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Male Factors: An Ignored Etiology in Recurrent Pregnancy Loss

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ABSTRACT [ENGLISH/ANGLAIS]

The etiologies of recurrent pregnancy loses could be chromosomal aberrations, uterine defects, immunological problems, endocrinological problems, advanced reproductive age, sperm deformities and gene mutations. But most often the male factors are neglected and not consider as major factor in Pregnancy loss. In male partner of the patients with recurrent pregnancy loss RPL have a significant increase in sperm DNA fragmentation and decreased sperm functions. This study was conducted to evaluate the correlation between male factors and pregnancy loss. In our study with 50 idiopathic RPL cases 30 cases were identified with female factors responsible for RPL, 7 cases with both male and female eitiologiesand rest 13 were idiopathic. Randomly selected males were taken as controls with proven fertility rate and compared with RPL group. The semen samples of the respective male partners were analysed along with basic semen analysis procedures. Sperm function test was carried out by Hypo-osmotic swelling test (HOS), Acrosomal Reaction (AR) and Nuclear decondensation test (NCD). Sperm motility, viability and sperm function tests scores were significantly lower in the RPL group when compared to the control group. Through this pilot study it is significant that male factor might be a possible contributing factor towards RPL. Therefore both the partners should be evaluated and treated simultaneously in order to achieve successful pregnancy.

Keywords: Recurrent pregnancy loss, hypo-osmotic swelling test, acrosome reaction, nuclear decondensation test

RÉSUMÉ [FRANÇAIS/FRENCH]

Les étiologies de la grossesse pourrait être récurrente perd des aberrations chromosomiques, les malformations utérines, des problèmes immunologiques, endocrinologiques problèmes, avancé en âge de procréer, des malformations des spermatozoïdes et des mutations du gène. Mais le plus souvent les facteurs masculins sont négligés et ne pas considérer comme un facteur important dans la perte de grossesse. Dans partenaire masculin des patients avec RPL perte de grossesse récurrentes ont une augmentation significative de fragmentation de l'ADN des spermatozoïdes et une diminution des fonctions des spermatozoïdes. Cette étude a été menée pour évaluer la corrélation entre les facteurs masculins et la perte de grossesse. Dans notre étude avec 50 cas idiopathiques RPL 30 cas ont été identifiés avec des facteurs féminins responsables de RPL, 7 cas avec les deux autres eitiologiesand mâle et femelle 13 étaient idiopathiques. Hommes choisis au hasard ont été prises comme contrôle avec un taux de fécondité éprouvée et comparée à un groupe RLP. Les échantillons de sperme des partenaires respectifs des hommes ont été analysés avec les procédures de base d'analyse du sperme. Test de la fonction du sperme a été réalisé par hypo-osmotique d'essai (HOS) gonflement, réaction acrosomique (RA) et le test nucléaire décondensation (MNT). Scores motilité des spermatozoïdes, la viabilité et tests de la fonction des spermatozoïdes étaient significativement plus faibles dans le groupe RPL lorsque comparé au groupe contrôle. Grâce à cette étude pilote, il est significatif que facteur masculin pourrait être un facteur contributif possible vers la reconnaissance des acquis. Par conséquent les deux partenaires doivent être évalués et traités simultanément afin d'obtenir une grossesse réussie.

Mots-clés: Perte de grossesse récurrente, hypo-osmotique essai de gonflement, réaction acrosomique, tester décondensation nucléaire

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INTRODUCTION

Pregnancy loss (PL) is defined as the spontaneous or operative expulsion of the conceptus outside the uterine environment before fetal viability or before the fetus becomes capable of extrauterine survival [1]. Recurrent Pregnancy Loss (RPL) or habitual abortion is the occurrence of three or more consecutive losses of clinically recognized pregnancies prior to the 20th week of gestation. Pregnancy loss can be divided into four stages threatened, inevitable, incomplete and complete pregnancy loss based on the type of expulsion of the conceptus [2-5]. In general population it is difficult to

record the incidences for PL as it is often mistaken for heavy bleeding. It is estimated that about 10 to 15% of confirmed pregnancies result in RPL [7, 8].

