

## CAPACITATION OF GOLDEN HAMSTER SPERMATOZOA DURING INCUBATION IN CULTURE MEDIUM

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(Received 30th April 1973)

Golden hamster spermatozoa recovered from the cauda epididymidis can penetrate eggs *in vitro* after a delay of 2 to 3 hr, which is attributed to the need for sperm capacitation (Barros, 1968). During an investigation into the optimal conditions for fertilization of hamster eggs *in vitro* (Bavister, 1971), I found that this delay could be substantially reduced by preincubating epididymal spermatozoa in a simple culture medium (a modification of Tyrode's solution, containing bovine serum albumin, designated Tyrode-B; Bavister, 1969). The preliminary findings in this work have already been reported (Austin, Bavister & Edwards, 1973); the present communication describes the techniques used and contains results of a larger series of experiments. Since there is some controversy over the meaning of the term 'capacitation', it is used below to denote only those changes undergone by spermatozoa after leaving the male reproductive tract and before the occurrence of the acrosome reaction.

Epididymal tails, recovered from 3- to 4-month-old golden hamsters killed by cervical dislocation, were placed in a plastic tissue culture dish (Falcon Plastics) and covered with sterile paraffin oil. Epididymal fluid was released by making several incisions in the epididymal tubule in the region of the ductus deferens, and 0.5 ml Tyrode-B was added. The resulting sperm suspensions were incubated at 25 to 30° C; the motility of spermatozoa in this type of suspension rapidly deteriorated at 37° C. At intervals during this preincubation period, samples of the suspension were withdrawn in a fine Pasteur pipette and used (a) to estimate the proportion of spermatozoa showing the acrosome reaction, as judged under phase contrast microscopy (100 motile spermatozoa examined), and (b) to inseminate 20- to 30- $\mu$ l droplets of Tyrode-B under paraffin oil containing eggs in cumulus freshly recovered from superovulated female hamsters that had been given HCG 15 to 16 hr previously. The dosage and timing of gonadotrophin injections and the method of egg recovery were as described by Yanagimachi & Chang (1964). The gametes were incubated at 37° C under 5% CO<sub>2</sub> in air, in a continuous-flow gas incubator, at pH 7.6, which is optimal for fertilization of hamster eggs *in vitro* (Bavister, 1969). Eggs were removed from the culture droplets at intervals after insemination and scored under phase contrast microscopy for evidence of sperm penetration (Yanagimachi & Chang, 1964); only eggs with a sperm head in the perivitelline space or within the egg cytoplasm were counted as penetrated.

The results of these experiments are shown in Table 1. Under the conditions described, penetration of eggs by untreated epididymal spermatozoa did not occur within 2 hr. Preincubation of spermatozoa in Tyrode-B shortened the interval between insemination and penetration of eggs; this interval was progressively reduced by increasing duration of preincubation. The effect reached a maximum after preincubation for 3½ to 5 hr. Penetration was never observed within 1 hr after insemination, regardless of the duration of preincubation.

Preincubation of spermatozoa in Tyrode-B did not effectively induce the acrosome reaction, since only 1 to 2% of motile spermatozoa showed the acrosome reaction, even when preincubation was continued for 18 hr on several occasions. After incubation for 1 to 2 hr with the products of ovulation, the majority of motile spermatozoa exhibited the acrosome reaction. The motility of spermatozoa in the suspension increased very markedly after preincubation for 2 to 3 hr.

**Table 1.** Timing of penetration of hamster eggs *in vitro* by preincubated epididymal spermatozoa

Preincubation period (hr)	No. of eggs penetrated/no. of eggs inseminated following incubation with spermatozoa for					
	1 hr	1¼ to 1½ hr	1¾ to 2 hr	2¼ to 2½ hr	2¾ to 3 hr	3¼ to 3½ hr
0*			0/121 (0)	22/203 (11)	189/810 (23)	252/417 (60)
2†	0/45 (0)	0/20 (0)	7/85 (8)	42/72 (58)	20/45 (44)	12/21 (57)
3½ to 5†	0/52 (0)	11/72 (15)	58/141 (41)	78/96 (81)		
6 to 9½†	0/77 (0)	36/168 (21)	39/98 (40)	47/52 (90)		

Figures in parentheses are approximate percentages of eggs penetrated.

