CASE REPORT

Failure of pregnancy after intracytoplasmic sperm injection with decapitated spermatozoa

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Case report

At the time of consultation in our centre, the patient and his wife were 33 and 31 years old respectively; they had been unable to conceive over a period of 5 years. The woman had a normal clinical history and presented normal characteristics on examination, with regular menses, normal hysterosalpingography and hormonal assessment. The karyotypes of both individuals were normal. The man presented a normal male phenotype and no history of significant illness. Physical examination revealed no particular abnormality. Analysis of two semen samples collected by masturbation after 3 days of sexual abstinence showed sperm concentrations of 12.6 and 29.8 x 10⁶ spermatozoa/ml. Direct light microscopic analysis showed numerous isolated motile tails and fewer isolated heads. In the two samples respectively, among intact spermatozoa, 60% and 80% were motile, with 5% and 20% showing rapid progressive motility. Most of the intact spermatozoa had a bent tail. Morphological examination of spermatozoa after Shorr staining confirmed a high rate of teratozoospermy (84% and 99%, according to the 1992 WHO criteria) with a predominance of isolated heads (46% and 84%) and bent tails (32% and 20%). Seemingly normal-shaped acrosomes were seen in the great majority of spermatozoa analysed (81% and 87%). Aniline blue staining revealed a normally condensed chromatin.

Ultrastructural investigations revealed the presence of numerous headless tails, which in all cases carried the proximal centriole and the segmented columns (Figure 1a); the break always occurred between the caputulum and the posterior nuclear pole. The rare intact spermatozoa all had a bent tail (Figure 1b). Longitudinal sections through the heads showed a normal shaped nucleus, with the exception of the caudal pole, which revealed an absence of the implantation fossa and most obvious ultrastructural anomaly is the absence of the implantation fossa and of the basal plate. This type of pathology causes the sperm neck to weaken and break, which in turn prevents the spermatozoon from progressing along the female genital tract or fertilizing eggs.

Here we report the case of an infertile couple in which the male presented with decapitated spermatozoa. This couple underwent one conventional in-vitro fertilization (IVF), three subzonal inseminations (SUZI) and four intracytoplasmic sperm injection (ICSI) attempts. Each ICSI attempt resulted in the transfer of embryos with good morphological characteristics, but no pregnancy occurred. The significance of these results is discussed here.

Introduction

The presence of a structural abnormality which affects the whole sperm population, or a high proportion of it, is relatively uncommon in man. Among these abnormalities, only 10 cases of decapitated spermatozoa have already been described (Luders, 1976; Perotti et al., 1981; Holstein et al., 1986; Chemes et al., 1987; Bacceti et al., 1989; Toyama et al., 1995). The pathogenesis of this syndrome is not clear, but the

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basal plate. Longitudinal and transverse sections of the tails appeared to be normal.

The couple had previously undergone five IVF attempts in an other centre, including one classic IVF attempt where 10 mature oocytes were retrieved but no embryo was obtained. The other IVF attempts comprised three SUZI and one ICSI. In data pooled from these four attempts, 26 oocytes were retrieved, 24 of which were mature and therefore injected.

Seven embryos were in this way obtained and transferred, but no pregnancy was achieved. The quality of the transferred embryos is not known.

Three ICSI operations were performed in our reproduction centre (Table I). After pituitary desensitization with leuprolcline (Enantone 3.75 mg; Takeda, Puteaux, France), the patient’s wife was stimulated using follicle stimulating hormone (FSH) (Metrodine HP, 48 ampoules; Serono, France). Oestradiol plasma levels and follicle growth were monitored every 2 days and human chorionic gonadotrophin (HCG) (Organon, St Denis, France) was administered after 12 days of stimulation. Oocyte retrieval was performed 36 h after HCG injection. In all, 27 oocytes were retrieved (Table I). Spermatozoa were separated from the seminal plasma after a mini swim-up procedure, which consisted of two washes of the spermatozoa in Eppendorf tubes with 1 ml of M1 medium (Bicef, l’Aigle, France), followed by a 15 min semi-horizontal migration in 20 µl of B2 medium (Bio Merieux, Montalieu, France). The suspension contained a few complete spermatozoa with bent tails, but numerous isolated tails with progressive motility. The excessive weakness of the sperm neck frequently caused the spermatozoon to break in the microinjection pipette. With gentle motions, however, we successfully injected an entire spermatozoon into each of 20 oocytes. Five oocytes were injected with a spermatozoon whose head and tail separated in the microinjection pipette. In the three attempts conducted in our centre, 18 embryos were obtained, 10 were transferred and three were frozen at the seven- to eight-cell stage, after 72 h. These three embryos were thawed and transferred in one induction cycle later, using clomifene (Clomid 500 mg; Marion Merrel, Puteaux, France) and FSH (Metrodine, three ampoules; Serono). At transfer, the embryos were graded according to morphological criteria previously described (Saıás-Magnan et al., 1993). All embryos transferred were of a regular size and shape, with 0–10% blastomeric fragmentation, the number of blastomeres ranging from four to eight. After each embryo transfer, the HCG plasma concentration was always negative.

**Discussion**

The present case is the first report of ICSI using spermatozoa from a man presenting with the decapitated spermatozoa syndrome. This pathology is responsible for male infertility, and embryos cannot be obtained without assisted fertilization. In the three ICSI attempts performed in our laboratory, only head and tail from the same spermatozoon were injected, thus giving a cleavage rate similar to that of our other ICSI cases.