In females egg quality and quantity are together known as ovarian reserve which decreases steadily with age and aged ova are often associated with higher frequency of chromosomal abnormalities as a result, the infertility rate and pregnancy loss rate increases with age [9]. The frequency of sperm chromosomal anomalies, such as aneuploidy or DNA strand breaks, has been suggested to increase with age [10, 11]. About 15% to 20% of pregnancies end in spontaneous pregnancy loss (SPL), mostly in the first trimester, the most frequent cause being represented by chromosomal abnormalities [12] are such as It is estimated that approximately 8 to 12% of all pregnancy losses are the result of endocrine factors such as insulin-dependent diabetes mellitus [13], thyroid disorders [14, 15], luteal phase defects [16] and hypersecretion of luteinizing hormone (LH) with polycystic ovaries [17] . Thrombosis in decidual vessels is reported to be one of the major causes of RPL. The incidence of uterine anomalies in patients with RPL are rbetween 15 and 27% [18]. About 22% of women with RPL have antiphospholipid antibodies [19].. Ten percent of women with infertility, implantation failures and RPL have produced antibodies to sperm. The role of infectious agents in recurrent loss is proposed with an incidence of 0.5% to 5%. [20] Environmental exposures should not be overlooked when trying to achieve a successful pregnancy [21]. 22, 23. Few studies have indicated that low sperm quality increases the risk of idiopathic RPL [24, 25]. So far only few studies are available to show the association of male factor with recurrent pregnancy loss. But in south Indian no such data is available focusing the male factors for pregnancy losses. In this view the present study was designed to find out the influence of male factors in causing recurrent pregnancy loss in Mysore, South India.

MATERIALS AND METHODS

In this study fifty couples with a history of confirmed three or more consecutive pregnancy loss in the first or early second trimester were recruited from IVF center in Mysore. Ethical clearance was taken from the university ethical clearance committee and also from the concerned IVF centers. Informed consent letters were taken from the participants. Information regarding the family, medical and reproductive histories were collected using genetic register. Pedigree was constructed to the occurrence of this condition in the family and to know the pattern of the expression.

Subjects with less than 2 consecutive abortions or with abortions occurring after 20 weeks or with history of preterm labor and known diabetics,diagnosed uterine, hormonal, immune abnormalities and infective pathologiewere excluded from the study. Age range was 20-40 for females and 20-45 years for males. Females with s Couples with recently proven fertility and unassisted pregnancies were taken as controls. Semen samples from the male partners were collected through masturbation after 3–4 days of sexual abstinence according to the WHO protocol [26]. The semen samples were allowed to liquefy at 37°C for 30 minutes. Physical examination such as liquefaction time, colour, odour, and pH were recorded after 30 minutes. Basic microscopic examination was carried out to record the count, density and motility of the sperm according to WHO protocol. Functional capacity of the sperms were examined by sperm function tests through hypo-osmotic swelling test (HOS), Acrosomal Reaction (AR) and Nuclear decondensation test (NCD) [27].

RESULTS

In this study with 50 idiopathic RPL cases 30 cases were identified with female factors responsible for RPL, 7 cases with both male and female eitiologies and rest 13 were idiopathic with respect to female factors(Table1) . Further when the semen samples of the respective male partners were analyzed abnormal sperm morphologies were observed, which were suspected to be the major cause for these idiopathic cases. In this study the PL above 3 were considered and the losses ranged from 3-8. In one of the cases 8 PL were observed which occurred between 5-20th of gestation (Table 1). Table 2 represents the routine semen analysis of males with idiopathic RPL group and the control group. The semen volume in 2 cases was less than 1.5 ml in the RPL group and in three of the cases the count was less than 20 million with reduced motility. The vitality in the RPL group was similar to the normal range of 60-70% of 6 expect 3 cases where the vitality was less 50%. In the RPL group motility of sperms were observed to be reduce where rapid linear were of 25%, Linear progressive were of 35%, Sluggish linear of 25% and immotile of 15% (Table 3)

In this study the mean HOS tests scores of the RPL group were subnormal and 10 cases of RPL group had low HOS scores (table 4). Figure 1 represents the HOS test in the

control and RPL group respectively shows the less response of spermatozoa for HOS in RPL group. Similarly nuclear decondensation was least in spermatozoans from RPL partners (Figure 2). Even otherwise acrosome reaction shows the subnormal conditions in the RPL group when compared to the normal (figure 3). Mean test scores for HOS and NCD were below normal range in the RPL group. All sperm function scores in the control group were within normal range.

