

Ultrastructure of human sperm in men with congenital absence of the vas deferens: clinical implications

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Objective: To determine the ultrastructural features of epididymal and vasa efferentia sperm in men with congenital absence of the vas deferens.

Design: Prospective.

Setting: University of California Irvine Center for Reproductive Health.

Patients: Thirteen men with surgical diagnosis of congenital absence of the vas deferens undergoing epididymal and vasa efferentia aspiration for assisted reproductive technology procedures.

Results: The morphological quality and the integrity of the spermatozoa aspirated from the extratesticular segment of the rete testis, the vasa efferentia, and the caput epididymis were always markedly superior to those of sperm aspirated from the corpus and cauda epididymis, where the vast majority, if not all, were degenerating or frankly necrotic. The aspirates obtained from the distal segments of the epididymis also contained large numbers of sperm-laden macrophages; these were instead absent or exceptional in the fluids aspirated from the pre-epididymal portions of the excurrent pathways and from the caput of the epididymis.

Conclusions: This study demonstrates that the ultrastructural morphology of spermatozoa obtained by aspiration from the rete testis, vasa efferentia, and caput epididymis of individuals with congenital absence of the vas deferens is indistinguishable from that of spermatozoa in the semen. *Fertil Steril* 1992;58:190-3

Key Words: Congenital absence of the vas deferens, sperm ultrastructure, epididymis

The numerous studies that have been performed during the last 25 years have consistently demonstrated that the mammalian epididymis plays an important role in the maturation of the spermatozoon (1). It is generally accepted that at the time they leave the testes, spermatozoa, even though fully differen-

tiated, are neither mature nor motile nor do they possess any fertilizing competence. Although the latter is acquired in the female reproductive tract through capacitation, motility and maturity are attained during epididymal transit. Evidence of acquisition of maturity are the shedding of the cytoplasmic droplet and, more importantly, physicochemical modifications of the plasma membrane that are thought to be essential to prepare the spermatozoon to undergo capacitation (2). Completely immotile or ineffectively motile at the time they enter the caput epididymis, spermatozoa in the cauda epididymis display vigorous, forward progressive motility (3). Contributory to the development of motility is the shedding of the cytoplasmic droplet enabling the flagellum to beat freely, and possibly, also, the plasma membrane may change as described above.

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Recently, we have reported that human spermatozoa aspirated from the caput epididymis and vasa efferentia of individuals with congenital agenesis of the vas deferens can be used to inseminate human oocytes in vitro during in vitro fertilization and gamete intrafallopian transfer procedures (4, 5). Not only are these spermatozoa motile but they also demonstrate reproductive competence as shown by their fertilization capability and the subsequent birth of children. This appears to contradict the prevailing concept that epididymal sperm transit is necessary for acquisition of motility and maturity and that mature and motile sperm can be found only in the most distal segments of the epididymis. In an attempt to clarify this apparent discrepancy, we decided to perform a detailed ultrastructural study of spermatozoa aspirated from different segments of the testicular excurrent pathways of patients with congenital absence of the vas deferens who underwent microsurgical procedures to obtain spermatozoa for insemination of their wife's oocytes in vitro.

MATERIALS AND METHODS

The spermatozoa were collected by microsurgical aspiration of fluid from different segments of the epididymis, the vasa efferentia, and the rete testis, as reported elsewhere (5). They were collected from 13 men with surgical diagnosis of congenital absence of the vas deferens. Upon aspiration, aliquots of sperm suspensions were fixed in picric acid formaldehyde solution (6) in phosphate buffer for 1 hour; the mixture was then gently centrifuged to concentrate the spermatozoa, the supernatant decanted, and the pellet, after two rinses in phosphate buffer, postfixed for 30 minutes in 1% OsO₄ (Poly Science, Warrington, PA). The pellet was subsequently dehydrated in increasing ethanol concentrations and subdivided in small fragments that were individually embedded in Epon 812 (Poly Science). The plastic blocks were initially sectioned at 1 μ m thickness and the sections stained by flotation on 1% aqueous solution of toluidine blue; they were then placed on microscopic slides and examined to assess the quality of the preparation and obtain an overall view of the cellular density of the samples. The blocks containing the highest number of spermatozoa were appropriately trimmed, and ultrathin sections were prepared. After staining with lead hydroxide, the sections were then examined with Hitachi HU11E and Hitachi 600 (Hitachi Co., Tokyo, Japan) microscopes.