\* Each result in the series with non-preincubated (i.e. fresh) epididymal spermatozoa represents the combined values from at least eight experiments.

† Each result obtained with spermatozoa preincubated for varying lengths of time represents combined values from at least three experiments.

I infer from these results that hamster epididymal spermatozoa were capacitated by incubation in Tyrode-B, reaching an end-point after incubation for 3½ to 5 hr, without a significant proportion of spermatozoa undergoing the acrosome reaction. This inference is supported by indirect evidence: even after the end-point was reached, preincubated spermatozoa always required incubation for 1 to 1½ hr with the products of ovulation before penetrating the eggs. This interval is the same as that found by Yanagimachi (1966) to be necessary for capacitated hamster spermatozoa to penetrate eggs *in vivo*, and probably represents the time course of the acrosome reaction, attachment of spermatozoa to the zona pellucida and its penetration. In addition, in the present work, the majority of spermatozoa in the suspensions underwent a fairly rapid and substantial increase in motility after preincubation for several hours. Observing a similar phenomenon after incubating hamster spermatozoa with follicular

fluid, Yanagimachi (1969a) concluded that 'a sharp increase in sperm motility is a characteristic feature of the capacitated spermatozoa'. Moreover, Yanagimachi also observed that the largest proportion of vigorously motile spermatozoa appeared after incubation for 4 to 5 hr with follicular fluid, an interval that corresponds closely to the time required in the present work for preincubation *in vitro* to be fully effective (3½ to 5 hr).

Capacitation of mouse spermatozoa by preincubation in a culture medium has already been reported (Toyoda, Yokoyama & Hosi, 1971). It is not clear at the moment whether, in the mouse, the acrosome reaction occurs during or after the preincubation or 'capacitation' period, but it is not easy to discriminate between these changes in this species.

In the experiments reported here, capacitation was achieved during preincubation of spermatozoa in culture, but the acrosome reaction was only induced in the great majority of spermatozoa following the addition of the products of ovulation. Capacitation and the acrosome reaction may thus occur under quite different conditions in the hamster, and may be considered and investigated separately, just as in the rabbit (Bedford, 1970). In this way, the rôle of follicular fluid components, which have been implicated in capacitation (Yanagimachi, 1969a, b), can perhaps be clarified.

This study was supported in part by a Medical Research Council Scholarship for Training in Research Methods, and partly by a grant from the World Health Organisation.

#### REFERENCES

- AUSTIN, C. R., BAVISTER, B. D. & EDWARDS, R. G. (1973) *Components of capacitation*. In: The Regulation of Mammalian Reproduction, p. 247. Eds. S. J. Segal, R. Crozier, P. A. Corfman and P. G. Condliffe. C. C. Thomas, Springfield, Illinois.
- BARROS, C. (1968) *In vitro* capacitation of golden hamster spermatozoa. *Anat. Rec.* **160**, 310 (Abstr.).
- BAVISTER, B. D. (1969) Environmental factors important for *in vitro* fertilization in the hamster. *J. Reprod. Fert.* **18**, 544 (Abstr.).
- BAVISTER, B. D. (1971) *A study of in vitro fertilization and capacitation in the hamster*. Ph.D. thesis, Cambridge University.
- BEDFORD, J. M. (1970) Sperm capacitation and fertilization in mammals. *Biol. Reprod. Suppl.* **2**, 128.
- TOYODA, Y., YOKOYAMA, M. & HOSI, T. (1971) Studies on the fertilization of mouse eggs *in vitro*. II. Effects of *in vitro* pre-incubation of spermatozoa on time of sperm penetration of mouse eggs *in vitro*. *Jap. J. Anim. Reprod.* **16**, 152.
- YANAGIMACHI, R. (1966) Time and process of sperm penetration into hamster ova *in vivo* and *in vitro*. *J. Reprod. Fert.* **11**, 359.
- YANAGIMACHI, R. (1969a) *In vitro* capacitation of hamster spermatozoa by follicular fluid. *J. Reprod. Fert.* **18**, 275.
- YANAGIMACHI, R. (1969b) *In vitro* acrosome reaction and capacitation of golden hamster spermatozoa by bovine follicular fluid and its fractions. *J. exp. Zool.* **170**, 269.
- YANAGIMACHI, R. & CHANG, M. C. (1964) *In vitro* fertilization of golden hamster ova. *J. exp. Zool.* **200**, 281.