# Effect Of Primary Obstructive Infertility On Sperm Morphology

A Sarda, S Bhalla, N Sarda, P Pal, S Salhan

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**Abstract**

Due to epithelial breach secondary to obstruction, small, intermittent exposure of the epididymal interstitium to sperms is likely to induce a local humoral response. It is possible that the presence of local antibodies may affect the sperm maturation and morphology in the epididymis. Thirty patients of primary obstructive infertility and five controls were studied. The supernatant of the centrifuged epididymal fluid was studied for analysis of antisperm antibody by ELISA and the sediment was used for study of sperm morphology along with smears of epididymal fluid by light and scanning electron microscope. ELISA for antisperm antibodies in the epididymal fluid was positive in 16/30 (53.3%) patients. In 23 patients sperms could be isolated in the epididymal fluid for examination by SEM. In 16/23 (70%) of the patients the normal sperms were < 60% and 62.5% of the sixteen patients had a significant ELISA. In 7 patients no sperms were seen in the epididymal fluid pellets and 71.4% of them had significant ELISA. The deleterious effects of obstruction and the local immune response lead to changes in the sperm morphology which can cause failure of assisted reproduction techniques.

## INTRODUCTION

There are contradictory reports regarding changes in sperm morphology in obstructive azoospermia, which becomes of paramount importance with the use of epididymal sperms for assisted reproduction. In a light microscopy comparison of epididymal sperms from men with obstructive azoospermia and ejaculated sperms from fertile men, no significant difference was found in the morphology of sperms in the two groups.[1] Rajalakshmi et al performed SEM studies on ejaculated spermatozoa in men who had undergone vasoepididymal anastomosis (VEA), and showed that ejaculated spermatozoa from men with obstruction, at the corpus or the caput epididymidis and who had undergone VEA showed a significantly higher percentage of morphological abnormalities compared to men of proven fertility.[2] In men who had undergone VEA due to obstruction at the cauda epididymidis the percentage abnormality was lower.

Although ISCI is a highly predictable technique in terms of achieving fertilization and embryo transfer, fertilization failures occur. Fertilization failure after ICSI is due mainly to defective oocyte activation.[3] A review of the fertilization failures occurring after ICSI showed that no fertilization ensued in only 3% of the cycles (76/2732 cycles).[4] In 49% of these failed cycles, fertilization failure was related to a sperm factor, i.e. the absence of motile spermatozoa for injection, or for the injection of morphologically abnormal spermatozoa, such as those without acrosomes. Sperm factor has also been cited as an important reason for low pregnancy rates even after a successful vasoepididymal anastomosis to bypass obstruction.[5]

The process of sperm maturation, whereby sperms gain their ability to fertilize eggs occurs in the proximal epididymis and is associated with many physiological, morphological and biochemical changes in the spermatozoa. It is also possible that in obstructive infertility breach in the epithelium causes extravasation of sperms into the interstitium and brings them in contact with the immunocompetent cells in insufficient amounts to induce detectable ASA.[6,7] These local changes may affect sperm morphology manifested as swollen sperm heads in 28-33% of vasectomized males within a year following the operation in contrast to less than 2% of nonvasectomized male population.[8] Wen et al report that the sperm concentration in the proximal vas of vasectomized males was significantly higher than that of fertile men; however, the means of sperm motility (in 46.4% all sperms were immotile with the condition being more common with the [passage of time postoperatively), sperm viability and sperm function tests were statistically lower than the respective values for normal

fertile men.[9] However, other reports suggest that there is little evidence that suggests a cause/effect relationship between antisperm antibodies and abnormalities of principal semen parameters (sperm count, motility and morphology).[10]

The present study of the morphology of sperms in the epididymal fluid in the patients of obstructive azoospermia related to the presence of a local immune response may be extrapolated to explain not only the persistence of infertility after surgical removal of obstruction but also to explain failure of assisted reproduction.

## MATERIALS AND METHODS

Thirty patients of primary obstructive infertility were studied. None of these patients had any history or clinical evidence of infection, trauma or surgery; none of the patients had undergone vasectomy. Clinical findings were recorded in a predetermined proforma. The selection criteria for the study group were primary obstructive azoospermia, clinically palpable epididymis, fine needle aspiration cytology showing spermatogenesis. The patients were subjected to vasoepididymostomy under magnification. During surgery epididymis was identified and its features noted — distension, appearance and amount of discharge on incision over epididymis. Patency of the vas was confirmed by injecting about 20 ml of saline in the vas using a 26 G needle and a 20 ml syringe and asking the patient whether he has an urge to micturate. If the vas was patent, the epididymis was incised at the point of maximal dilatation as evidenced by the presence of yellowish inspissated material or, if such obvious evidence was lacking, in the body of the epididymis, and tissue taken for histological examination. Character of the fluid coming out of the epididymis was noted and smears of this fluid were made for observing sperm morphology. Fluid was also taken in a syringe diluted with normal saline for determination of anti-sperm antibodies in the supernatant fluid with the precipitated sperms being used to study sperm morphology and other studies. A side-to-side anastomosis between the vas deferens and epididymis was performed.