TABLE 1

Table 1 shows the summary of the analyzed cases in the present study

Number of Cases	Number of Pregnancy Loses	Weeks
38	3	5 – 12
8	4	5 – 15
4	More than 4	5 – 20

TABLE 2

Table 2 shows the comparison of different semen parameters in male partners of recurrent pregnancy loss (RPL) and control group

Parameters	N = 13	Mean	SD	SEM	Correlation	Sig
Volume	RPL	2.3000	0.8972	0.2488	0.980	0.00
	Counts	3.6846	0.6694	0.1857		
Count	RPL	24.8462	11.8170	3.2774	0.877	0.00
	Counts	44.4615	12.4741	3.4597		
Viability	RPL	41.4615	10.3087	2.8591	0.678	0.11
	Counts	57.5385	9.2070	2.5536		

TABLE 3

Table 3 shows the Co-paritive results of the sperm motility in Recurrent pregnancy loss group and control (A- rapid linear, B- progressive linear, C- Sluggish linear, D-Immotile, RPL: Recurrent pregnancy loss)

Parameters / Motility / N = 13		Mean	SD	SEM	Correlation	Sig
A	RPL	24.1538	6.9982	1.9409	0.081	0.0001
	Counts	35.9231	4.0096	1.1121		
B	RPL	21.8462	7.6250	2.1148	0.21	0.0001
	Counts	35.9231	4.7339	1.3130		
C	RPL	24.2308	3.9403	1.0928	0.97	0.01
	Counts	16.0000	5.6862	1.5771		
D	RPL	21.0769	6.7015	1.8587	-0.224	0.001
	Counts	11.6923	3.0382	0.8427		

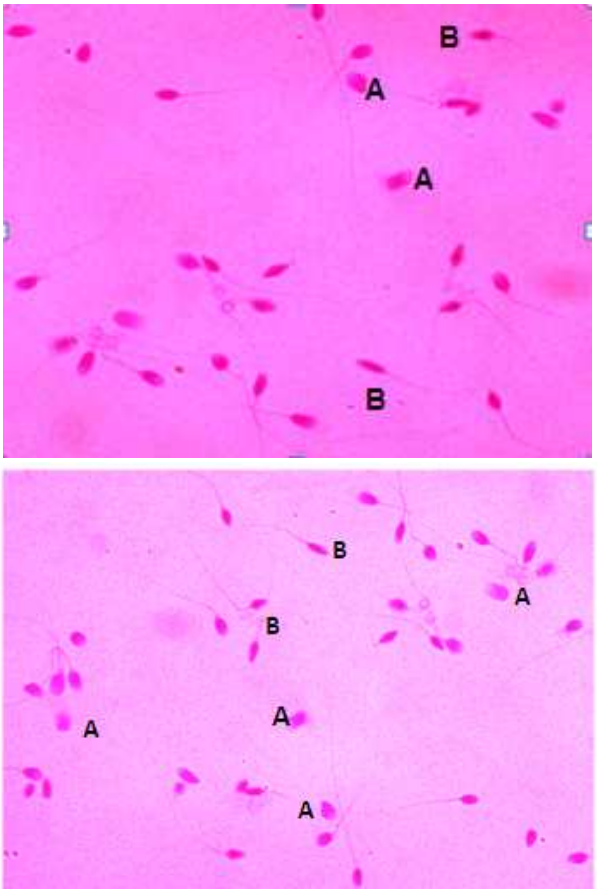
TABLE 4

Table 4 shows the Sperm function test in male partners of Recurrent pregnancy loss group and control (HOS- Hypo Osmotic swelling test, NCD- Nuclear chromatin decondensation test, AIT-Acrosome intactness test)

Parameters / N = 13		Mean	SD	SEM	Correlation	Sig
Volume	RPL	45.1538	6.6313	1.8392	0.492	0.0001
	Counts	71.6923	5.8649	1.6266		
Count	RPL	63.0000	11.9373	3.3108	0.388	0.0001
	Counts	80.0000	5.9161	1.6408		
Viability	RPL	34.7692	5.8617	1. 6257	0.981	0.001
	Counts	64.1538	9.3439	2.5915		

