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Penetration of Human Eggs by Human Spermatozoa in vitro

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ABSTRACT

The timing of sperm penetration through the zona pellucida, and fertilization of human eggs had received little attention in the literature. Edwards et al. (1969) inseminated eggs matured *in vitro* with ejaculated spermatozoa which had been washed and found that spermatozoa did not penetrate the zona pellucida earlier than 7 h after insemination. A similar time interval between insemination and zona penetration by spermatozoa has been reported by others (Bavister et al., 1969; Overstreet and Hembree, 1976). This time interval has been considered to represent the time required for sperm capacitation, acrosome reaction and passage of the reacted spermatozoa through the zona pellucida (Austin, 1969; Austin et al., 1973). We report here that under our experimental conditions, sperm penetration into human eggs can occur much faster than previously reported.

MATERIALS AND METHODS

Immature oocytes were collected at laparoscopy by flushing ovarian follicles with Dulbecco's phosphate buffered saline supplemented with 5.56 mM glucose and 0.1% bovine serum albumin. The recovery rate and criteria used for selection of viable oocytes were as reported by Lopata and coworkers (1974). The selected oocytes were thoroughly rinsed with the same buffer and transferred into 0.2 ml of a culture medium under liquid paraffin in a plastic petri dish. The culture medium was Ham's F-10 supplemented with 1 mM sodium pyruvate, 10 mM sodium lactate, penicillin G (100 units/ml) and 15% heat-inactivated human serum. The oocytes were incubated for 2 days at 37°C under 5% CO_2 in air. When examined at the end of incubation, the follicle cells that were originally surrounding each oocyte were found as a compact cell mass attached to an almost naked oocyte. The oocytes were mechanically freed from the follicle cell mass and then washed twice with BWW medium (Biggers et al., 1971), supplemented with 15% heat-inactivated human serum and placed in 0.2 ml of fresh, BWW medium (serum-supplemented) under paraffin oil in a petri dish. Freshly ejaculated spermatozoa were washed 3 times with BWW medium by centrifugation (1,000 rpm for 10 min each) and added to the droplet containing the oocytes. The final concentration of spermatozoa in the droplet was about 5×10^6 spermatozoa/ml. The dish was incubated at 37°C under 5% CO_2 in air. Two oocytes, selected at random, were removed from the dish at 2, 4 or 7 h after insemination (total number of oocytes studied = 6) and were fixed in 5% glutaraldehyde, postfixed with 2% osmium tetroxide, dehydrated in graded ethanol and embedded in an epon-araldite mixture. The oocytes were studied using both light and transmission electron microscopy for signs of zona penetration and fertilization.

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RESULTS

The results of this study are summarized in Table 1. The heads of spermatozoa were embedded deep in the zonae pellucida of all the oocytes examined at 2, 4 or 7 h after insemination (Figs. 1a, b). A spermatozoon was observed in the perivitelline space of 1 oocyte 2 h after insemination (Fig. 2) and 3 pronuclear-like bodies were found in the vitellus of 1 oocyte 7 h after insemination (Fig. 3). Due to technical difficulties, only a small part of the latter oocyte was studied. No sperm tails were found in the sections viewed. Figure 4 illustrates the heads of spermatozoa undergoing the acrosome reaction. There was a progressive increase with time in the proportion of acrosome-reacted spermatozoa on and in the vicinity of the zona pellucida; all the spermatozoa within the zona pellucida were fully or partially acrosome-reacted (Table 2).

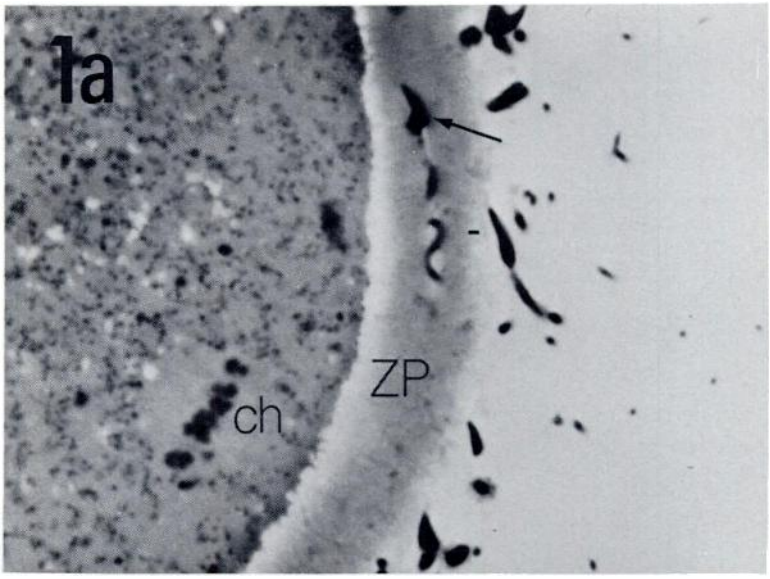

FIG. 1a. Spermatozoon (arrow) in zona pellucida (ZP) 2 h after insemination. Note metaphase chromosomes (ch). X 1500.