The epididymal fluid was centrifuged and the supernatant was stored at -20° C for analysis of antisperm antibody by ELISA. The sediment was made into pellets for study of sperm morphology along with smears of epididymal fluid.

Five controls were also studied. They were of proven fertility. Testicular biopsy and epididymal wedge biopsy were taken at the time of autopsy (organ donation from four subjects dead as a result of trauma) or from a fifty year old patient who underwent orchiectomy for prostatic malignancy with skeletal metastases.

Epididymal fluid was also aspirated for cytology for sperm morphology and also for analysis of antisperm antibodies.

Grade of spermatogenesis in the testicular tissue was done according to the accepted criteria.[11] The interstitium was specially screened for presence of lymphocytes, plasma cells, histiocytes. Mature spermatids were identified as oval cells with dark densely stained chromatin. The number of mature spermatids was totaled in twenty tubules and the total divided by twenty to give an estimate of number of mature spermatids per tubule for quantitation of mature spermatids per tubule.[12] By studying the proportion of Sertoli cells versus spermatogenetic cells, it was possible to give a cytological diagnosis in histological terms by the semiquatitative analysis of a combination of the various cells seen on the smear. For the study, spermatogenesis was graded into four grades on the basis of spermatogenetic cells and Sertoli cells.

- Normal spermatogenesis was considered when both the spermatogenetic and Sertoli cells were seen in normal proportion.
- Hypo spermatogenesis was considered when the ratio of spermatogenetic to Sertoli cells was decreased.
- Maturation arrest was considered when there was a significant increase in the percentage of more immature cells with low percentage of other cells and an absence of spermatozoa.
- Sertoli cell only syndrome was considered when the aspirate reveals predominantly Sertoli cells with a few Leydig cells but no germ cells or spermatozoa.

The histologic features observed in the epididymal tissue included epithelial flattening, lining cells (cilia, pigment, nuclei, sperm ingestion), ducts (whether normal or dilated), ductal contents (macrophages, sperms, desquamated epithelial cells), macrophages (vacuolation, sperm ingestion, pigment), interstitium [extravasation of sperm with epithelial breach, macrophages (with or without pigment), other cells (lymphocytes, plasma cells)].

Sperm morphology was studied by light microscope study of slides made from epididymal fluid sperm pellets and fixed in

difluorotetrachloroethane using Papanicolaou staining procedure[13] and counting and expressing the normal and abnormal spermatozoa in percentage. For SEM the spermatozoa were pelletted at 1500 rpm for 15 minutes and the sperm pellets were fixed in Karnovsky's fluid[14] for 30 minutes and washed twice in 0.I M cacodylate buffer followed by double distilled water. A thin film of spermatozoa was smeared on a clean glass slide, air dried, mounted on SEM stub with silver paint, sputter coated at 300 Å with gold and observed under SEM (Philips 501). The strict (Tygerberg) criteria were used for morphology evaluation.[15] In contrast to other authors, so called 'borderline normal' spermatozoa are classified as abnormal. According to this the whole sperm was evaluated and classified as normal if the head was oval and had smooth contour, acrosome was 40 - 70% of the head, had homogeneous staining with no neck/midpiece and tail defects and no cytoplasmic droplets (>30%).

ELISA technique was used to detect antisperm antibodies by utilizing a commercially available kit. The kit is an indirect noncompetitive enzyme immunoassay for the semiquantitative and qualitative determination of antibodies directed to spermatozoa surface antigen. The detection limit of the assay is 0.2 U/ml and permits the determination of lgG, IgM and lgA antibodies directed against sperm surface antigens.

The Chi square test was used for statistical analysis with Yates modification wherever applicable.

