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Correspondence

Sperm DNA damage & oxidative stress in recurrent spontaneous abortion (RSA)

Sir,

Recurrent spontaneous abortion (RSA) is defined as three or more consecutive pregnancy losses of less than or equal to 20 wk of gestation. The aetiology in about 60 per cent cases is not known. Till date, apart from chromosomal analysis of both partners, the male factor is largely ignored and only women are investigated to understand aetiology RSA. However, recent studies by Shamsi *et al*[1] have reported that sperm factors play an important in fertilization, implantation and embryogenesis. Thus, sperm is not a mere vector of paternal DNA but, plays a critical and dynamic role which extends beyond fertilization. This study was planned to understand the role of sperm factors [sperm oxidative stress (OS) and DNA damage] in idiopathic cases of RSA.

After obtaining ethical clearance of study protocol from the ethics committee of All India Institute of Medical Sciences (AIIMS), New Delhi, 25 couples with idiopathic RSA attending antenatal clinic and 25 proven controls (men who had fathered a child in last one year and had normal sperm parameters) were enrolled in the study from February to August 2010. The male partner of couple with idiopathic RSA were enrolled consecutively. Informed consent was obtained from both cases and controls. The mean age of cases was 28 ± 4.2 yr and that of controls was 26 ± 3.8 yr. The individuals were evaluated to rule out immunological, endocrinal, infections and anatomical defects. The absence of antiphospholipid syndrome was confirmed by ruling out the presence of lupus anticoagulant (LA) and anticardiolipin (aCL) antibody. After detailed gynaecological and laboratory investigation it was found that female partner was normal. Both male and female partners were cytogenetically normal. The semen samples were collected after abstinence of 4 days in a non toxic vial and the analysis was done immediately after liquefaction (30-40 min after ejaculation). Neat semen was analysed for ROS levels by chemiluminescence[2] and DNA damages was assessed by comet assay[3]. Of the 25 cases, 19 had normal sperm count (54.97 ± 12.35 million per ml) and six had oligozoospermia (14.54 ± 2.4 million per ml). ROS in neat semen was elevated in 62.5 per cent cases and was in normal range in 32.5 per cent cases. The mean ROS levels observed were 55,096.4 relative light unit (RLU)/min/20 million sperms in male partner as compared to 5 924.8 RLU/min/20 million sperms in controls (Table).

The mean of number of sperm with high DNA damage (Grade C and D comet) was 39.38 ± 3.05 in male partner as compared to 10.80 ± 2.28 in controls.

Recent studies have shown that sperm DNA damage correlates with infertility, increased risk of early pregnancy loss, defective embryogenesis, major and minor congenital malformations, genetic and epigenetic abnormality and prenatal morbidity[4,5]. Sperm DNA damage also correlates with pregnancy loss after *in vitro* fertilization introcytoplasmic sperm injection (IVF/ICSI). There are several causes of DNA damage, main among these are oxidative stress, abortive apoptosis, aberrant DNA packaging, altered histone-protamine ratio, high temperature, xenobiotics, electromagnetic radiation and smoking, drugs and varicocele[6,7]. High

Table. Details of sperm parameters, ROS levels in neat semen and DNA damage in sperms of patients and controls

	Semen volume (ml)	Sperm concentration (million/ml)	% abnormal sperm morphology	ROS (RLU/ (min/20million sperms)	% sperms with DNA damage
Patients	1.5	45.2	47.9	55 096.4	39.3
Controls	2.1	73.5	53.1	5 924.8	10.8

ROS level is one of the main causes of DNA damage and are produced by morphologically abnormal sperms and leucocytes; 75-80 per cent infertile men have high ROS levels[8,9]. Oxidative stress induces peroxidative damage to cell membrane and causes mitochondrial mutation and nuclear DNA damage (fragmentation and denaturation)[10-12]. Sperm DNA is bound to protamines and thus is highly compact and condensed, however, about 15 per cent DNA is bound to histones and is peripherally located in the nucleus and is susceptible to environmental insults especially oxidative damage.

This study highlights the role of paternal factors in RSA. Thus male factors (sperm OS and DNA damage) should be evaluated in diagnostic workup of idiopathic cases of RSA. It is important to mention here that standard semen parameters (SSP) are just modest predictors of fertility potential and normozoospermic men may have sperm DNA damage which cannot be assessed by routine semen analysis. Thus evaluation of seminal ROS levels and sperm DNA damage has both diagnostic and prognostic capabilities and would aid in understanding aetiology and providing most adapted therapeutics to couples experiencing RSA.

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