# Scanning electron microscopy of a rare case of total multi-flagellar large-headed tetraploid spermatozoa: Impact on fertilization and embryogenesis

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## Abstract

**Introduction**
The objective of this report is to communicate the fertilization and embryogenesis characteristics in a case of total tetraploid multi-flagellar spermatozoa.
**Case Report**
The female (21yrs) had unremarkable gynecological history. The male (26yrs) has had left varicocoelectomy previously. 10 oocytes were retrieved, 5 from each side. 4 were at the matured metaphase II stage and remaining were immature. The sperm count was 800,000 per ml, 25% was moderately/sluggishly motile. ICSI was attempted on 4 matured oocytes. The sperm head was too large for the ICSI pipette. Those sperm that could be sucked into the ICSI pipette were used. During ICSI the abnormal head appeared as if it actually was a fused mass of more than one head but this could not be confirmed. Subsequent scanning electron microscopy (SEM) noted the sperm head to be completely abnormal (100%) and the tail-abnormality of above 90%. The tail was thick and either fused or appeared to be 2 to 4 tails.
**Results**
Three oocytes fertilized. All of them had 1 very large abnormal sized pronucleus (PN; ~40µ in diameter) with another 1PN of normal size of about 19 to 22 µ in diameter or 2 PNs with normal size or smaller. Scanning electron microscopy showed the spermatozoa to be multiflagellar with large irregular head. By 44 hours following ICSI, 2 zygotes were at the 2- and 3-cell stages of good quality but one did not cleave. By day 3 all embryos had cleaved to the 8-, 5- and 4-cell stages of good quality but thereafter arrested. The embryos were not transferred to the patient because of embryo abnormality.
**Discussion and Conclusion**
This case report suggests that ICSI may not be possible to treat infertility in some cases of total sperm morphological abnormality. It is clear ovulation induction in such patients should not be attempted.

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## Introduction

The process of spermatogenesis in humans differs from that of most other mammals qualitatively and quantitatively. Human spermatogenesis is distinguished from that of most other animals by lower quantitative production with higher degree of aberrant sperm

morphology. In general, human spermatogenesis is seen to be more chaotic than ordered. One interesting concept that has resulted from recent studies suggests genetic abnormalities to be common in severe male infertility (Schlegel et al., 2022).

Chromosome abnormalities are extremely common in human gametes with roughly 21% of oocytes and 9% of spermatozoa being defective. An intriguing feature of these anomalies is that most defective oocytes are aneuploid whereas most abnormal spermatozoa exhibit structural abnormality (Martin, 2008). A series of disease-causing genes are implicated as causative factors of male infertility due to teratozoospermia; which include multiple morphological abnormalities of the flagella (MMAF; Zhang et al., 2021).
One of the most typical forms of male infertility is oligoasthenoteratozoospermia (OAT). According to Jungwirth et al. (2012), it is characterized by a mix of qualitative and quantitative sperm abnormalities (Jungwirth et al., 2012). The ejaculate of patients with large-headed multiflagellar spermatozoa (MIM 243060), also known as macrozoospermia or macrocephalic sperm head syndrome, contains 100% abnormal spermatozoa, which are characterized by an oversized irregular head, an abnormal midpiece and acrosome, and multiple flagella. These patients present with primary infertility (Coutton et al., 2015).

The objective of this report is to communicate the observations on the fertilization and embryogenesis characteristics following assisted fertilization in a case of 100% sperm abnormality characterized by a large irregular head with abnormal midpiece, acrosome and multi-flaagellar spermatozoa.

## Case Report

The couple presented with primary infertility after 4 years of marriage. The female had unremarkable gynecological history whereas the male has had left varicocoelectomy two years previously. The semen report performed elsewhere indicated a 98% morphological abnormality. The couple reported having been treated twice unsuccessfully elsewhere with Clomid-induced ovulation induction. The couple sought assisted reproduction treatment at our centre recently. Following standard ovulation stimulation 10 oocytes were retrieved, 5 from each side of which 4 were at the matured metaphase II stage and the remaining were immature at metaphase I (n=2) and GV- (n=4) stages.

Semen was collected by masturbation on day of oocyte retrieval. The volume of semen was 2ml. The sperm count was 800,000 per ml of which only 25% was moderately or sluggishly motile.

