Reproductive BioMedicine Online (2015) 31, 89–99

www.sciencedirect.com
www.rbmonline.com

ARTICLE

Evaluation of sperm nuclear integrity in patients with different percentages of decapitated sperm in ejaculates

Christine Rondanino [a,b,*], Véronique Duchesne [a,b], Denise Escalier [c], Fanny Jumeau [a,b], France Verhaeghe [a,b], Marie-Claire Peers [d], Valérie Mitchell [a,d], Nathalie Rives [a,b]

[a] *EA 4308 'Gamétogenèse et qualité du gamète', Rouen, France;* [b] *Laboratoire de Biologie de la Reproduction – CECOS, CHU – Hôpitaux de Rouen, Institut de Recherche et d'Innovation Biomédicale, Université de Rouen, Rouen, France;* [c] *Institut National de la Santé et de la Recherche Médicale U933, Hôpital Armand-Trousseau, Université Pierre et Marie Curie-Paris 6 and Assistance Publique–Hôpitaux de Paris, Paris, France;* [d] *Institut de Biologie de la Reproduction – Spermiologie – CECOS, CHRU de Lille, Lille, France*

* Corresponding author. *E-mail address:* christine.rondanino@chu-rouen.fr (C Rondanino).

Dr. Christine Rondanino is currently research engineer in the Reproductive Biology Laboratory at Rouen University Hospital (France). She obtained her Ph.D. degree in 2003 at the University of Orléans (France). Her research interests focus on male infertility and spermatogenesis since 2011.

Abstract The decapitated sperm defect is a rare type of teratozoospermia responsible for male infertility. Spermatozoa from patients affected by this syndrome are used for intracytoplasmic sperm injection (ICSI) although little is known about their DNA integrity. This study evaluated sperm nuclear alterations in four patients and ten fertile men (control group). Sperm samples were examined by light, transmission electron and high-magnification contrast microscopy and analysed after terminal deoxynucleotidyltransferase-mediated dUTP nick end labelling, aniline blue staining and fluorescence in-situ hybridization. Spermatozoa from patients presented varying degrees of decapitation, along with morphological and ultrastructural head abnormalities. Whereas the proportion of spermatozoa with fragmented DNA and numerical chromosome abnormalities was similar in patients 1–3 and controls, the percentage of spermatozoa with hypocondensed chromatin was higher in patients 1–3 than in fertile men. Patient 4 presented a distinct phenotype, with an increased proportion of flagellated spermatozoa with DNA strand breaks as well as increased aneuploidy and diploidy rates compared with controls and with patients 1–3. No successful pregnancy resulted from ICSI although embryos were obtained for three patients. The morphological defects and the nuclear alterations observed in spermatozoa of patients with the decapitated sperm syndrome may have contributed to ICSI failures.

http://dx.doi.org/10.1016/j.rbmo.2015.04.002
1472-6483/

KEYWORDS: aneuploidy rates, chromatin condensation, decapitated spermatozoa, DNA fragmentation, sperm nucleus

Introduction

Teratozoospermia, defined as the presence of a high percentage of morphologically abnormal spermatozoa in semen (WHO, 2010), is frequently responsible for male infertility. There are two main types of teratozoospermia: (i) polymorphic teratozoospermia, characterized by numerous and heterogeneous alterations in the shape of the sperm head, midpiece and/or tail; and (ii) monomorphic teratozoospermia, in which a systematic and homogenous defect is present in most spermatozoa. To this category belong the dysplasia of the fibrous sheath, primary ciliary dyskinesia, sperm macrocephaly syndrome, globozoospermia, miniacrosome sperm defect and decapitated sperm defect.

The decapitated sperm syndrome, in which numerous isolated flagella are found in semen, has been reported in bulls (Blom and Birch-Andersen, 1970), boars (Toyama and Itoh, 1996) and in several cases of infertile men (Baccetti et al., 1984; Chemes et al., 1987, 1999; Gambera et al., 2010; Holstein et al., 1986; Perotti and Gioria, 1981; Perotti et al., 1981; Porcu et al., 2003; Saïas-Magnan et al., 1999; Toyama et al., 1995). The syndrome was called decapitated and decaudated sperm defect when both isolated tails and heads were observed in semen (Baccetti et al., 1989; Toyama et al., 2000). In some patients, spermatozoa with abnormal head-midpiece alignment were present in the ejaculates (Chemes et al., 1999; Porcu et al., 2003; Rawe et al., 2002). In others, semen analysis was normal but spermatozoa became easily decapitated when micromanipulated (Emery et al., 2004; Kamal et al., 1999). It has been proposed that all these variants are related and represent different degrees of alterations of the head-neck junction, the decapitation of most or all spermatozoa being the most extreme situation (Chemes and Rawe, 2010; Chemes et al., 1999). Since these disorders have been reported in brothers and in consanguineous patients (Baccetti et al., 1989, 2001; Chemes et al., 1999; Porcu et al., 2003), they are likely to be of genetic origin. The presence of mosaic forms, e.g. association of abnormal head-neck connection with round heads and poor acrosome formation (Aughey and Orr, 1978) or with dysplasia of the fibrous sheath (Moretti et al., 2011; Rawe et al., 2002), have also been described.

