Evidence supporting the existence of sperm maturation in the human epididymis

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Summary. Zona-free hamster oocytes were used to evaluate the penetrating capacity of spermatozoa recovered from the caput, corpus and cauda segments of 5 human epididymides. The ability of spermatozoa to bind tightly to oocytes increased in spermatozoa from successive segments. However, only spermatozoa recovered from the cauda epididymidis were able to penetrate the oocytes (26%). These results suggest that, in man, a gradual process of sperm maturation occurs in the epididymis, as has been observed in numerous other species of mammals.

Introduction

Spermatozoa from a variety of species, guinea-pig, rat, rabbit, hamster, ram (Bedford, 1975), bull (Igboeli & Foote, 1968), pig (Holtz & Smidt, 1976), undergo a maturation process during their passage through the epididymis which results in the development of fertilizing capacity as they reach the distal segments of the organ. It has now become clear that the epididymis actively contributes to sperm maturation with factors produced by the epithelium under the influence of androgens (Orgebin-Crist, Danzo & Davies, 1975).

Technical and ethical difficulties have precluded experiments to ascertain whether sperm maturation occurs in man. Belonoschkin (1942) and Bedford, Calvin & Cooper (1973) examined the motility of spermatozoa from different epididymal segments and found an increased capacity for progressive motility as spermatozoa pass through the epididymis. Furthermore, Bedford *et al.* (1973) also noted an increase in the formation of disulphide bonds as well as changes in the ability to bind positively charged colloidal ferric oxide to the surface of spermatozoa from different segments of the epididymis in man. These changes are similar to those that occur in laboratory and domestic animals and which are considered a facet of sperm maturation (Bedford, 1975). However, Young (1970) reported fertility in patients with obstructive azoospermia after anastomosis of the vas deferens to the caput epididymidis, suggesting that spermatozoa are already fertile in the human caput epididymidis or that maturation can occur in the vas deferens. Both of these possibilities seem unlikely in view of the results obtained from laboratory animals whereby spermatozoa artificially retained in the caput epididymidis failed to develop fertilizing capacity (Gaddum & Glover, 1965; Bedford, 1967; Cummins, 1976). The environment of the vas deferens is not conducive to sperm survival, as shown for the hamster by Lubicz-Nawrocki & Chang (1978).

In view of these facts, it seemed pertinent to re-examine the question of the existence of sperm maturation in man by using the techniques developed by Yanagimachi, Yanagimachi & Rogers (1976) and Barros, González, Herrera & Bustos-Obregón (1978) to assess the ability of human spermatozoa to penetrate zona-free hamster oocytes.

Materials and Methods

The epididymides used throughout this study were obtained from patients undergoing castration or epididymectomy as treatment for testicular cysts (Patient 1, aged 26 years), prostatic carcinoma (Patient 2, aged 63 years), carcinoma of the penis (Patient 3, aged 49 years), and paraepididymal cysts (Patients 4 and 5, aged 54 and 57 years, respectively). Immediately after removal, the epididymides were placed in sterile culture medium (Eagle's MEM with Earle's salts, Gibco) at room temperature and taken to the laboratory. The tissues were carefully dissected under sterile conditions and divided into the caput, corpus and cauda epididymidis (segments 2a and 2b, segment 3b, and segments 4b and 4c, respectively, in the diagram of Bedford *et al.*, 1973). The caput and corpus epididymidis were each minced in tubes containing 1 ml modified Tyrode's medium (TMPA) (Barros, González, Herrera & Bustos-Obregón, 1979). The tissues were allowed to sediment and the supernatant containing the spermatozoa was aspirated. Spermatozoa from the cauda epididymidis were obtained by retrograde flushing of this segment by means of a needle introduced into the vas deferens. Sperm suspensions, diluted with TMPA to a concentration of $1-2 \times 10^6/\text{ml}$ were maintained for 1 h at 37°C, under mineral oil, before the addition of zona-free golden hamster oocytes.