The wet unstained spermatozoa preparation at time of ICSI did not reveal the actual morphology of the patient. The head appeared rather large. Many spermatozoa had either abnormally thick tails or were multi-flagellar, usually four flagella. ICSI was attempted on the 4 matured oocytes with sperm that appeared to least abnormal. The sperm head was mostly too large for the ICSI pipette. Those sperm that could be sucked into the ICSI pipette were used for ICSI. These spermatozoa had thick tails with somewhat large heads. During ICSI the abnormal head appeared as if it actually were a fused mass of more than one head and thick tails. Most spermatozoa appeared to have two heads some even four heads. Attempts to separate the fused spermatozoa using the ICSI injection pipette were not successful. The 4 injected oocytes were cultured overnight following ICSI with spermatozoa described above.

A stained specimen of the spermatozoa revealed the real condition of the specimen. The heads were completely abnormal (100%), while neck abnormality was about 30% and a very high level of tail-abnormality of above 90% (Fig. 1). The tail was either thick or the spermatozoa had 2 to 4 tails, and were grossly abnormal in comparison to control apparently normal spermatozoa specimen. The specimen was subsequently prepared for scanning electron microscopy (SEM).

## Results

The SEM revealed gross morphological abnormalities. All SEM scans were abnormal. SEM revealed severe abnormalities in the head, neck and tail of the spermatozoa units. Some spermatozoa had thick sperm tails. SEM confirmed this was due to the fusion of more

than one tail which contributed to its thick appearance. Tails were either fused together or were separate (Fig.2). Furthermore individual sperm units appeared to have more than one head fused together. Most spermatozoa had large irregular heads with up to 4 tails that were either fused or were separated.

Three of 4 oocytes fertilized. However, all of them had 1 very large abnormal sized pronucleus (PN) of about 40u in diameter plus additional PN or PNs as follows: The first zygote had one additional PN of normal size of about 19 to 22u in diameter, the second zygote had 2 additional PNs of normal size and the third zygote had additional smaller PNs probably fragments which were not clearly discernible (Fig. 4).

By 44 hours following ICSI, 2 zygotes were at the 2- and 3-cell stages of excellent to moderate quality (Range: 1=poor; 4=excellent) but 1 did not cleave. By day 3 the embryos had attained the 8-cell compacted, the 5- and 4-cell stages of excellent to moderate quality. The embryos subsequently underwent developmental arrest. No further embryo development was noted on subsequent days until day 6.

## Discussion

This is a unique case report of a male with 100% spermatozoa abnormality where more than one sperm is fused together which behaves and moves about as if it is a single sperm unit. However from the large size of the PN and also because the injected sperm had two or possibly more tails it is obvious that more than one to four spermatozoa heads were present in a single sperm unit injected into the oocyte. The very large PN noted in all three zygotes could be 4 PNs from the four spermatozoa fused together. Due to ethical consideration we could not perform SEM on human embryos to study the embryogenesis in greater detail in these embryos.

The embryos that developed following ICSI were not transferred to the patient because of (i) abnormal PNs detected, (ii) the probable polypoidy and (ii) because embryo had undergone developmental arrest. The couple was counseled as appropriate.

**Fig 1: Stained specimen of abnormal sperm with abnormal morphology**
[Most sperm have more than one tail and head fused together some as many as four tails and possibly four heads ]

