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Original Article

Impact of spermiogram and sperm function test in idiopathic recurrent pregnancy loss

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ABSTRACT

Aims: To evaluate the role of sperm factors in idiopathic recurrent pregnancy loss cases. **Methods:** We recruited 100 male subjects where their female partners experienced 2 or more idiopathic pregnancy losses. Recurrent pregnancy loss (RPL) males were grouped into two groups RPL1 (individuals having 2 abortion) and RPL2 (individuals having more than 2 abortions). Fifty volunteers who had fathered child/children prior to the study without the history of recurrent pregnancy loss and unassisted pregnancies were considered as control group. We grouped RPL males into two categories age below 35 years and above 35 years to test whether age plays any role on RPL. Routine semen parameters and sperm function test were performed for all the subjects. Statistical analysis was performed using Independent-Samples T test. **Results:** Insignificant differences were observed in seminal volume, pH, motility and sperm count but vitality test scores were significantly lesser in both RPL groups when compared to control group. For all three sperm function test both the RPL group showed lesser scores when compare to control. RPL2 group showed more abnormalities when compared to RPL1 group. We did not identify any significant difference for any of the parameters between two age group of RPL males. **Conclusion:** Our results recommend the screening of both partners simultaneous in RPL cases for the better diagnosis and treatment.

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Case report

Recurrent pregnancy loss (RPL) also known as recurrent miscarriage or recurrent abortion, is traditionally defined as occurrence of three or more consecutive pregnancy failures in the first or early second trimester of gestation. [1] RPL is growing clinical problem for clinicians and patients that needs adequate attention and investigations. Approximately 15-20% of clinically recognized pregnancies end in spontaneous abortions. Most losses occur at the time of implantation. Only 30% of all conceptions result in a live birth. [2] The risk of miscarriage is 43.7% after two previous losses, 44.6% after three and 61.9% after four losses. [3] This suggests a need for evaluation after two losses in patients with no prior live births. An estimated 0.5 to 3% of the women in reproductive age experiences RPL and between 50-60% of these losses are idiopathic. [4]

The etiologies of RPL include parental chromosomal anomalies, endocrine, uterine malformations, haematological and

autoimmune factors. Several studies have related these condition with women owing that the relation exist only between mother and the developing embryo. Attempts have been made to show the relation between RPL and sperm quality but it remains controversial. [5]

Evidences are available in literature that paternal genome effects early embryonic development, sperm integrity vital for sperm-egg interaction and fertilization, and it provides the centromere in the first mitotic division after fertilization. [6, 7] Though one half of the genetic material is contributed by paternal genome to embryo it has been poorly evaluated in RPL subjects. Evaluation of paternal factor in RPL involves chromosomal analysis alone. Dewan et al [8] has reported that semen analysis is not usually part of the initial assessment for RPL because it is generally carried out in infertility evaluations. Basic semen analyses do not provide the much information regarding the functional competence and reproductive potential.

Hence in this study we made an attempt to evaluate and compare standard semen parameters and sperm function test in ejaculates from the men whose partners had two or more idiopathic pregnancy loss and from a control group of men with proven fertility.

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MATERIALS AND METHODS

Study subjects:

The study was approved by Institutional ethical review board and the hospital ethical committee. All the subjects were recruited after obtaining a informed written consent. Genetic register was taken to collect information regarding the family, medical and reproductive histories.

For the present study 100 males subjects aged 20-45 years where their female partners experienced two or more consecutive idiopathic pregnancy losses were recruited. Subjects were ascertained through gynecologist of different hospitals in and around Mysore. In this study the pregnancy losses ranged from 2-7. RPL were grouped into two groups RPL1 (individuals having 2 abortion) and RPL2 (individuals having more than 2 abortions). There were 50 males in each RPL group. Fifty volunteers who had fathered child/children prior to the study without the history of recurrent pregnancy loss and unassisted pregnancies were considered as control group.

Laboratory procedures:

Semen samples were obtained by means of masturbation from both subject and control after recommending 3 days of sexual abstinence according to the WHO guidelines [9]. Samples were collected in sterile plastic container and allowed to liquefy at 37°C for 30 minutes and was analysed within one hour of retrieval. The analysis includes physical examination like color, odor, pH, liquefaction time and volume. Initial microscopic investigations were made to estimate sperm concentration, agglutination of spermatozoan and the presence of cellular elements other than spermatozoa. Sperm count were performed using Neubauer counting chamber (expressed in millions of spermatozoa per milliliter), percentage of rapidly progressive motile spermatozoa (a) and the total percentage of progressively motile spermatozoa (a+b) were analyzed, viability was recorded using Eosin-nigrosin stains (a modified Blom's technique) and morphology was examined by Papanicolaou staining method. All the samples were evaluated according to WHO (1999) criterion. Apart from these routine semen analysis sperm quality was analysed through sperm function tests namely hypo-osmotic swelling test (HOST) [10], nuclear chromatin decondensation test [11] and acrosomal intactness test [12] with modification.

Statistical Analysis

Independent- Samples T test was used to find out whether the difference is significant between groups using statistical program SPSS (version 14.0). Results were reported as mean ± standard deviation and error. Differences were considered significant when p value is less than 0.05.

