was 50-60% in both cases. Such changes in sperm motility have been observed in boar (Nagai et al., 1984) and bull (Iritani et al., 1984) spermatozoa preincubated in m-KRB solution after preservation for 18-20 h and 14-18 h under a semi-aerobic condition.

As shown in Table 1, zona-free hamster eggs were not penetrated after insemination either with ejaculated spermatozoa which were preserved for 6 h before preincuabation or with epididymal spermatozoa which were not preserved before preincubation. However, 12 and 29% of eggs were penetrated by ejaculated spermatozoa preincubated in m-KRB solution after preservation for 12 and 18 h under a semi-aerobic condition, and only 4% of the eggs were penetrated by epididymal sperma-

Table 1. *In vitro* penetration of zona-free hamster eggs by goat spermatozoa preserved for different periods and preincubated for 5 – 6h.

Source of sperm	Duration of preservation (h)	No. of eggs examined	No. of eggs penetrated* (%)	No. of eggs activated (%)
Ejaculated	0	75	0+0/75 (0)	0/0 (0)
	6	55	0+0/55 (0)	0/0 (0)
	12	74	4+5/74 (12)	4/9 (44)
	18	42	7+5/42 (29)	6/12 (50)
Epididymal	0	52	0+0/52 (0)	0/0 (0)
	18	47	1+1/47 (4)	1/2 (50)

* The first figure denotes the number of eggs with enlarged sperm head and the second denotes the number of eggs with male pronucleus.

tozoa which were preserved for 18 h before preincubation. Half of the penetrated eggs were activated thereafter. These results suggested that both ejaculated and epididymal spermatozoa acquired the ability of penetration during the preincubation when they were preserved for 12-18 h in a semi-aerobic condition. The present results suggested at an early stage of capacitation, such as loosening of coating factors and plasma membrane of the spermatozoa, might be initiated during the storage period at 20-25°C, and this indicated the possibility for *in vitro* capacitation of goat spermatozoa in m-KRB solution. Iritani et al. (1984) reported that 58% cattle follicular oocytes matured in culture were fertilized after insemination with bull spermatozoa preserved for 14-18 h at 20°C in a test tube before preincubation.

Table 2. Effects of washing and concentrations during preincubation of ejaculated goat spermatozoa preincubated in m-KRB solution on penetration *in vitro* of zona-free hamster eggs

Times of washing	Sperm concentration at preincubation ($\times 10^7$/ml)	No. of eggs examined	No. of eggs penetrated* (%)	No. of eggs activated (%)
	3 − 5	71	0+ 0/71 (0)	0/ 0 (0)
0	32 − 47	68	9+ 9/68 (27)	10/18 (56)
	110 − 140	73	7+13/73 (27)	13/20 (65)
	3 − 5	92	0+ 1/92 (1)	1/ 1 (100)
1	25 − 48	83	15+11/83 (31)	11/26 (42)
	105 − 190	84	18+ 6/84 (29)	6/24 (25)
	3 − 5	60	0+ 0/60 (0)	0/ 0 (0)
2	27 − 48	75	0+ 0/75 (0)	0/ 0 (0)
	105 − 190	108	4+ 2/108 (6)	2/ 6 (33)

* The first figure denotes the number of eggs with enlarged sperm head and the second denotes the number of eggs with male pronucleus.

Table 2 shows the penetration rates of zona-free hamster eggs by goat spermatozoa preincubated in m-KRB solution under different conditions. When ejaculated spermatozoa were preincubated at a low concentration (3-5 x 10^7 spermatozoa/ml) after washing once or twice, or without washing, the penetration rates were extremely low (0, 1 and 0%). But when the sperm concentration at preincubation was increased to 25-48 x 10^7/ml, the rates were 31, 0 and 27%, and 29, 6 and 27% at the concentration of 105-190 x 10^7/ml, respectively. The highest penetration rate (31%) was obtained when spermatozoa were preincubated at 25-48 x 10^7/ml after washing once. Of the 95 penetrated eggs, 43 (45%) were activated to early anaphase of the second meiotic division or they extruded the second polar body (Plate, a-d). Ritar and Salamon (1982) reported that removal of seminal plasma by centrifugation was beneficial for the survival of goat spermatozoa. In the present experiments, twice washing of spermatozoa was not advantageous for capacitation of spermatozoa compared to once washing or not washing at all. These results were agreeable with the report by Iritani et al. (1984) in which washing once was better than twice for capacitation of ejaculated bull spermatozoa.

In the present experiments, none of zona-free hamster eggs were penetrated when they were inseminated with spermatozoa preincubated at the concentration of 3-5 x 10^7/ml in m-KRB solution in a CO_2

incubator. However, 27-31% of eggs were penetrated at the concentrations of 25-48 and 105-190 x 10^7/ml. The present results suggested that the sperm concentration during preincubation might be an important factor for capacitation. Pavlok (1981) reported that all the zona-free hamster and pig eggs were penetrated by ejaculated boar spermatozoa preincubated for 4 h at a high concentration (7-8 x 10^8/ml), and Nagai et al. (1984) also reported that about 70% of pig oocytes were fertilized with spermatozoa preincubated for 4 h at a high concentration (4-16 x 10^8/ml).

The present results suggested that some physiological change similar to sperm capacitation might be induced during preincubation at a high concentration (more than 25 x 10^7/ml) in m-KRB solution and zona-free hamster eggs could be used for the assessment of the fertilizing capacity of goat spermatozoa *in vitro.* Although the mechanism of the occurrence of this change is not clear, these results also indicate that ejaculated goat spermatozoa can be capacitated with m-KRB solution instead of Ionophore A-23187 treatment that was successfully used by Pao and Hanada (1983).

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Explanation of Plates

Zona-free hamster eggs penetrated by goat spermatozpa preincubated in m-KRB solution and photographed under a phase-contrast microscope after being stained with aceto-lacmoid.

1) An egg 5 h after insemination with spermatozoa preincubated for 6 h in m-KRB solution, showing one enlarged sperm head (large arrow) and tail (small arrow), but egg chromosomes (Ch) are still unactivated at metaphase of second meiotic division. x 600.

b) and c). An egg(b) 5 h after insemination with spermatozoa preincubated for 6 h in m-KRB solution, showing female chromosomes at early telophase of the second meiotic division (arrow) and an egg(c) showing female pronculeus (FP) and the second polar body(PB). :x 480.

d) An egg 5 h after insemination with spermatozoa preincubated for 6 h in m-KRB solution, showing one female pronucleus (FP) and one male pronucleus (MP) with their corresponding sperm tail (arrow) and the second polar body (PB). :x 480.