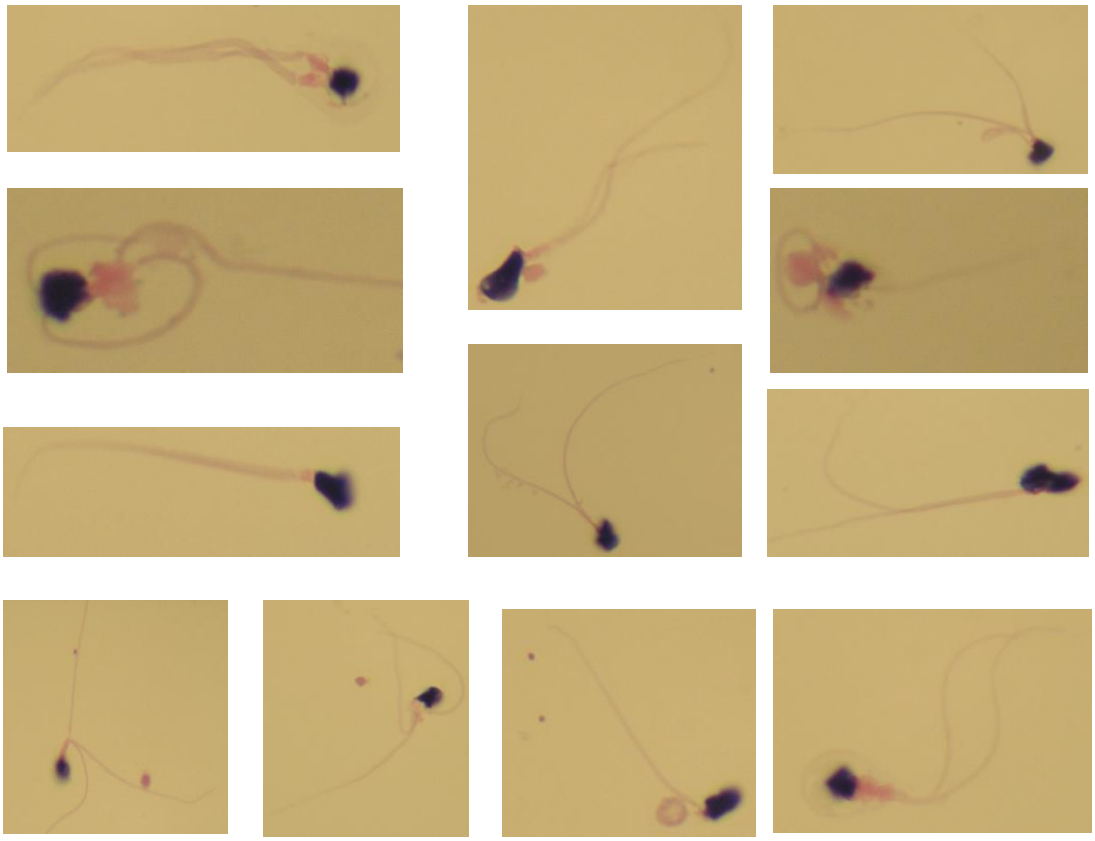


**Fig 2: Electron microcography of abnormal sperm morphology**
[Most sperm have more than one tail and head fused together some as many as four or more tails and possibly four heads as seen in the bottom three images]

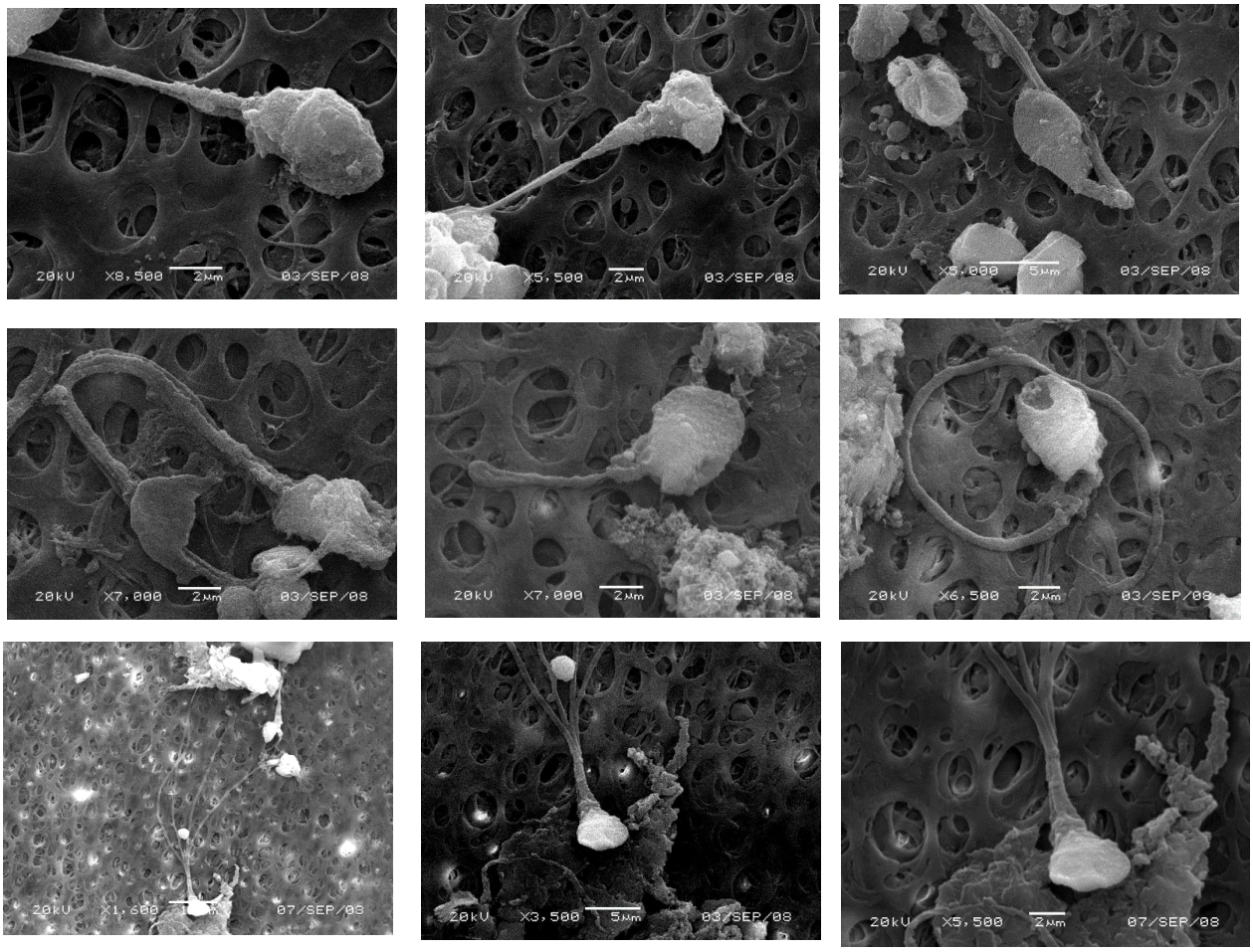
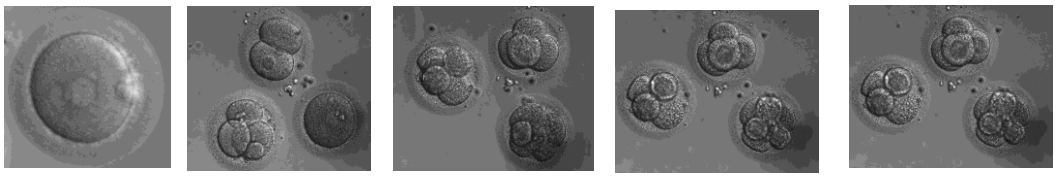



