CASE REPORT

Congenital malformations after intracytoplasmic injection of spermatids

H. Zech1,5, P. Vanderzwalmen2, Y. Prapas3, B. Lejeune2, E. Duba4 and R. Schoysman2

1 Institut für Reproduktionsmedizin und Endokrinologie, Bregenz, Austria, 2 Schoysman Infertility Management Foundation, Vilvoorde, Belgium, 3 4th University Clinic of Obstetrics and Gynecology, Hippocratie General Hospital, Thessaloniki, Greece, and 4 Institut für Medizinische Biologie und Humangenetik der Universität Innsbruck, Innsbruck, Austria

5 To whom correspondence should be addressed at: Römerstrasse 2, 6900 Bregenz, Austria

Spermatid microinjection into oocytes was applied in cases of intracytoplasmic sperm injection (ICSI)/testicular sperm extraction (TESE) where no spermatozoa could be found in numerous testicular samples. Although several pregnancies were obtained with this procedure, serious concerns remain regarding its safety. Although the relevance of the injection of spermatids is by no means certain, we wish to report that from four pregnancies obtained after injection of elongated spermatids, two cases of major malformation resulted.

Key words: azoospermia/genetics/ICSI/ malformation/ spermatids

Introduction

As has already been suggested (Edwards et al., 1994), one option to treat patients with total absence of spermatozoa in the ejaculate or in the testicular tissue consists in the injection of spermatids into the cytoplasm of metaphase II oocytes.

This technique has generated worldwide interest. Until now, the births of only three and nine healthy babies respectively after injection of round (ROS) and elongated spermatids (ELS) have been reported (Fishel et al., 1995, 1997; Vanderzwalmen et al., 1995, 1997; Chen et al., 1996; Mansour et al., 1996; Tesarik et al., 1996; Antinori et al., 1997a,b; Araki et al., 1997; Kahraman et al., 1998; Sofikitis et al., 1998). Aside from the low success rate obtained after human spermatid injection, one of the least known problems with this procedure concerns its safety. We report two major malformations out of four pregnancies obtained after intracytoplasmic injection (ICSI) of ELS retrieved from 14 patients suffering from non-obstructive azoospermia.

Case report I

The first couple (woman aged 31 years and man aged 38 years) was referred to the centre because preliminary examination showed fluctuating sperm production in the past with occasionally no sperm production. At the time of oocyte aspiration, no spermatozoa could be found either in the ejaculate or in several testicular biopsies. In the latter, ELS (at Sd1 stage of spermatid development) (de Kretser and Kerr, 1988) were isolated and injected into seven metaphase II oocytes. After 18 h, all the oocytes were normally fertilized, and four good quality embryos (grade A) were obtained and transferred on day 2. After a positive pregnancy test, the pregnancy progressed normally and the ultrasound showed a normally developing fetal sac. Amniocentesis was proposed to the couple but they refused, in spite of extensive genetic counselling. On week 20 of the gestation, a hydrocephalus was diagnosed at ultrasound and the pregnancy was terminated. Despite a normal karyotype in the parents and a family history free of chromosomal anomalies, cytogenetic analysis of the fetal tissues showed a male with trisomy 9 (47,XY,+9) in all amniotic cells. The histopathological examination showed a hydrocephalus, spina bifida and diaphragmatocele.

Case report II

The second couple with a normal family medical history (woman 25, man 38 years old, both with normal karyotype), was referred because of azoospermia with tubular atrophy. A preliminary testicular biopsy of both testes showed diffuse tubular atrophy with a stop of the spermatogenesis at the stage of spermatocytes (mean Johnson score: 5.22 right and 5.26 left). As no spermatozoa could be found in several testicular biopsies at the time of oocyte aspiration, elongated spermatids were injected, on this occasion into 10 metaphase II oocytes. After 18 h, six oocytes showed two pronuclei, four of them cleaved (two grade A, one grade B and one grade C) and were transferred on day 2. Despite genetic counselling, the couple did not agree to an amniocentesis. Three ultrasounds were performed away from our institute. After an uneventful pregnancy, a boy (46, XY) was born at term (weight 2800 g, length 48 cm) by spontaneous delivery (Apgar score: 8–9–9) and had an open lumbosacral myelomeningocele (Arnold Chiari Syndrome type II) which had not been detected during the pregnancy.

Discussion

This is a first report on malformations observed in pregnancies obtained through the injection of ELS, but no firm conclusions can be drawn since both aberrations have
also been observed after spontaneous conception and in IVF with or without ICSI. It is not yet known if spermatid injection itself or the basic cause of infertility in these male patients or an unknown genetic disposition might have given rise to our observations.

To be sure that the result is of any importance in relation to ICSI, for the first case we have to find out by DNA studies whether the additional chromosome 9 is of paternal origin. We might suggest that probably the multiple congenital anomalies are the result of a rare de-novo chromosomal aneuploidy. In the second case, no underlying cause was identified for the neural tube defect.

Data from animal studies have shown that chromosomes of spermatids retrieved from fertile and healthy animals are potentially capable of pairing with chromosomes of oocytes and participating in syngamy and full embryonic development (Ogura et al., 1994; Kimura and Yanagimachi, 1995; Ogura and Yanagimachi, 1995; Sofikitis et al., 1996), suggesting that such cells can provide the paternally imprinted genes needed for embryonic development. It remains mandatory that careful investigations on gene expression and genomic imprinting of cells at various stages of spermatogenesis should be undertaken, especially with immature cells from infertile patients. Caution is still needed with the use of immature testicular cells since diseases linked to imprinting would be detectable only during later development.

During spermatogenesis in fertile animal models, the transition of histone to protamine allows a better protection of the ELS DNA from chemical or physical denaturation. However, in patients with spermiogenesis anomalies, abnormal DNA packaging is observed due to a lack of exchange of histone to protamine and, in consequence, problems with chromatin remodelling, abnormal DNA methylation and increased sensitivity of DNA to damage (Jurisicova et al., 1999).

Even when the somatic karyotype appears normal, we are obliged to inform the couple as clearly as possible of the risks to be expected. It would be helpful to analyse the chromosome status of the germ cells on a routine basis in order to avoid high-risk situations. In fact, there can still be risks for meiotic errors arising in the germinal cells of men with severely impaired spermatogenesis (Chandley and Hargreave, 1996; Lange et al., 1997).

Our own accumulated ICSI results (unpublished) show that the incidence of malformation with ejaculated (1.7%) or testicular (0%) spermatids is similar to that of conventional IVF. In spite of its potential risks, ICSI still seems to be remarkably safe (Edwards, 1999). Recently, a follow-up study of children born after ICSI with epididymal and testicular spermatids showed no additional risk when compared with standard IVF procedures (Bonduelle et al., 1998). In the case of spermatid injection, further data are needed to determine whether congenital malformations occur more often.

Before going ahead with an attempt, the patients should be advised of the risks and the extremely low efficacy of the procedure and convinced about the need for an amniocentesis. The risk of transmission of chromosomal aberrations, of de-novo chromosomal aberrations, of genetic transmission of Y chromosome deletions and of genomic imprinting anomalies should not be overlooked and care should be taken to avoid the serious consequences of such pathologies.

After un promising results and in consideration of potentially high rates of malformation, we have postponed attempts for this treatment for the time being. We suggest that any continuation of work in this area should be managed very carefully (Vanderzwalmen et al., 1998).

**References**


Congenital malformations after injection of spermatids


Received on May 25, 1999; accepted January 18, 2000