Reproductive BioMedicine Online (2016) ■■, ■■–■■

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ARTICLE

Sperm DNA fragmentation in Italian couples with recurrent pregnancy loss

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Abstract The aetiopathogenesis of recurrent pregnancy loss (RPL) is heterogeneous. The aim of this study was to investigate the male factor in Italian couples experiencing RPL following natural conception. The study investigated 112 men from RPL couples and two control groups: 114 infertile men with one or more impaired semen parameters and 114 fertile men with high-quality semen parameters. Semen parameters were examined according to WHO criteria. Sperm DNA fragmentation (SDF) was evaluated using TdT-mediated dUDP nick-end labelling (TUNEL) assay. With the exception of ejaculate volume, the seminal profile of patients with RPL was similar to that of fertile patients and better than the infertile ones. Despite good spermatogenesis, however, sperm DNA integrity was impaired in the RPL group, with SDF values significantly higher than in fertile controls (18.8 ± 7.0 versus 12.8 ± 5.3, $P < 0.001$) and similar to those of infertile patients. SDF also showed a positive correlation with the age of patients with RPL and number of miscarriages. The results suggest a correlation between increased SDF and impaired reproductive capacity in terms of both fertilization and pregnancies carried to term, but high SDF cannot yet be considered a predictive factor for the risk of RPL.

KEYWORDS: male infertility, miscarriage, semen quality, sperm DNA integrity, spermatozoa

http://dx.doi.org/10.1016/j.rbmo.2016.09.014
1472-6483/

Please cite this article in press as: Tania Carlini, et al., Sperm DNA fragmentation in Italian couples with recurrent pregnancy loss, Reproductive BioMedicine Online (2016), doi: 10.1016/j.rbmo.2016.09.014

Introduction

Miscarriage is the most common obstetric complication, occurring in 15% of all clinically recognized pregnancies. This figure rises to about 50% for preclinical miscarriages (Chard, 1991; Wilcox et al., 1998). It has been estimated that just one-third of conceptions lead to the birth of a baby (Wang et al., 2003; Zinaman et al., 1996). Recurrent pregnancy loss (RPL) is defined as two or more consecutive miscarriages diagnosed before the 14th week (ASRM, 2013). It affects about 1% of couples attempting to have a child (Porter and Scott, 2005). Its aetiopathogenesis is heterogeneous and multiple factors may be involved, complicating the identification of predisposing factors.

Despite thorough investigation of the female partner of RPL couples, it is estimated that the cause is never found in 50% of cases (Lee and Silver, 2000). It is therefore plausible to suppose that in some of these so-called idiopathic cases, the cause may be due to the male partner, which to date has been little investigated. Studies of the correlation between sperm quality and RPL have produced conflicting results (Gopalkrishnan et al., 2000; Saxena et al., 2008; Sbracia et al., 1996). Impaired semen parameters have been associated with infertility and reduced reproductive capacity, with failed fertilization and embryonal division following IVF (Oehninger, 2011). Although it is unclear as to what extent the male factor is involved in RPL, a high percentage of morphological sperm abnormalities has been associated with an increased risk of miscarriage in couples undergoing assisted reproduction treatments (Kobayashi et al., 1991). Patients with karyotype 46XY, who present impaired semen parameters, also have a high percentage of spermatozoa with aneuploidies (Vicari et al., 2003); although these are capable of fertilizing the oocyte, they give rise to an embryo with chromosome damage, which may result in spontaneous abortion.

As both the spermatozoa and the oocyte contribute equally to the genetic makeup of the embryonic DNA, it is reasonable to presume that genetic and epigenetic sperm damage may compromise the development of the embryo and placenta and thus cause miscarriage. There has been great interest in the study of sperm DNA fragmentation in recent years, as the integrity of sperm DNA is crucial for the accurate transmission of genetic information to the embryo. A meta-analysis conducted by Evenson and Wixon (2008) found that the lower the sperm DNA damage, the greater the successful natural pregnancy rate, while major sperm chromatin damage increases the risk of congenital abnormalities (Kumar et al., 2012) and predisposition to childhood cancers in the offspring (Aitken and Krausz, 2001). Various studies in the literature have investigated the relationship between sperm DNA fragmentation and RPL. Systematic review and meta-analysis (Robinson et al., 2012; Zini et al., 2008) found that sperm DNA damage is significantly correlated with an increased risk of miscarriage following IVF and intracytoplasmic sperm injection. Some studies have found high sperm DNA fragmentation in the male partners of RPL couples following natural conception (Brahem et al., 2011; Carrell et al., 2003; Imam et al., 2011; Kumar et al., 2012), while others (Coughlan et al., 2015; Gil-Villa et al., 2010) contradict the theory that sperm DNA damage is one of the factors involved in RPL.

Given the growing interest in the study of sperm DNA fragmentation, the aim of this study was to investigate the male factor in Italian couples experiencing RPL following natural conception. It focused on quality of spermatogenesis and sperm chromatin integrity, to establish any paternal contribution to the aetiopathogenesis of RPL.

Materials and methods

Ethics statement

The study was approved by our University Hospital's institutional review board (number 182/11, 18 February 2011). Written informed consent was obtained from all study participants.

Patients

The study recruited 112 men from Caucasian couples reporting two or more spontaneous abortions (**Figure 1**) attending the RPL Sterility Unit at ASL Roma C who underwent a semen examination at the Seminology Laboratory–Sperm Bank ('La Sapienza' University of Rome, Department of Experimental Medicine). The miscarriage was diagnosed before the 14th week but fetal heartbeat was documented between the eighth and 12th week.

Patients with an abnormal chromosome number or structure, blood relationship with their partner or miscarriage after exposure to radiotherapy or chemotherapeutic treatments were excluded. Both male and female partners had normal karyotypes.

A full screening of the RPL women was carried out, including physical examination, testing for immunological, acquired or inherited thrombophilia and reproductive hormonal assays. The presence of any infectious or parasitic disease was excluded. The female partners (<38 years old) presented a normal uterus, as confirmed by vaginal ultrasound, and showed

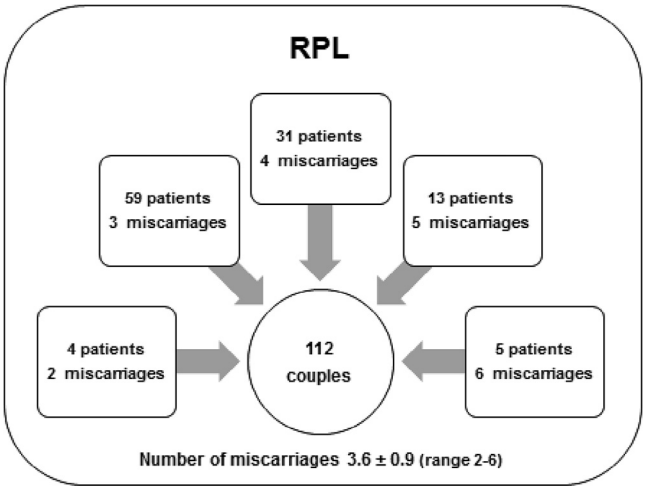

Figure 1 RPL couples categorized by number of miscarriages. RPL = men from couples with recurrent pregnancy loss.

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