Sperm deoxyribonucleic acid fragmentation assessment in normozoospermic male partners of couples with unexplained recurrent pregnancy loss: a prospective study

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Objective: To determine whether sperm DNA integrity in normozoospermic male partners plays a role in idiopathic recurrent pregnancy loss (RPL).
Design: Prospective, cohort study.
Setting: Academic tertiary care center.
Patient(s): Group I: 26 male partners of women with unexplained RPL. Group II: 31 normozoospermic males with proven fertility.
Intervention(s): Semen samples were collected by masturbation after 48–72 hours of abstinence. After liquefaction at room temperature, semen analysis was performed according to World Health Organization standards. Only samples with $>20 \times 10^6$ spermatozoa/mL with at least 50% progressive sperm motility and 30 % normal morphology were selected for the study. DNA fragmentation of the sperm was assessed with TUNEL assay followed by flow cytometric analysis.
Main Outcome Measure(s): Sperm DNA fragmentation in both groups.
Result(s): Mean DNA fragmentation (mean ± SD) was significantly more in men with RPL (36.8 ± 5) compared with controls (9.4 ± 2.7).
Conclusion(s): Sperm DNA fragmentation may play a role in unexplained RPL despite normal semen analysis parameters. (Fertil Steril® 2016;105:329–36. ©2016 by American Society for Reproductive Medicine.)
Key Words: Sperm DNA, recurrent pregnancy loss, unexplained infertility, TUNEL assay

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Semen analysis parameters are used to assess fertility potential; however, they exhibit significant variability and are poor predictors of reproductive outcome (1, 2). The male gamete contributes 50% of the genomic material to the embryo and placenta (3–5). The integrity of the paternal genome is paramount for the initiation and maintenance of pregnancy (6). Standard semen analyses fail to provide information about the sperm genome integrity, which is a prerequisite for successful fertilization, normal embryonic development, and fetal well-being (7, 8). Tests of sperm DNA integrity may be useful in the diagnosis of male infertility (1, 9, 10). Terminal uridine nick-end labeling assay and sperm chromatin structure assay have been used for the identification of significant DNA fragmentation

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in couples with infertility and recurrent pregnancy loss (RPL) (9,11–13).

The etiology of RPL is multifactorial. Studies on RPL usually focus on genetics and maternal factors including endocrine, anatomic, infectious, and immune disorders. The etiology is unexplained in approximately 50% of affected couples (14, 15). Genetic and epigenetic alterations of the sperm could affect embryonic development leading to pregnancy loss (16). The presence of a genetic abnormality or sperm DNA fragmentation has profound implications on embryogenesis, prenatal and postnatal growth (5, 17). Sperm DNA fragmentation is associated with increased preimplantation and postimplantation losses, congenital malformations, and childhood cancers (18, 19). In the present study, we assessed sperm DNA fragmentation with normal semen parameters in couples with unexplained RPL.

MATERIALS AND METHODS

Study Subjects

This study was conducted at the University of Texas Health Science Center, San Antonio, Texas, USA. The procedures and protocols were approved by the Institutional Review Board.

Informed consent was obtained from all participants. Two groups were included: group I, male partners of women with a history of unexplained RPL (N = 26) and group II, healthy men with proven fertility (N = 31) (Fig. 1).

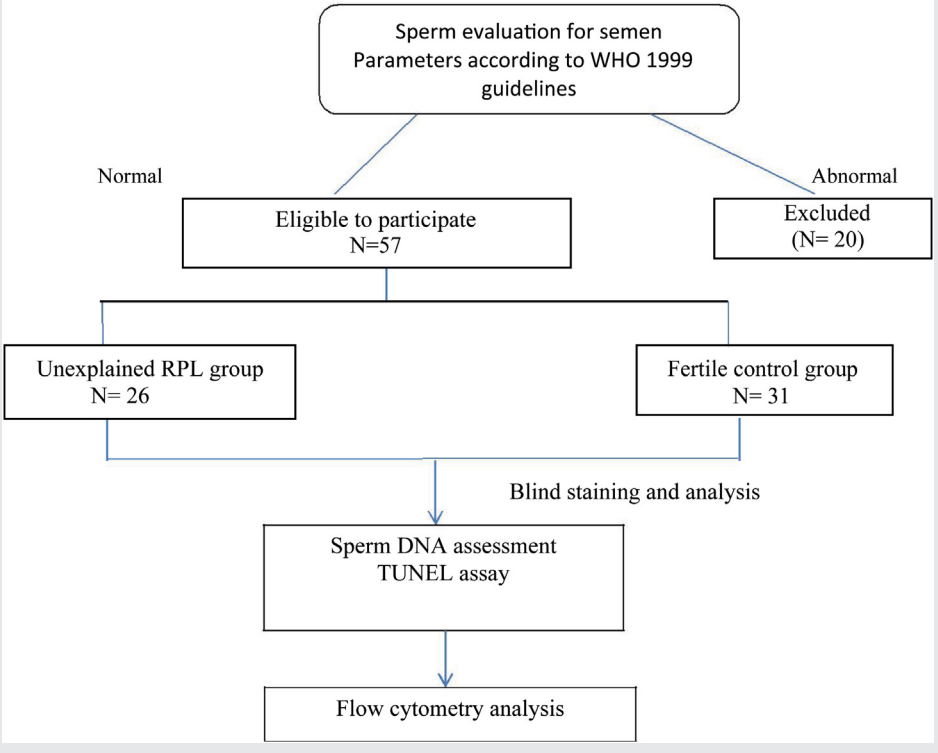
A detailed medical history, including reproductive history and infertility evaluation was obtained from all participants. Subjects who had any evidence of infection on semen analysis (leukocytospermia) were excluded. Also subjects who were currently smokers, drank >2 alcoholic beverages per week, or who used any recreational drugs were excluded. Obese subjects (body mass index [BMI] >30) or subjects on any medications for chronic medical disorders were also excluded from the study.

Recurrent pregnancy loss was defined as at least two prior pregnancy losses at <20 weeks of gestation, with all pregnancies fathered by the same partner. All female partners had a routine RPL evaluation including physical examination, hysterosalpingogram (HSG) or sonohysterogram (SHG), thyroid function tests, PRL level, blood glucose screening, lupus anticoagulant, anticardiolipin antibody, and anti-β-2 microglobulin. All couples had normal karyotypes.

Sample Collection and Preparation

Semen samples were collected by masturbation after 48–72 hours of abstinence. After approximately 30 minutes of liquefaction at room temperature, semen analysis was performed according to the 1999 World Health Organization standards (20). Only samples with $>20 \times 10^6$ spermatozoa/mL, at least 50% progressive sperm motility and 30% or more normal morphology were selected for the study. Two semen samples on two different occasions were

FIGURE 1



Study design. RPL = recurrent pregnancy loss; WHO = World Health Organization.

Bareh. Sperm DNA and RPL. Fertil Steril 2016.