

## IDENTIFICATION OF THE MIDPIECE AND TAIL OF THE SPERMATOZOON DURING FERTILIZATION OF HUMAN EGGS *IN VITRO*

B. D. BAVISTER,\* R. G. EDWARDS\* AND P. C. STEPTOE†

\**Physiological Laboratory Cambridge, and †Oldham General Hospital, Lancashire*

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Positive identification of the sperm midpiece and tail in the vitellus establishes beyond reasonable doubt that pronucleate eggs are undergoing fertilization. Previously Edwards, Bavister & Steptoe (1969) tentatively identified sperm midpieces in pronucleate human eggs fertilized *in vitro*. Unequivocal evidence of midpieces and tails in eggs undergoing fertilization is now presented.

Oocytes recovered from two ovaries excised 3 hr previously were cultured in a mixture containing three parts follicular fluid: one part of Bavister's medium (Bavister, 1969). Four samples of follicular fluid were used, three being straw-coloured and a fourth, which was slightly pink, probably came from an atretic follicle. After 36 to 37 hr in culture, the eggs were washed through two or three changes of Bavister's medium, the dilution of the follicular fluid being approximately 1:100. Ejaculated spermatozoa were washed twice in this medium and then added to some of the eggs at a concentration of  $10^6$ /ml. In some droplets, additional samples of follicular fluid were added. Eggs were examined by phase-contrast microscopy between 10½ and 24 hr later, and then fixed and stained according to the method described by Dickmann, Clewe, Bonney & Noyes (1965).

Twenty-one eggs were obtained from one of the ovaries. One droplet clotted, and the two eggs in it were discarded. Of the remaining eleven eggs used for fertilization, four had not matured or were degenerate. Six of the other seven eggs were undergoing monospermic fertilization (Table 1A). In one, a spermatozoon was half-way through the zona. In the second, a midpiece, tail and swelling spermhead were seen, and the egg was in telophase of the second meiotic division (Pl. 1, Fig. 1a, b). In the third egg, the male and female elements were developing asynchronously: the sperm head was enlarging in the vitellus (Pl. 1, Fig. 2), the midpiece lay on the side of the egg and the tip of the tail protruded through the zona to the outside; a female pronucleus and first and second polar bodies were identified. Three remaining eggs had two pronuclei each (Pl. 1, Fig. 3a, b). One egg contained a distinct midpiece and tail and at least one polar body. In another, the midpiece, tail and first and second polar bodies were identified. The third pronucleate egg was damaged during handling and details were obscured.

Eight control eggs (i.e. without added spermatozoa) from this ovary were examined at equivalent times: four had failed to mature, three were at metaphase-II, and one was either at metaphase-I or metaphase-II.

TABLE I  
FERTILIZATION OF HUMAN EGGS IN VITRO

Time after spermatozoa added to eggs (hr)	Details of the eggs				
	Unpenetrated	Spermatozoon in zona	Spermatozoon in perivitelline space	Enlarging sperm head in vitellus	Pronucleate*
A. Present data					
10½		1			
11 to 11½					2†
12½					1
13½	1			1	
14½					1
24	3				
B. Combined data					
6 to 6½	3				
7 to 10½	5	2	4		
11 and later	12	5	1	1	11
Total	20	7	5	1	11

\* The four pronucleate eggs were cultured in clear follicular fluid. The remaining three eggs from the same ovary were in earlier stages of fertilization or unfertilized.

† One of these eggs possessed a female pronucleus and an enlarging sperm head in the vitellus; the other possessed two pronuclei.

The three eggs obtained from the second ovary were all unfertilized 24 hr after adding spermatozoa.

Adoption of a different fixation and staining method has made sperm mid-piece and tail readily recognizable, and has confirmed that fertilization is progressing normally in these oocytes. The authors' earlier observations are thus extended; the combined data on human fertilization is given in Table 1B.