**Fig. 3 Abnormal Preimplantation Embryo Development (fertilized by abnormal sperm**)



Day 1 Day 2 Day 3 Day 4 Day 5

The egg when fertilized by spermatozoa with more than one head and tail results in zygotes with one very large PN [about 40µ] and another 1 normal sized PN [about 22µ] and one or more smaller faint PNs. The abnormal zygotes developed normally albeit slowly until day 3. These abnormal embryos did not develop beyond day 3.

It is of interest to note that in spite of gross morphological abnormalities motor activity of the sperm was still present in 25% of the sperm population which may have misled previous treatment options elsewhere into considering ovulation induction which is inappropriate in this case. Of relevance to the present report is the observation of Cantu et al. (1981) that noted the presence of maturation arrest in 3 of 13 brothers from a consanguineous marriage, suggesting the possibility of an autosomal recessive genetic defect that could be causal for disordered spermatogenesis (Cantu et al., 1981). Consanguineous marriage is common in the Arab population.

The ultrastructural studies of Escalier revealed a 3-fold increase in nuclear volume. It also noted on an average 3.6 flagella per sperm head (Escalier, 1983). This defect was first reported by Nistal and coworkers (Nistal et al., 1977). There do not appear to be any way by which this condition can be ameliorated in the present times. However, the removal of the extra PNs by microsurgical enucleation in zygotes fertilized by these tetraploid spermatozoa may help overcome polyploidy in the zygote, as previously reported for 3PN zygotes (Kattera and Chen, 2003; Sultan et al., 2004) but its safety is questionable. This condition may be a manifestation of consanguineous marriage.

## Conclusion

This case reports suggest that ICSI may not be helpful in alleviating infertility in some cases of total sperm morphological abnormality, in particular in spermatozoa exhibiting polyploidy due to fusion of more than one spermatozoa. It is also very clear ovulation induction in such patients should not be attempted until such time this condition could be ameliorated. The present report contributes to our knowledge base on the sperm morphological abnormalities that occur in the human and cellular processes that occur during abnormal embryogenesis that occur as a consequence of fertilization with abnormal polyploid fused spermatozoa.

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