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CASE REPORT:

Pregnancy with spermatozoa from a globozoospermic man after intracytoplasmic sperm injection treatment

K.M.Trokoudes[1,3], N.Danos[1], L.Kalogirou[1], R.Vlachou[1], T.Lysiotis[1], N.Georghiades[1], S.Lerios[1] and K.Kyriacou[2]

[1]'Pedieos' IVF Center, 8 Karaoli St., Suite 201, Nicosia 162, and [2]The Cyprus Institute of Neurology and Genetics, P.O. Box 3462, Nicosia, Cyprus

[3]To whom correspondence should be addressed

We present a case of third trimester pregnancy occurring in a 29 year old woman after intracytoplasmic sperm injection (ICSI) treatment with spermatozoa from a globozoospermic man. We believe this is the first reported case. A 37 year old man was diagnosed with globozoospermia with normal sperm count and motility. In-vitro fertilization and subzonal insemination treatments failed to achieve fertilization of any eggs. The ICSI method produced 50% fertilization, 75% cleavage rate and a singleton pregnancy with a female fetus. No pregnancy or fetal abnormalities have been noted after >7 months of gestation.

Key words: globozoospermia/ICSI/infertility/normal pregnancy/transmission electron microscopy

Introduction

Globozoospermia is a rare condition of unknown mode of inheritance which is characterized by the complete absence of the acrosome, disorganized mid-piece, lack of zona binding and infertility (Aitken *et al.*, 1990). Because of the above, classical in-vitro fertilization (IVF) has not been successful in fertilization. Subzonal insemination (SUZI) has similarly been unsuccessful in producing sperm–oocyte fusion (Dale *et al.*, 1994). The new technique of intracytoplasmic sperm injection (ICSI) involves the direct deposition of the spermatozoon in the cytoplasm of the oocyte (Palermo *et al.*, 1993). It is a much more powerful method of fertilization than IVF or SUZI in cases of severe sperm problems. It was therefore natural for us to apply this method of fertilization to globozoospermic cases once the opportunity arose.

Case report

History

This couple presented to us with a 4 year history of infertility. The 27 year old woman had a complete infertility work-up: regular ovulating cycles, normal hormone profile, normal hysterosalpingogram and laparoscopy.

The 36 year old husband underwent a varicocelectomy for a left-sided varicocele in 1991. Various non-specific drug treatments were administered from time to time, such as mesterolone, folic acid and multi-vitamins. He had a negative medical history and a normal physical examination, including normal-sized testicles.

Laboratory studies

The initial spermogram showed a sperm count of 25×10^6/ml with 50% motility. About 40% of the spermatozoa had a grade 3 and 4 motility rating, grade 4 being excellent. Sperm antibody testing with the mixed agglutination reaction (MAR) method was negative for any antibodies. Morphological studies using light and transmission electron microscopy (TEM) and cytological differentiation of seminal smears showed 100% round-headed spermatozoa. Mini-Percoll washing produced grade 4 motility spermatozooa which were also all round-headed.

For morphological analysis two sperm samples, collected on separate occasions, were examined by both light and TEM. For ultrastructural examination, fresh ejaculate was fixed in 3% glutaraldehyde and post-fixed in osmium tetroxide.

The most consistent morphological finding noted was the uniform presence of rounded heads and the complete loss of acrosome (Figures 1 and 2). No evidence of an acrosomal structure, or remnants of it, was obtained following the ultrastructural examination of several hundred spermatozoa. Other abnormalities included deranged inner and outer nuclear membranes, swelling of midpieces, frequent lack of mitochondria and disturbances in the microtubular structure. Clear vacuoles were frequently seen in the nuclei (Figure 2).

Fertilization attempts

Classical IVF and SUZI were both attempted once, without any fertilization of the oocytes. No binding of the spermatozoa to the zona was noted in IVF.