RESULTS

The results of the morphological appearance of sperm from different sources of the male genital tract and the ultrastructure features were identical among the 13 congenital absence of the vas deferens patients studied. Of importance for the primary objective of the study was the observation that the morphological quality and the integrity of the spermatozoa aspirated from the extratesticular segment of the rete testis, the vasa efferentia, and the caput epididymis were always markedly superior to those of sperm aspirated from the corpus and cauda epididymis, where the vast majority, if not all, were degenerating or frankly necrotic. The aspirates obtained from the distal segments of the epididymis also contained large numbers of sperm-laden macrophages; these were instead absent or exceptional in the fluids aspirated from the pre-epididymal portions of the excurrent pathways and from the caput of the epididymis.

Most of the sperm aspirated from the rete testis, vasa efferentia, and caput of the epididymis were morphologically identical to ejaculated spermatozoa of normospermic subjects, and no differences were noted in the ultrastructural organization of their nuclei, acrosomes, and flagella (Figs. 1A and B). Some of them possessed a small cytoplasmic droplet around the most posterior portions of the head and the initial segment of the flagellum. These cytoplasmic remnants appeared to contain scattered mitochondria, small vesicles, and stacks of membranes. Their presence evidently did not interfere with sperm motility because the aliquots of the aspirates used for oocyte insemination all contained vigorously motile spermatozoa. Very few spermatozoa in the aspirates from these pre-epididymal segments or from the caput of the epididymis appeared to be degenerative. Degenerating sperm from these proximal regions showed less regressive changes than sperm from the more distal regions of the epididymis. They displayed regressive changes of the acrosome mostly consisting of swelling of the organelle, lack of homogeneity of the matrix and formation of vesicles within its content, separation of the acrosome from the underlying nucleus, and fragmentation of acrosomal membranes. The degenerative changes of the nucleus were represented mostly by karyorrhexis and karyolysis; in the flagellum, there was swelling of the mitochondria with reduction and dilation of their cristae and filamentous transformation of the axonemal microtubules. The plasma membranes were frequently fragmented or absent.

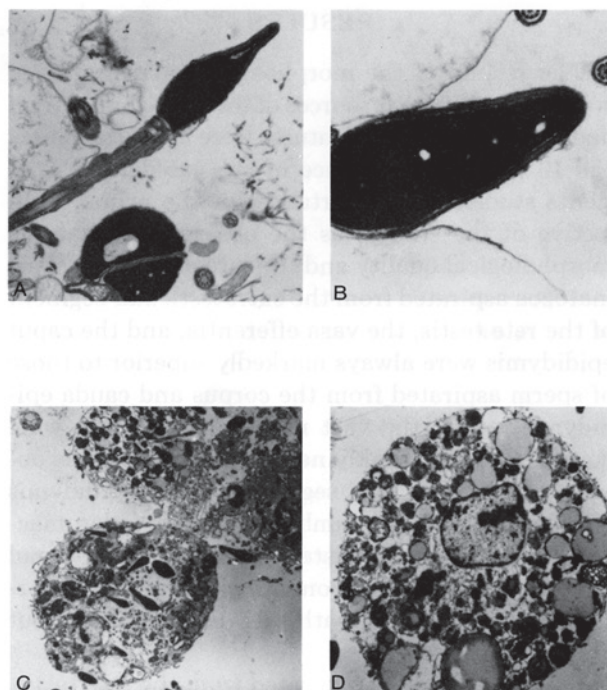


Figure 1 (A), Ultrastructure of sperm obtained from the rete testis from a man with congenital absence of the vas deferens. Note the similarities in the organization of the nucleus acrosome and flagella to normal ejaculated sperm. (B), Ultrastructure of sperm obtained from the vasa efferentia from a man with congenital absence of the vas deferens. Note the similarities in the organization of the nucleus acrosome and flagella to normal ejaculated sperm. (C), Ultrastructure of cells obtained from the corpus epididymis in a man with congenital absence of the vas deferens. Note the macrophages with their cytoplasm occupied by very large amounts of phagocytized sperm remnants in different degrees of degradation and digestion. (D), Ultrastructure of macrophage obtained from the cauda epididymis from a man with congenital absence of the vas deferens. Note the presence of prominent whorls of membranes and numerous lipid droplets indicating advanced stages of sperm degeneration.