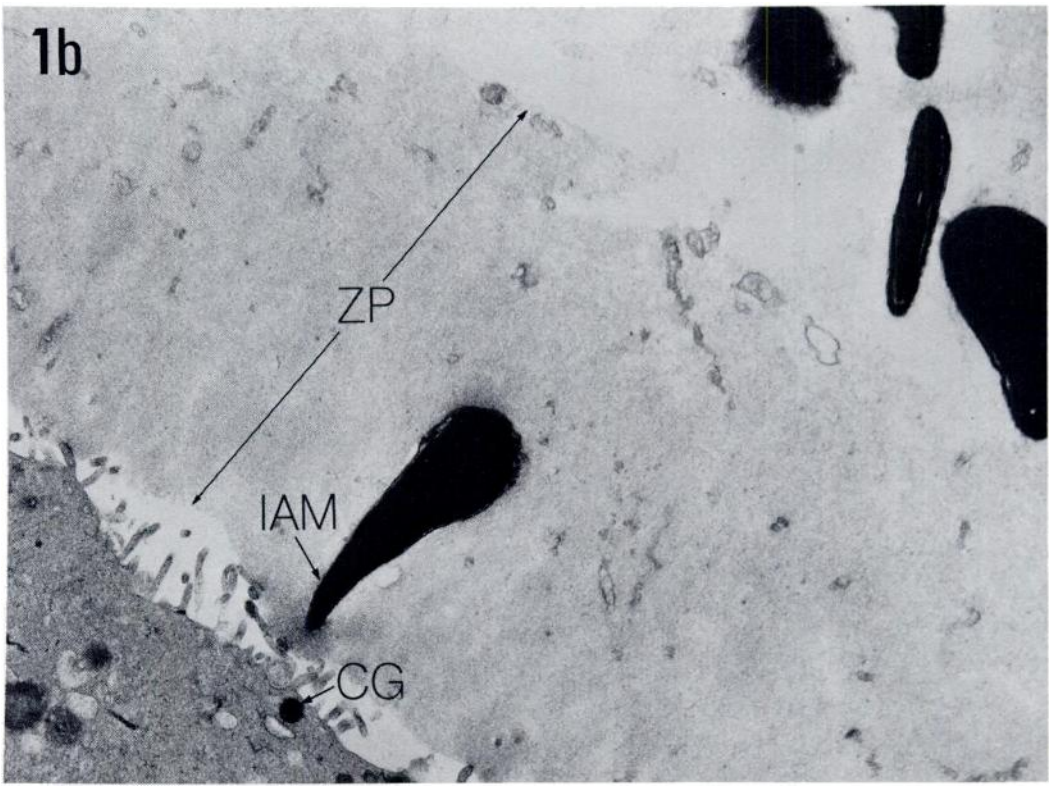

FIG. 1b. Spermatozoon at inner edge of zona pellucida (ZP) 2 h after insemination. Note inner acrosomal membrane (IAM) in contact with zona pellucida. Cortical granule (CG) in cortical ooplasm. X 8000.

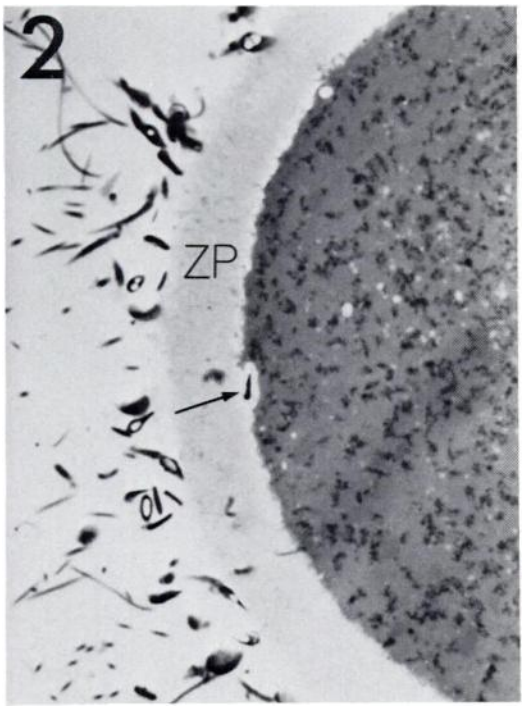

FIG. 2. Spermatozoon (arrow) in perivitelline space 2 h after insemination. X1000.