In February 1994, fertilization with ICSI was attempted. The ovaries were stimulated using the ultra-short protocol (buserelin 0.5 ml s.c. on days 2, 3, 4 and 5 of the present menstrual cycle). Six oocytes were prepared for ICSI according to the published method of Palermo *et al.* (1993). No tail-touching of the spermatozoa was done prior to aspiration and injection into the ovum cytoplasm. The culture medium after the microinjection was Earle's medium supplemented with 10% patient's serum.

Three oocytes were fertilized, two cleaved normally and developed to the 6-cell and 8-cell stage respectively at 48 h. The embryo quality was excellent: no fragments and equal

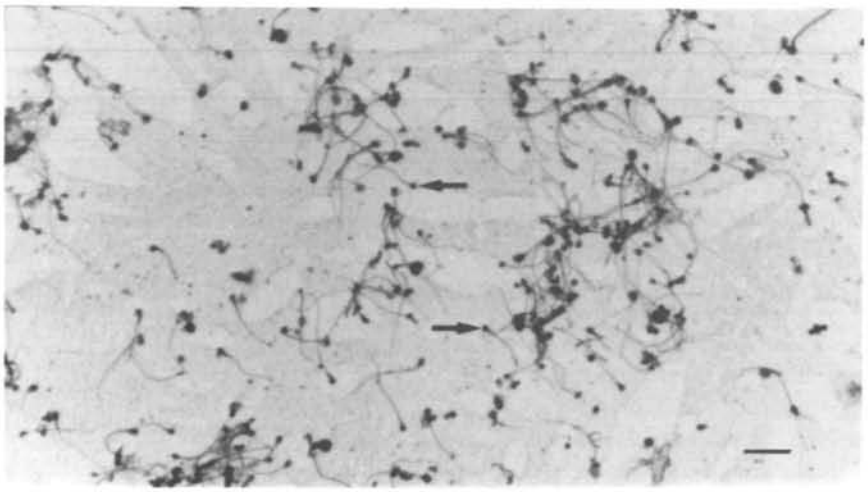

Figure 1. Light micrograph showing round-headed spermatozoa. Note absence of acrosomes (arrows). Bar = 8 μm.

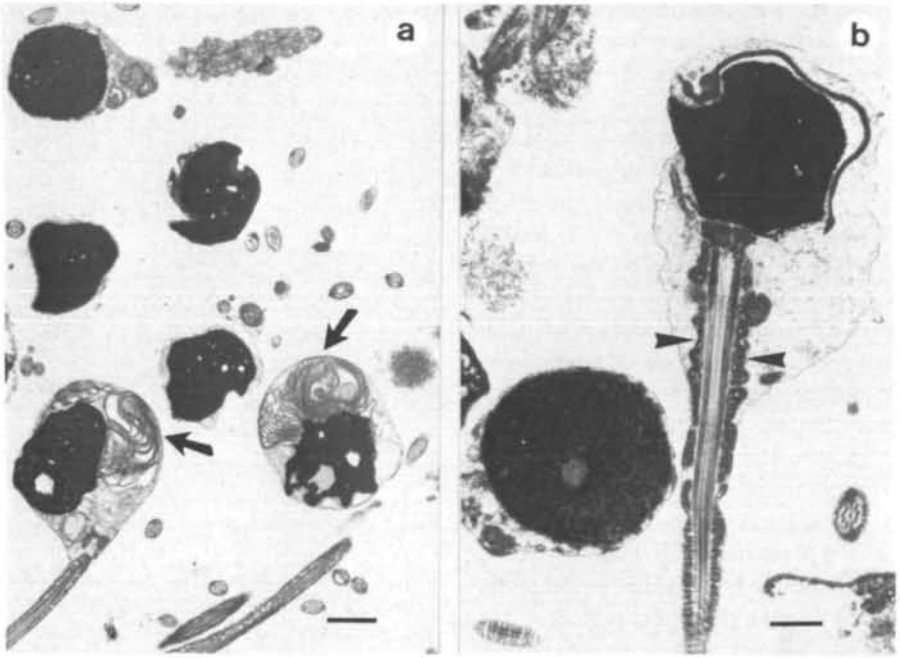

Figure 2. Transmission electron micrographs showing several acrosomeless spermatozoa. (**a**) In some heads the nuclear membranes are swollen and appear highly laminated (arrows). Bar = 0.9 μm. (**b**) Round-headed spermatozoa with one exhibiting a normal midpiece enclosed by mitochondria (arrowheads). Bar = 0.6 μm.