In the aspirates from the corpus and cauda epididymis, the situation was drastically different in that normal appearing spermatozoa were either absent or exceptional. Almost all of the sperm were in such advanced stages of degeneration and necrosis as to be barely recognizable. Macrophages were abundant and generally of an impressive size and multinucleated. Their cytoplasm was occupied to capacity by very large amounts of phagocytized sperm remnants in different degrees of degradation and digestion (Fig. 1C). Prominent whorls of membranes (myelin figures) and numerous lipid droplets was also frequently noted (Fig. 1D). Many of the macrophages were degenerating. The sperm motility in the 13 cases at aspiration from the vasa efferentia or caput epididymis ranged from 7% to 40% with

an average of 16.5% and a progression of 1 to 2. One hundred fifty-four oocytes were inseminated, of which 52 developed into embryos for a fertilization rate of 33.8% per oocyte and 61.5% per patient (8 of 13).

DISCUSSION

The extremely good correlation between maturity, motility, and functional competence of the sperm obtained from the pre-epididymal segments of the excurrent pathways and the caput epididymis on the one hand and their ultrastructural appearance on the other is a further demonstration of the important role of electron microscopy for a precise assessment of sperm quality (7). The observations made in this study confirm our previous findings that spermatozoa aspirated from the most proximal segments of the testicular excurrent system are motile and reproductively competent. Electron microscopic examination has, in fact, shown that these spermatozoa are identical to those in the semen, possibly with the exception of the presence of cytoplasmic droplets that may be occasionally present also in ejaculated spermatozoa.

These observations differ from the concept that upon leaving the testes and entering the testicular excurrent pathways, spermatozoa are functionally immature and that to attain maturity, expressed as the ability to move and to undergo capacitation, they must transit through the epididymis. This discrepancy is difficult to explain. However, it is important to remember that the conditions that were evaluated in this study are unlike normal ones: we studied spermatozoa obtained from different portions of the extratesticular excurrent system of congenital absence of the vas deferens patient and not of normal individuals. Thus, it would be unjustifiable to draw from this study conclusions undermining the validity of the concept, demonstrated by an abundant volume of studies all based on physiological conditions, that epididymal transit is important to sperm maturation and that the least mature spermatozoa are in the initial segments of the epididymis and the most mature in the most distal. The inapplicability of the observations made in this study for an understanding of the physiological process of sperm maturation is also vividly demonstrated by the presence of rampant sperm degeneration in the corpus and cauda of the epididymides of the individuals in our protocol and of impressive numbers of macrophages involved in the disposal of the necrotic sperm by phagocytosis;

this situation, in fact, would not be present under physiological conditions.

The presence of massive numbers of necrotic spermatozoa in the lower epididymis and the absence or the exceptionality of degenerating spermatozoa in the upper epididymis and in the pre-epididymal segments of the excurrent pathways are of significance. Because the latter had not yet, or had only recently, entered the epididymal environment, it is reasonable to conclude that the longer the sperm are stored in an abnormal epididymal environment, the higher the degree of sperm degeneration and necrosis. This deduction is similar to previous findings in vasectomized men (8). This hypothesis would also explain the seemingly paradoxical observation that from a functional point of view (viability, motility, reproductive competence), the spermatozoa aspirated from the proximal segments of the excurrent pathways are superior to those obtained from the middle and low epididymis. Incidentally, these observations support the concept that necrostermia is generally the expression of an epididymal pathology and corroborate the finding that the quality of the semen in some of these cases may be improved by increasing ejaculation frequency and thus decreasing sperm exposure to the unfavorable epididymal environment (9).

To the best of our knowledge, our study provides original information concerning the fate of spermatozoa entering an excurrent pathway blocked by congenital absence of the vas deferens. It is evident that they degenerate and then are disposed of by scavenger cells through phagocytosis and subsequent degradation. Our observations have shown also that upon having saturated their phagocytic capabilities, as demonstrated by the presence of impressive amounts of ingested sperm remnants in their cytoplasm, the macrophages degenerate, becoming subsequently prey to the phagocytic activity of other, fresher macrophagic cells continuously introduced in the system. Immunological studies should be performed to establish whether autoimmune conditions develop in patients with blocked excurrent pathways because of congenital absence of the vas deferens or

other causes as a result of continuous assimilation of products of sperm degradation.

In conclusion, this study demonstrates that the ultrastructural morphology of spermatozoa obtained by aspiration from the rete testis, vasa efferentia, and caput epididymis of individuals with congenital absence of the vas deferens is indistinguishable from that of spermatozoa in the semen. This observation provides the structural bases for the already demonstrated reproductive competence of these sperm. The apparent discrepancy between our observations and the concept that sperm maturation and partial functional competence are acquired only at the end of epididymal transit remains to be reconciled.

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