## RESULTS

Forty three percent of the patients were > 30 years old at presentation (mean: 31.1 years; median: 30 years; range 24-49 years). The serum FSH value was < 20 (mean 8.2 U/L; range 0.8 – 35.2 U/L) in 26/30 patients and 22/26 of these patients had a grade I spermatogenesis. Quantitative testicular biopsy, i.e., number of mature spermatids per tubule, which is very useful in predicting what the post operative sperm count should be in patients of obstructive infertility, revealed that 23/30 (76.7%) patients had > 30 spermatids per tubule and all these patients had Grade I spermatogenesis on testicular biopsy ($p < 0.001$). Further, 23/30 patients showed sperms in epididymal ducts and 20/23 of these patients had a grade I spermatogenesis. This was found to be statistically significant ($p < 0.05$). (Table 1)

Table 1 : Grade of spermatogenesis in testicular biopsy tissue obtained at operation

Grade I spermatogenesis 23 (11*)
Grade II spermatogenesis 3 (3*)
Grade III spermatogenesis 3 (2*)
Grade IV spermatogenesis 1 (1*)

* number of patients with a positive ELISA for antisperm antibodies in the epididymal fluid

Three patients had significant levels of antisperm antibody levels in the serum. However, only one of these patients had positive ELISA for antisperm antibodies in the epididymal fluid. On the other hand, 15 out of 27 patients with absence of antisperm bodies in blood had a positive ELISA for presence of antibodies in the epididymal fluid. This finding was not found to be statistically significant. ELISA for antisperm antibodies in the epididymal fluid was found to be positive in 16/30 (53.3%) patients. Negative results may have occurred in the remaining patients either because of the low concentration or the small amount of epididymal fluid available which was further diluted by the saline in which it was collected. Two of the patients with significant ELISA in the epididymal fluid had positive antisperm antibodies in the serum. .

The histology of the epididymis revealed dilatation of ducts (n=19; 63.3%), epithelial breach (n=14; 43.3%), sperm extravasation (n=8; 26.7%), presence of macrophages in ducts (n=18; 60%), loss of cilia (n=14; 46.7%), sperm ingestion by macrophages (n=10; 33.3%), and inflammatory exudation of lymphocytes/macrophages/plasma cells (n=25; 83.3%). Amongst the cases with inflammatory lymphocytic infiltration, 100% of the cases in whom epididymal fluid could be obtained had significant ELISA. In 12/14 patients with loss of cilia there was associated ductal dilatation; significantly 78.6% also had lymphocytic infiltration and in 60% the ELISA was significant.

In our study the percentage of abnormal sperms and morphology was performed with light microscopy. In 23 patients sperms could be isolated in the epididymal fluid for examination. We found that in 16/23 (70%) of patients the percentage of normal spermatozoa was less than 30%. The most common abnormality seen was spermatozoa with pyriform head which appeared in the shape of a tear drop with fine particles over the sperm head and tail. In 7 patients no sperms were seen in the epididymal fluid. In 40.5% of these patients (10/23) ELISA for antisperm antibodies was found to be positive in the epididymal fluid. In ten of the sixteen patients (62.5%) with less than 30% normal sperms, ELISA was positive for antisperm antibodies in the

epididymal fluid while it was positive in 2/7 (28.6%) patients with > 30% normal sperms.

None of the control group patients had any evidence of local cell mediated immune response in the epididymis and testis.

## DISCUSSION

Vasoepididymostomy is a technically challenging procedure. Even then, with the use of microsurgical single tubule-tubule anastomosis patency rates of 56% – 80% can be obtained. But the average spontaneous pregnancy rate with this procedure is 43.7% which remains disappointing.[16] The discrepancy between patency rates and spontaneous pregnancy rates after vasoepididymostomy needs to be explained. Two main factors implicated to explain this discrepancy are an irreversible damage to the epididymis due to prolonged obstruction and alteration in the morphology and function of spermatozoa. Immunological factors in the persistence of infertility after a patent vasoepididymostomy have not been widely studied. It is possible that the damage to epididymis and spermatozoa in cases of obstructive infertility are due to the development of a local autoimmune reaction to spermatozoal antigens.

Since epididymis is the major site of sperm maturation, when vasoepididymal anastomosis is done especially to the head of epididymis, sperms coming out would be bypassing the epididymis and not be mature enough to cause fertilization. This view has been challenged and evidences have been put forward to suggest that epididymal bypass may not have any such deleterious effect on the sperm maturation.[17] If vasoepididymostomy is done in cases with poor spermatogenesis the results are likely to be poor. In our study we selected patients who showed a normal spermatogenesis on FNAC of testes, although six patients were later found to have poor spermatogenesis (grade II, III or IV) on testicular biopsy obtained during the surgery.