<table>
<thead>
<tr>
<th>Year of ICSI attempt</th>
<th>Oocytes retrieved</th>
<th>Mature oocytes</th>
<th>Embryos obtained</th>
<th>Frozen</th>
<th>Transferred</th>
<th>Pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 (1995)</td>
<td>9</td>
<td>8</td>
<td>4</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>T2 (1996)</td>
<td>9</td>
<td>9</td>
<td>8</td>
<td>3</td>
<td>3 + 3*</td>
<td>0</td>
</tr>
<tr>
<td>T3 (1997)</td>
<td>9</td>
<td>8</td>
<td>6</td>
<td>0</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

*Embryo frozen and transferred after thawing one stimulation cycle later.
Pregnancy failure with decapitated spermatozoa

(75%). No pregnancy occurred, despite the transfer of good quality embryos. The reasons for these failures are unclear, but are probably related to the sperm anomaly. Indeed, this pathology partially confirms a previous experiment (Palermo et al., 1997), in which the authors assessed the ability of oocytes injected with a physically separated sperm segment (head only, head and tail separated, or tail only) to undergo normal embryonic development. In one study (Palermo et al., 1997), oocytes were monitored for cleavage for up to 72 h, and embryos were analysed by fluorescent in-situ hybridization (FISH). All of the embryos obtained after injection of a separated head and tail showed chromosome mosaicism.

One explanation for the absence of pregnancy in the present couple is that the embryos transferred may carry a chromosomal imbalance and are therefore incapable of progressing to the blastocyst stage. The mechanism that causes this chromosomal mosaicism among the blastomeres is unclear. The most likely explanation is that the spine becomes defective as a result of the abnormal behaviour of the centrosome, and cannot therefore ensure normal segregation of the chromosomes at each mitotic division of the embryo. Indeed, this study (Palermo et al., 1994) provides evidence that the human sperm centrosome controls the first mitotic divisions after fertilization. The interaction of the centrosome and nucleus appears to be important during spermatogenesis and fertilization, where the centriole often remains in close contact with the decondensing sperm nucleus. This structural association has obvious functional implications for cell polarity, as well as for cell divisions (Omura and Fukui, 1985). The fact that fertilization of oocytes with physically separated sperm segments leads to mosaic embryos (Palermo et al., 1997) suggests that physical disruption of the sperm neck in decapitated spermatozoa compromises the ability of the centrosome to function normally in the zygote.

The origin of this syndrome is very probably genetic, since a case of two brothers bearing the same pathology has been reported (Baccetti et al., 1989). The same defect has also been reported in cattle (Blom and Birch-Andersen, 1970) and pigs (Toyema and Itoh, 1996). The mechanism which gives rise to this anomaly is discussed. It has been suggested (Holstein et al., 1988) that the proximal centriole, which induces the development of the basal plate and the implantation fossa, is responsible for its own anchorage to the nucleus. An overproduction of vesicles arising from the Golgi complex was described (Bacetti et al., 1984) in a human with decapitated spermatozoa, and it was proposed that this had interfered with the attachment of the centriole to the nucleus. This overproduction of vesicles was not observed in the present case, and thus the sperm defect observed may be due to the dysfunction of the sperm centrosome (Van Blerkom and Henry, 1991). Our observation support previous conclusions (Perotti and Gioria, 1981; Perotti et al., 1981; Chemes et al., 1987) that the proximal centriole may be unable to induce development of the basal plate and the implantation fossa, either because it is prevented from moving to the caudal pole of the nucleus, or because of a functional anomaly.

Thus, the arrest of embryonic development could be related either to the presence of a functionally abnormal centriole, or to the loss of interaction between the nucleus and the proximal centriole during spermatogenesis and fertilization. In the first hypothesis, infertility may result from a defect in centrosome reconstitution following fertilization: the centrosome may be unable to bind γ-tubulin and other centrosome-associated proteins for some, but not all, division cycles (Simerly et al., 1995). In the second hypothesis, the developmental arrest may result from the disturbance of the sperm aster function, which is essential for the physical union of the male and female pronuclei. Abnormal distribution of chromosomes between blastomeres could result from this abnormal spindle and would explain the precocious degeneration of the embryos before implantation.

The most likely explanation for the absence of pregnancy in this couple is that the embryos’ chromosomal imbalance prevents their progression to the blastocyst stage. A high incidence of mosaicism in humans has been reported (Palermo et al., 1998) in other conditions. FISH analysis of the embryos derived from the reactivation of unfertilized oocytes by injection of sperm cytosolic factor showed an abnormal chromosomal complement of the blastomeres. Nevertheless, it is impossible to exclude the possibility that the wife is responsible for the implantation failure. Indeed, a case has been reported where pregnancy and delivery of a healthy baby resulted after two ICSI cycles using only sperm heads (Tucker et al., 1996), but these contained the centrosome. Despite seven unsuccessful assisted fertilization attempts, the patient and his wife remain highly motivated, and an ICSI with co-culture is underway. This will enable us to evaluate the ability of the embryos to progress to the blastocyst stage. Further reports of similar ICSI cases using decapitated spermatozoa will be required, in order to elucidate the relationship between the sperm defect and the reproductive failure of these patients. In the large majority of cases, severe sperm defects have no predictive value with regard to the success of ICSI and do not impair the fertilization process (Küpker et al., 1998). Nevertheless, the present defect is perhaps an example of one of the rare limits to the ICSI process.

Since this pathology might have a genetic origin, patients must be informed of the transmission risk, but genetic counselling is difficult because the mutations involved are unknown. In order to allow further characterization of the molecular basis of this centriolar pathology, genomic DNA has been isolated from this patient.

References


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