Ultrastructural studies have shown that in the neck region, the proximal centriole fails to attach to the caudal pole of the sperm nucleus (Chemes et al., 1987, 1999; Perotti and Gioria, 1981; Porcu et al., 2003; Saïas-Magnan et al., 1999; Toyama et al., 2000). Indeed, the implantation fossa and the basal plate, which lodges and anchors the centriole, are lacking (Baccetti et al., 1989; Chemes et al., 1987, 1999; Kamal et al., 1999; Perotti and Gioria, 1981; Perotti et al., 1981; Saïas-Magnan et al., 1999; Toyama et al., 1995, 2000). As a result, heads and flagella develop independently (Chemes et al., 1987, 1999; Holstein et al., 1986; Perotti and Gioria, 1981) and eventually separate. In most cases, the cleavage occurs between the nucleus and the centriolar region (Baccetti et al., 1989; Chemes et al., 1987, 1999; Perotti and Gioria, 1981; Perotti et al., 1981; Saïas-Magnan et al., 1999; Toyama et al., 1995, 2000). The proximal centriole appears structurally normal (Chemes et al., 1987; Rawe et al., 2008), suggesting that its inability to migrate towards the nucleus or its dysfunction may be responsible for the sperm defect. Tailless heads present in the testis are probably phagocytosed by Sertoli cells (Chemes et al., 1999), explaining why they are rarely found in semen (Chemes et al., 1987, 1999; Perotti et al., 1981; Toyama et al., 1995).

Whereas successful pregnancies have been obtained by intracytoplasmic sperm injection (ICSI) with easily decapitated spermatozoa (Emery et al., 2004; Kamal et al., 1999), the first attempts using spermatozoa with head-midpiece misalignment led to fertilized oocytes that failed to undergo normal syngamy and cleavage (Chemes et al., 1999; Rawe et al., 2002; Saïas-Magnan et al., 1999). The arrest of early embryo development could be a consequence of a functional failure of the centriole to nucleate a sperm aster in the zygote (Chemes et al., 1999; Rawe et al., 2002; Saïas-Magnan et al., 1999) or of an impaired release of the centriole into the oocyte cytoplasm after fertilization (Rawe et al., 2008). Later studies, however, reported pregnancy achievements after injection of spermatozoa with misaligned heads and midpieces (Gambera et al., 2010; Porcu et al., 2003).

Several types of monomorphic teratozoospermia have been associated with an increase in the frequency of sperm chromosome abnormalities (Brahem et al., 2012; Collodel and Moretti, 2006; Moretti and Collodel, 2006; Rives et al., 2005; Sun et al., 2006; Vozdova et al., 2014). Although sperm DNA alterations can negatively affect ICSI outcomes, there are few data regarding the nuclear integrity of spermatozoa with defective head-neck attachments. The levels of DNA fragmentation have never been measured and the results on sperm chromatin compaction are controversial (Baccetti et al., 1984, 1989; Gambera et al., 2010; Perotti et al., 1981; Saïas-Magnan et al., 1999). Moreover, the normal aneuploidy and diploidy frequencies determined in three men needs to be further confirmed (Gambera et al., 2010; Moretti and Collodel, 2006).

The aim of the present study was to explore DNA fragmentation, chromatin condensation and chromosome meiotic segregation in the ejaculated sperm of four infertile men with the decapitated sperm defect.

Materials and methods

Patients and semen samples

Four men (26–43 years old) who consulted in the Reproductive Biology Laboratory (Rouen University Hospital) were included in this study. Patient 1 is Egyptian and the son of first-degree cousins. Patients 2 and 3 are brothers and the descendants of Algerian first-degree cousins. Patient 4 is European, with no history of consanguinity in his family. All patients presented a normal 46,XY constitutional karyotype. The control group comprised 10 healthy sperm donors of proven fertility having at least one child of their own. They were aged 24–43 years and had a normal karyotype. This study was approved by the local research ethics committee on 2 March 2015 (reference: E2015–05). All the participants have signed a