blastomeres. On 11 March, 1994, two embryos were transferred via the cervix; a singleton pregnancy was achieved.

Progress of pregnancy

Oestradiol, progesterone and β-human chorionic gonadotrophin (HCG) concentrations were followed almost weekly for 11 weeks. There was a normal rise of all three hormones. An ultrasound examination at 11 weeks of gestation was normal. The triple test (maternal serum biochemistry screening: β HCG, αFP, Estriol) was also normal. A new ultrasound at 21 weeks was also completely normal. The conceptus was female.

Discussion

Globozoospermia is not an incidental sperm abnormality but a well-defined clinical entity (Pederson and Pebbe, 1974). It is characterized by the complete absence of an acrosome vesicle, disorganization of the midpiece and inability to bind the human zona pellucida and produce sperm–oocyte fusion. These abnormalities are found in all the spermatozoa of the patient's ejaculate. Other sperm abnormalities have been described in the area of the nucleus (nuclear membrane and chromatin condensation disorders) and the tail. It is a rare condition, with only 20 cases reported up to 1988 (Lalonde *et al.*, 1988). It probably occurs in 0.1% of all andrological

patients. The mode of inheritance has not been established and various reports include a polygenic and polymorphic mode, a monogenic mode, a dominant inheritance and a homozygous autosomal gene defect. In view of the various structural and ultrastructural abnormalities in globozoospermia, there has been considerable scepticism on the possibility of achieving a pregnancy and a normal fetus. Indeed, attempts to produce fertilization using acrosomeless spermatozoa from our patient have failed using both the classical IVF and SUZI methods.

The ICSI method has revolutionized the treatment of male subfertility (Palermo *et al.*, 1993). Fertilization and pregnancies have been achieved using both normal and abnormal-looking spermatozoa (Van Steirteghem *et al.*, 1993), epididymal spermatozoa (Tournaye *et al.*, 1994) and also testicular spermatozoa (Schoysman *et al.*, 1993). No significant increase in fetal abnormalities has been recorded as yet (Bonduelle *et al.*, 1994). However, to our knowledge, no term pregnancies from such a distinct clinical abnormality as globozoospermia have yet been reported. In view of the well-described sperm abnormalities in this entity, it is significant to determine their effect on the genetic make-up of the embryo and its subsequent development.

Our patient had a completely normal progression of her pregnancy. The concentrations of oestradiol, progesterone and β-HCG were not different from the rest of our IVF pregnancies. Two very careful ultrasound assessments and triple testing showed a normally developing pregnancy up to the seventh month of gestation. Amniocentesis was refused by the patient.

Our patient became pregnant from the first attempt of ICSI. The fertilization and cleavage rates were not different from other ICSI treatments in our centre. The limited experience with globozoospermia means that the fertilization and cleavage rates after ICSI are not known. However, anecdotal reports from various centres point to rates not different from the general ICSI experience, and our case reinforces this notion. As this is the first time a globozoospermic man will have offspring, our attention is naturally now turned to detecting any abnormalities of this baby in the post-natal period.

In summary, a 29 year old woman became pregnant with the ICSI method using her husband's acrosomeless, round-headed spermatozoa, and progressed to term without evidence of any embryo abnormalities.

Addendum: On November 17, 1994, a completely normal baby girl of 2700 g was born by Caesarean section.

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