FIGURE 1

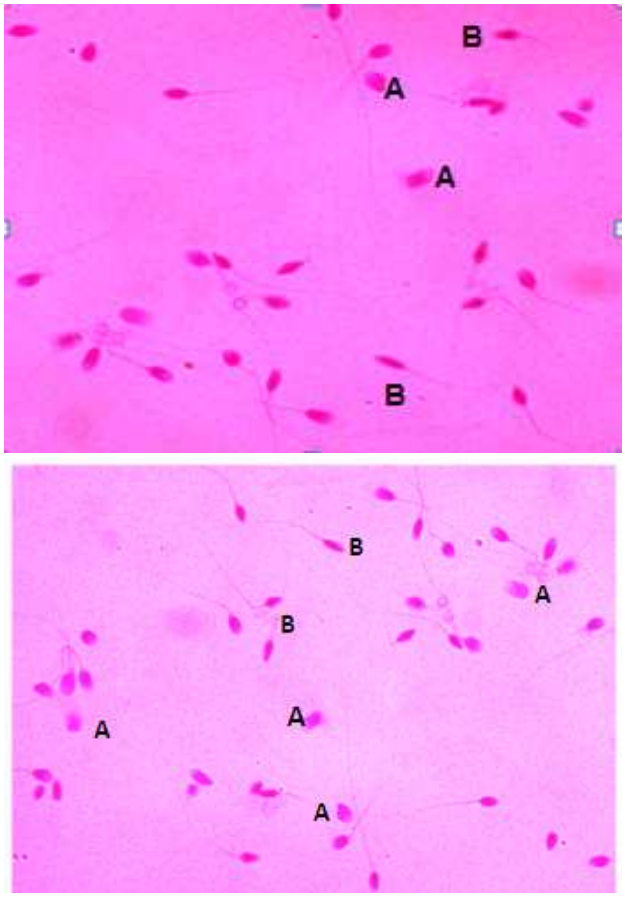
Figure 1 shows Hypo Osmotic Swelling test (HOS).



A: Positive response with coiled tail
B: Negative response with straight tail

FIGURE 2

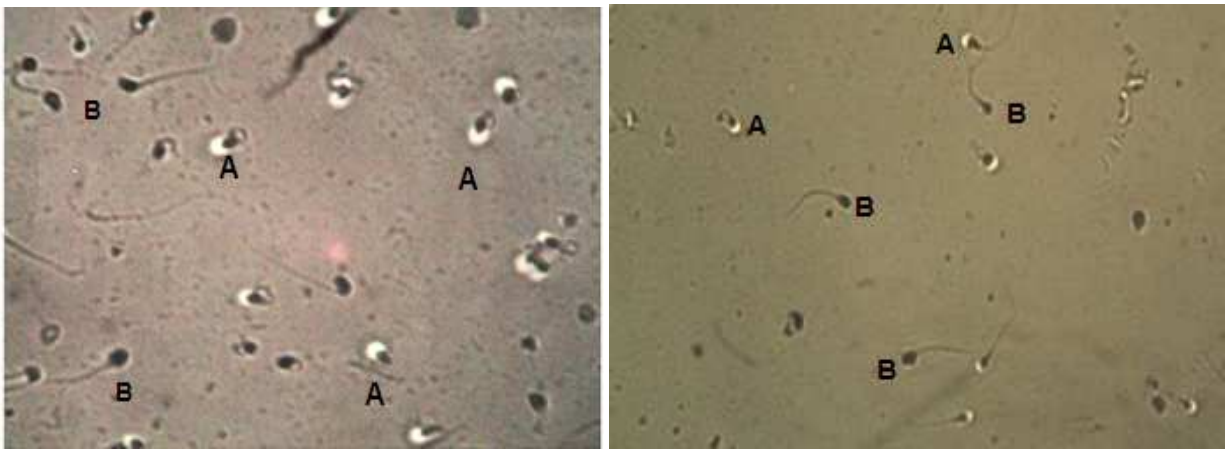
Figure 2 shows nuclear chromatin decondensation test



A: Positive response with swollen head,
B: Negative response narrow head

FIGURE 3

Figure 3 shows acrosomeintactness Test (AIT)