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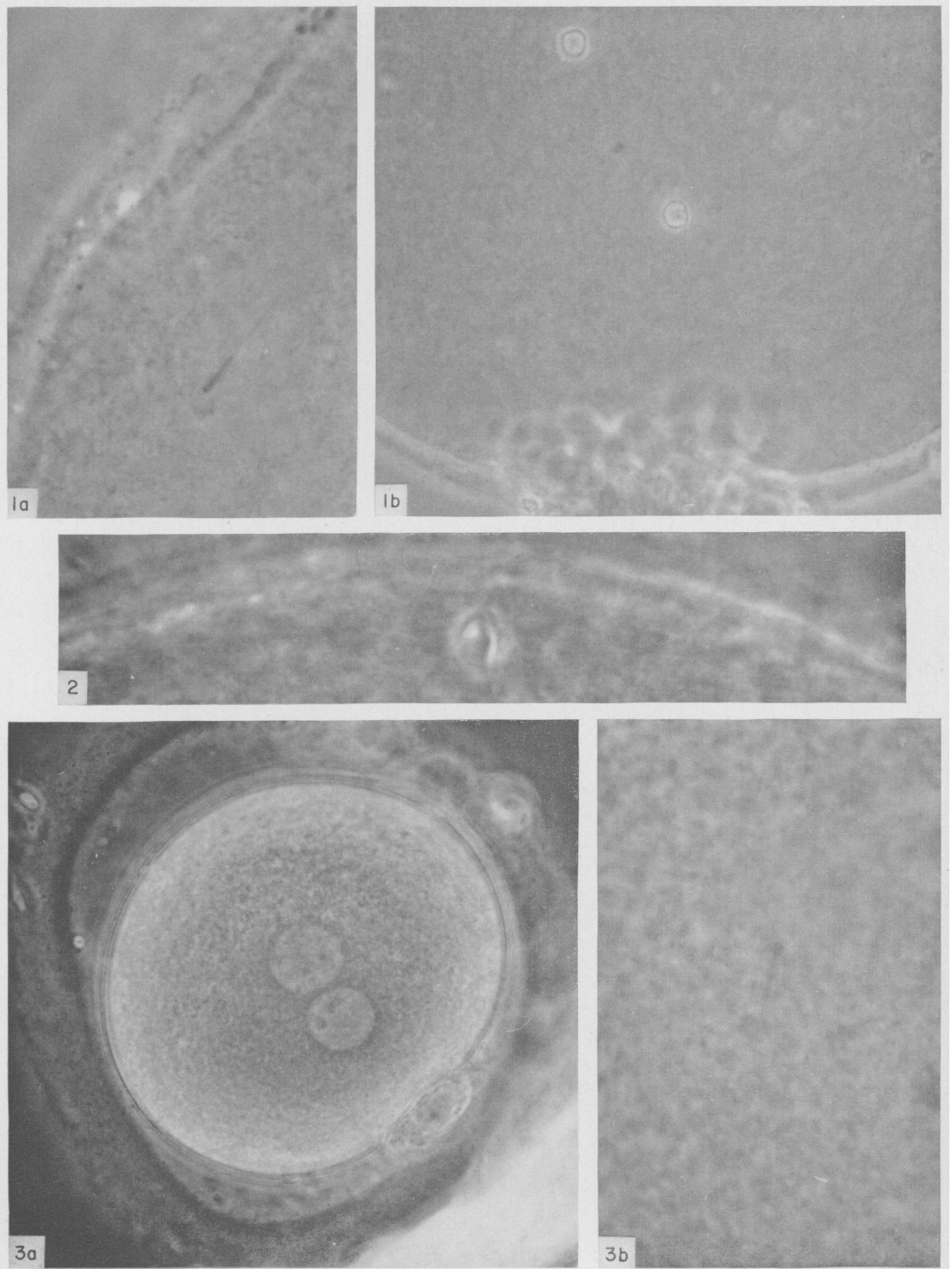
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#### EXPLANATION OF PLATE 1

- FIG. 1(a). Enlarging sperm head, midpiece and tail in a stained egg.  
 FIG. 1(b). Same egg showing telophase of the second meiotic division. The mid-body of the spindle can be seen.  
 FIG. 2. Enlarging sperm head in the vitellus. A female pronucleus and first and second polar bodies were found in this egg.  
 FIG. 3(a). Living egg with two pronuclei; polar bodies can clearly be seen.  
 FIG. 3(b). The midpiece of the fertilizing spermatozoon in the cytoplasm of this egg after staining.

PLATE 1



(Facing p. 160)