DISCUSSION

The results of this study indicate that human spermatozoa in BWW medium containing 15% human serum become capable of penetrating the zona pellucida of human oocytes in less than 2½ h (rinsing the spermatozoa in medium prior to insemination took 30 min). Previous workers (Edwards et al., 1969; Bavister et al., 1969; Overstreet and Hembree, 1976) used a modified Tyrode's solution with serum albumin as an insemination medium. The faster sperm penetration we observed (in less than 2½ h after insemination in our study compared with no less than 7 h after insemination reported by others) could be attributed to the difference in the composition of the medium used. It is unlikely that the time required for sperm capacitation and acrosome reaction of human spermatozoa is inherently fixed. The time must be greatly influenced or controlled by the environmental conditions to which the spermatozoa are exposed.

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TABLE 1. Timing of sperm penetration of human oocytes *in vitro*.

Time after insemination (h)	Details of events			
	Unpenetrated	Sperm deep in zona pellucida	Sperm in perivitelline space	Sperm in vitellus (pronuleate)
2	...	1	1	...
4	...	2	...	...
7	...	1	...	?1*

*3 pronuclear-like bodies in vitellus.

TABLE 2. The incidence of the acrosome reaction in spermatozoa found outside and within the zona pellucida.

Time after insemination (h)	Average no. of spermatozoa/ EM thin section	% of spermatozoa with acrosome reaction	
		Outside zona	Within zona
2	16.1	10 (11/108)	100 (134/134)
4	16.8	48 (45/94)	100 (74/74)
7	17.3	85 (50/59)	100 (131/131)

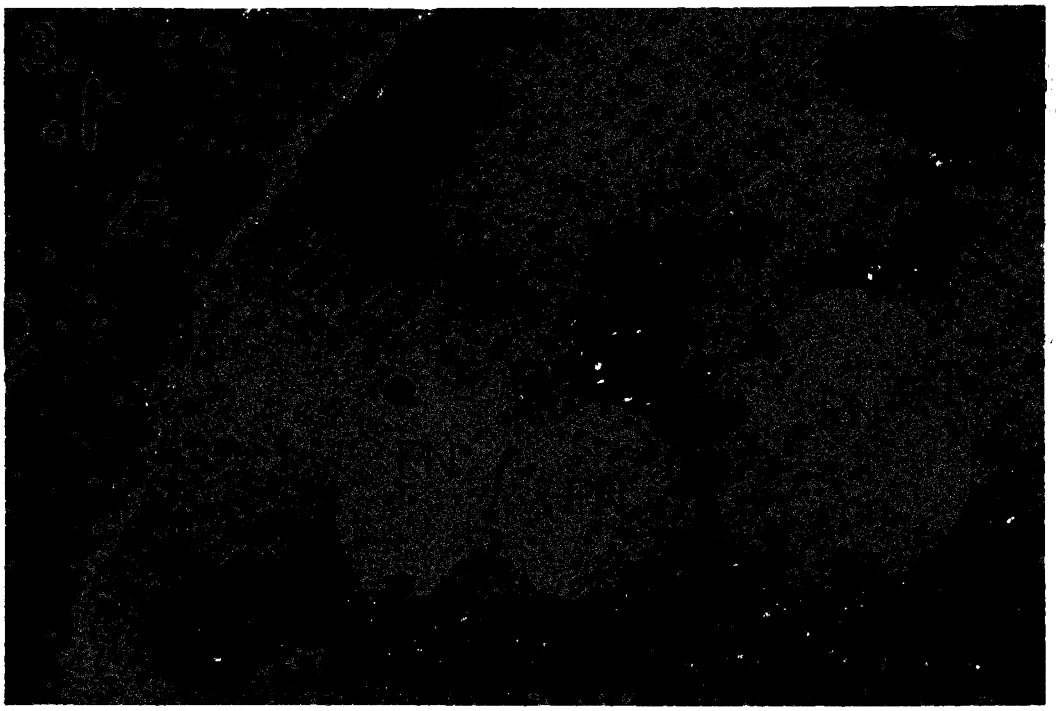

FIG. 3. Three (3) pronuclear-like bodies (PN) in ooplasm 7 h after insemination. Note presence of nucleoli (arrow) and chromatin (ch). No cortical granules visible. X 2200.

FIG. 4. The acrosome reaction; progressive stages illustrated by spermatozoa 1, 2 and 3. 1. Intact outer acrosomal membrane (arrow). 2. Vesiculated outer acrosomal membrane (arrow). 3. Exposed acrosome (arrow) in contact with zona pellucida. X 2600.

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