We studied the morphology of spermatozoa in epididymal fluid pellets both by light microscopy and SEM. Assessment of sperm morphology with light microscopy has not been viewed encouragingly due to its limited magnification. Application of scanning electron microscopy (SEM) for surface image at higher magnification have wide scope for the assessment of sperm morphology and the difference in the percentage of abnormal spermatozoa in the ejaculates is 20-30 per cent higher under the SEM than LM.[18,19] There are multiple advantages of SEM in the study of sperm morphology. It gives a large area for the scanning and direct assessment of sperm surface abnormalities from the video screen at different higher magnifications. Morphological abnormalities which are not feasible to be identified by LM can be detected by SEM.[20] Rajalakshmi et al performed SEM studies on ejaculated spermatozoa in men who had undergone VEA, and showed that ejaculated spermatozoa from men with obstruction, at the caput or the corpus epididymidis and who had undergone VEA showed a significantly higher ($P < 0.05$) percentage of morphological abnormalities ($71 \pm 9\%$ and $74 \pm 6\%$, respectively) compared to men of proven fertility ($37 \pm 6\%$; $n = 10$). In men who had undergone VEA due to obstruction at the cauda epididymidis the percentage abnormality was lower ($58 \pm 7\%$, $P > 0.05$).[2] The major type of abnormality in all groups was spermatozoa with pyriform head. Spermatozoa with round, tapering and large heads were also seen. These findings suggest that morphological abnormalities in spermatozoa are more common in patients with primary obstructive infertility and these abnormalities persist even after the reversal of obstruction.

We studied the morphology of sperms in the epididymal fluid in the patients of obstructive azoospermia included in our study and related it to the presence of local antisperm antibodies. In 16 / 23 (70%) of patients the percentage of normal spermatozoa was less than sixty percent. Ten out of sixteen of these patients had a positive ELISA for antisperm antibodies in the epididymal fluid, while only 2/5 patients with normal spermatozoa more than 60% had ELISA positive. This difference was found to be statistically significant ($p < 0.001$). In seven patients no spermatozoa could be seen in the epididymal fluid pellets. Rajalakshmi et al performed SEM studies on ejaculated spermatozoa in men who had undergone VEA, and obtained similar results showing that ejaculated spermatozoa from men with obstruction who had undergone VEA showed a significantly higher ($p < 0.05$) percentage of morphological abnormalities as compared to men of proven fertility.[2] The study of morphology of epididymal spermatozoa is of primary importance in the present context as epididymal sperms are being increasingly used for assisted reproduction techniques. In the recent past, there has been a widespread application of assisted reproductive techniques in the treatment of patients with obstructive azoospermia. Various new microsurgical techniques of sperm retrieval allow the use of not only epididymal (MESA, i.e., Microsurgical Epididymal Sperm Aspiration) but even testicular sperms (TESE, i.e., Testicular Sperm Extraction) for in vitro fertilization in these patients. In patients of obstructive azoospermia, the pregnancy rate with ICSI using sperms aspirated from the epididymis in

terms of pregnancies per cycle has been reported to be 41% - 47%.[21,22] Liu et al showed that in 49% of failed cycles of ICSI, fertilization failure was related to a sperm factor like the absence of motile spermatozoa for injection, or the injection of morphologically abnormal spermatozoa.[4] Thus, the study of function and morphology of spermatozoa in patients of primary obstructive infertility becomes imperative not only to explain the persistence of infertility after surgical removal of obstruction but also to explain failure of assisted reproduction. The present study goes a long way in explaining one important cause affecting sperm morphology which may be reversible with surgical bypass of blockage or with medication. There is a significant correlation between morphologically abnormal spermatozoa and the local presence of antisperm antibodies. This suggests the possibility that immunological factors may have a role to play in the failure of assisted reproduction techniques in these cases of obstructive azoospermia. In conclusion, therefore, and contrary to the trends presently in vogue, basic pathophysiologic changes due to causative and resultant factors need to be more extensively studied so as to increase the chances of success of any procedure for managing male infertility, especially the more advanced assisted reproductive techniques, by their proper application.

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## CORRESPONDENCE TO

Dr. A.K.Sarda 27 RPS, Triveni-1, New Delhi 110017 India Tel. : 0091-11-26011655 FAX : 0091-11-26672594 e-mail : aksarda@rediffmail.com

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## Author Information

**Anil K. Sarda, M.S.**
Professor, Departments of Surgery, Maulana Azad Medical College & Lok Nayak Hospital

**Shweta A. Bhalla, M.S.**
Resident, Departments of Surgery, Maulana Azad Medical College & Lok Nayak Hospital

**Nivedita Sarda, M.D.**
Senior Specialist, Department of Obstetrics and Gynaecology, V.M.M.College & Safdarjung Hospital

**Pramod C. Pal, Ph.D.**
Senior Research Officer, Department of Biochemistry, All India Institute of Medical Sciences

**Sudha Salhan, M.D.**
Consultant, Department of Obstetrics and Gynaecology, V.M.M.College & Safdarjung Hospital