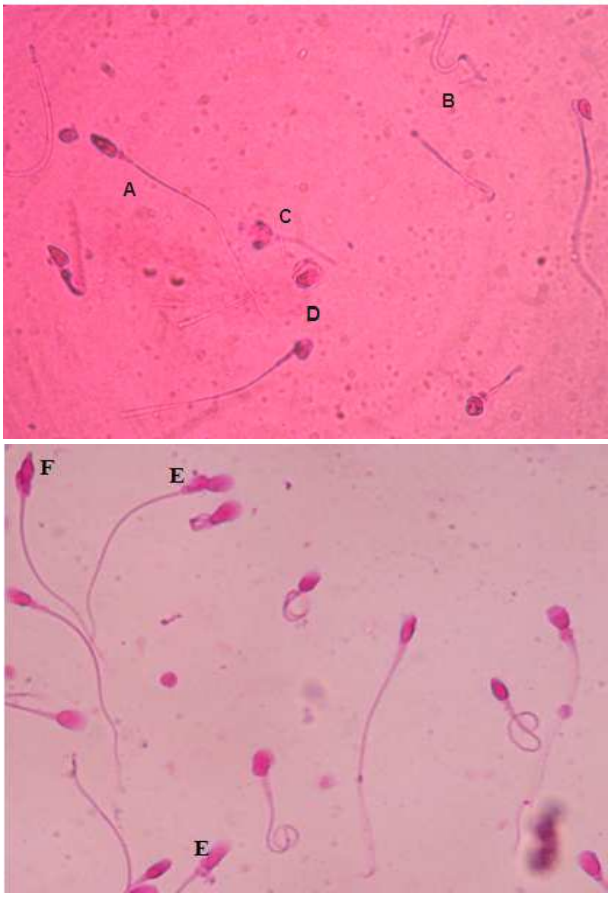


A: Positive response; B: Negative response

Comparison of sperm function tests of RPL and the control group revealed that HOS (p = 0.000), NCD (p = 0.000) and AR (p = 0.000) were significantly lower in the RPL group (Table 3). The papilinicolous stained slide were observed with increased rate of abnormal sperm morphology in the RPL group when compared to the control group (Figure 4).

FIGURE 4

Figure 4 shows sperms with different abnormalities



A: normal sperm; B: pin head; C: double head; D: swollen head; E: Cytoplasmic droplets; F: arrow head

DISCUSSION

During the fertilization sperm motility, viability, decondensation and penetration play a very important role. Sperm morphology therefore is an important criterion in the development of a healthy embryo. The abnormality in sperm morphology indicates some cytogentic defects in the sperm [27]. These sperms may succeed in fertilizing the healthy oocytes but results in abnormal embryos which might not be healthy and inviable and finally leads to pregnancy loss. Therefore sperm quality plays an important role in successful pregnancy. Abnormal sperm morphology has been associated with increased miscarriage rates in couples undergoing IVF-ET [28]. In the present study conventional semen analysis along with sperm function tests shows sperm abnormality in male partner with RPL cases. These results are supported by other studies [29, 30]. The possible etiology for the abnormal sperm could be due to increase of sperm DNA fragmentation, which may be major causative for RPL. Men who fathered normal pregnancies had 25% higher sperm counts and only 5% visually abnormal sperm. Sperm DNA fragmentation occurs in every ejaculate, and it can also be induced by various external and internal factors. Since sperms do not have the power of endogenous DNA repair, in contrast to the somatic cells, increased levels of impaired DNA integrity have high likelihood of transmission to the resulting zygote. This DNA damage in the sperm is a major source of the paternal contribution to embryonic mortality [29]. Thus, paternal genomic alterations may compromise embryo quality, embryo viability, and progression of pregnancy, ultimately leading to RPL.

In this study, reduced sperm function test supports the abnormal functioning of sperm in male partners with RPL wives (Figures 1 2 3). The morphological defects of the sperm might be due to subtle alterations in the membrane constitution of the sperm resulting in functional defects. These defects can be evaluated by performing HOS test, which checks the functional integrity and intactness of the sperm cell membrane. Recent studies indicated that low HOS test scores in couples undergoing IVF do not affect rates of fertilization or pregnancy, but are associated with higher rates of spontaneous miscarriage [34]. The acrosome of spermatozoa contains proteases, which help in penetration of spermatozoa through outer membranes of oocyte. Therefore the acrosome reaction test determines the activity of these enzymes is significantly reduced which supports the importance of acrosomal enzymes in normal reproduction. Significant decrease in nuclear chromatin to condense and irregular nuclei with vacuoles in *in vitro* studies along with sperm head abnormality has been reported in many studies [27]. Hence Loss of chromatin integrity as a possible contributing factor from males leading to early pregnancy loss has been well pointed out. The mean NCD score of the RPL group was below normal range and subnormal scores were recorded in the present study. So it is evident with the subnormal or decreased sperm functional rates which strongly correlate with the RPL.

CONCLUSION

Semen profile and sperm function tests scores were significantly lower in the RPL group when compared to the control group. Through this pilot study it is significant that male factor might be a possible contributing factor towards RPL. Apart from routine semen analysis, sperm

function tests may be an informative tool in cases of idiopathic RPL. Therefore both the partners should be evaluated and treated simultaneously in order to achieve successful pregnancy.

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CONFLICT OF INTEREST

No conflict of interest was declared by authors