Oocytes were obtained by flushing the oviducts of golden hamsters (*Mesocricetus auratus*) that had been induced to superovulate by the sequential s.c. injection of 30 i.u. PMSG (Sigma Chemical Co, St Louis, Missouri) and 30 i.u. hCG (Sigma) 62 and 16 h before being killed (Barros *et al.*, 1979). Oocytes were treated with 0.1% bovine testicular hyaluronidase (Sigma) for 15 min at 25°C to separate the cumulus cells. The zona pellucida was removed by incubation with 0.1% bovine pancreatic trypsin (Sigma) for 2-3 min at 25°C (Yanagimachi *et al.*, 1976). The oocytes were washed three times in fresh TMPA, added to the sperm suspensions and incubated for 5-7 h at 37°C under mineral oil equilibrated with air. At the end of the incubation period, oocytes were washed three times, by aspiration through a capillary pipette as indicated by Hartmann & Gwatkin (1971), mounted on slides and examined by phase-contrast microscopy. Tight sperm binding, indicated by the permanence of spermatozoa bound to the oocytes after washings (Hartmann & Gwatkin, 1971), was scored. Penetration was judged to have occurred if sperm chromatin had dispersed within the oocytes.

Statistical analysis was performed by the study of the binomial distribution of percentages (Richmond, 1964).

Results

Human spermatozoa recovered from different epididymal segments were able to bind to zona-free hamster oocytes (Table 1). No penetration of oocytes occurred after incubation with spermatozoa from the caput or corpus epididymidis but caudal spermatozoa penetrated 26% of the eggs (Table 2; $P < 0.001$). Of the 26 oocytes penetrated, 8 had fewer than 5 bound spermatozoa, 9 had 5-10 and 9 had more than 10 bound spermatozoa.

Table 1. Binding of spermatozoa, recovered from different segments of the human epididymis, to zona-free hamster oocytes

Epididymal segment	No. of oocytes	No. (%) of oocytes with		
		1-5 spermatozoa	5-10 spermatozoa	>10 spermatozoa
Caput	53	44 (83)	6 (11)	3 (6)
Corpus	62	40 (64)	14 (23)	8 (13)
Cauda	99	38 (38)*	27 (27)	34 (34)*

* Significantly different from values for caput and corpus spermatozoa ($P < 0.005$). 26/2023 01:38:08PM via free access

Table 2. Penetration* of zona-free hamster oocytes by human spermatozoa recovered from the cauda epididymidis

	Experiment					Total
	1	2	3	4	5	
Oocytes penetrated/total tested	8/35	2/12	7/18	5/13	4/21	26/99
%	23	17	39	38	19	27.19 ± 5.36†

* Judged to have occurred when one or more dispersed sperm nuclei were observed within an oocyte.

† Mean ± s.e.m.

Discussion

Although some mature spermatozoa are detectable in proximal (caput and proximal corpus) segments of the epididymis of several animal species (Bedford, 1975), most of the sperm population develops fertilizing ability abruptly in the distal regions (rabbit: Orgebin-Crist, 1969; hamster: Horan & Bedford, 1972; rat: Blandau & Rumery, 1964). Our results suggest that a similar phenomenon might occur in man and imply the existence of a maturation process, as was suspected from previous observations (Bedford *et al.*, 1973). This interpretation of our data is at variance with the results of Young (1970). Using epididymo-vasostomy for treatment of blockage of the vasa deferentia, he reported 121 successful results (spermatozoa in the ejaculate) out of 326 operations. However, only 21 of these 121 patients regained fertility. As the anastomosis of the vas and epididymis is performed at the caput segment, that result would imply that in approximately 19% of the population spermatozoa are already fertile in the caput epididymidis. However, the operation entails the formation of a sinus in the tissue and therefore does not preclude the presence in the ejaculate of spermatozoa from more distal region of the organ.

The percentage of penetration of zona-denuded oocytes by spermatozoa from the cauda epididymidis resembles the 25% obtained by Barros *et al.* (1979), for ejaculated spermatozoa from normal men, and this suggests that spermatozoa have fully matured by the time they reach the cauda epididymidis, as judged by the present tests.

Although there was an apparent absence of correlation between penetration of ova and the number of spermatozoa bound to them, significantly more spermatozoa from the cauda epididymidis than from the caput or corpus were attached to the eggs (Table 1). It is therefore concluded that spermatozoa in the human epididymis undergo morphological and physiological changes that enable them to interact with ova. Since sperm-egg fusion only takes place after the acrosomes have reacted (Cummins, 1976; Yanagimachi *et al.*, 1976), and as this reaction involves complete capacitation (Chang & Hunter, 1975; Cummins, 1976), our results suggest that human spermatozoa acquire the ability to capacitate as they reach the caudal portion of the epididymis. The ability to capacitate is one of the changes undergone by maturing spermatozoa in laboratory animals (Overstreet, 1970; Cummins, 1976).

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