RESULTS:

The mean age of males of the RPL1, RPL2 and control group was 32.8± 5.8, 34± 6.2 and 33.06 ± 5.75 respectively. Semen characters of RPL groups and control are depicted in table 1 we did not observe any significant differences in seminal volume, pH, motility and sperm count but vitality test scores were significantly lesser value in both RPL group when compare to control. For NCD test number of condensed and decondensed heads was counted. If more than 70% of spermatozoa show decondensed nuclear chromatin then it was considered as normal. Percentage of coiled (curled) tail was recorded, if more than 60% of spermatozoa, shows coiled tail then it was considered as normal for HOS test and for AIT percentage spermatozoa with halos surrounding the head

were recorded. Values more than 50% was considered as normal Scores of sperm function tests are shown in table 2. For all three function test both RPL group showed lesser scores when compare to control. Based on semen profile the different condition are depicted in Table 3 RPL2 showed more abnormalities when compared to RPL1. The effect of age on RPL was analyzed in two categories of RPL group, age below 35 and above 35 but no significant difference were observed for any of the parameters between these group.

Table 1: Comparison of semen characters between control and RPL groups

Parameters	Control (N=50) Mean±SD Error	RPL 1 (N=50) Mean±SD Error	RPL2 (N=50) Mean±SD Error
pH	7.78±0.34 0.04	7.89±0.35 0.04	8±0.43 0.06
Volume (ml)	2.39±1.07 0.14	2.19±1.05 0.14	2.36±1.37 0.19
SMA	34.96±16.45 2.23	29.01±15.81 2.17	27.72±19.44 2.72
SMB	28.79±13.10 1.78	30.05±12.54 1.72	29.49±13.46 1.88
Vitality	73.44±6.70 0.91	64.22±12.73** 1.74	62.19±14.36** 2.01
Sperm count (million/ml)	59.35±28.82 3.95	45.89±32.70 4.49	46.96±28.79 4.03

N=number of subjects, SMA=sperm motility grade A, SMB=sperm motility grade B, RPL=Recurrent pregnancy loss, **= statistically significant (p < 0.05).

Table 2: Sperm function test

Test	Control (N=50) Mean±SD Error	RPL 1 (N=50) Mean±SD Error	RPL2 (N=50) Mean±SD Error
NCD	76.83±7.68 1.04	59.58±18.20** 2.50	58.62±17.59** 2.46
HOS	75.24±7.11 0.96	66.11±15.23** 2.11	62.29±17.54** 2.45
AIT	74.0±11.20 3.73	43.16±21.18** 4.99	46.00±3.67** 3.67

N=number of subjects, NCD=Nuclear chromatin decondensation, HOS=Hypo osmotic swelling, AIT=Acrosome intactness test. **= statistically significant (p < 0.05).

Table 3: Semen condition based on sperm count and motility in RPL and control groups.

Group	Normal	Oligospermia	Asthenospermia	Oligoasthenospermia
RPL1(N=50)	42	2	3	3
RPL2(N=50)	38	3	4	5
Control(N=50)	50	0	0	0

Note: Numbers depicts number of individuals

Table 4: Comparison of basic semen parameters and sperm function test in RPL group based on age category

Parameters	Age below 35 years (N=60) Mean±SD	Age above 35 years (N=40) Mean±SD	P value
pH	7.9±0.37	7.8±0.4	0.137
Volume (ml)	2.1±1.24	2.4±1.1	0.603
SMA	26.9±18.08	31.09±17.3	0.709
SMB	30.1±12.8	29.3±13.6	0.528
Vitality	62.9±13.3	64.5±12.9	0.958
Sperm count (million/ml)	41.6±28.4	53.±32.8	0.404
NCD	56.5±17.4	62.8±17.9	0.655
HOS	65.7±15.8	61.3±17.3	0.131
AIT	43.1±20.8	44.7±19.2	0.242

DISCUSSION:

The role of male factors in recurrent spontaneous loss remains largely ignored and male were assessed only for cytogenetic studies. There are evidences that good quality sperm yield good quality embryo. Deviation from normal or sub standard sperm will affect its motility, penetration and decondensation finally affecting conception, implantation and nidation. The paternal genome is of paramount importance in normal embryo and fetal development. Few animal studies have showed that male genome contributes more to the placenta than the female genome which was proven by parthenogenode studies. [13]

The relation between standard semen parameters and recurrent pregnancy loss has been controversial subject. [5] Hence an attempt was made to evaluate the role of sperm factors in RPL through semen parameters and sperm function tests. This study strengthens the current literature associating sperm quality with RPL. Few reports state that paternal age is associated with gain-of function mutation within sperm that have detrimental effects on embryo. In our study we did not found any significant differences in the age of RPL group and control. We did not observe any variation in the semen parameters and sperm function in the individuals above the age of 35. control group. Our finding supports these previous reports.

Few studies have reported that higher rates of early pregnancy loss rates in couples undergoing assisted reproductive techniques like in vitro fertilization and intracytoplasmic sperm injection when high sperm abnormal morphology and sperm DNA fragmentation were present. [14, 15] Subnormal motility was reported in RPL group by Saxena and colleagues [16]. Our data showed subnormal motility in 15% (6% in RPL1 and 9% in RPL2) and subnormal count in 13% (5% in RPL1 and 8% in RPL2) of the RPL cases. RPL2 group individual showed higher subnormal scores than RPL1. Though the semen analysis is used as a surrogate measure of the man's fertility potential, it is not a direct measure of this. Clinical studies have shown that a normal semen analysis may not reflect defects in sperm function, and men with poor sperm parameters may cause spontaneous pregnancy loss. [17]

To overcome this along with routine semen analysis we performed sperm function tests like NCD, HOS and AIT to evaluate the functional capacity of sperm. NCD of spermatozoa and subsequent male pronucleus formation in essential for fertilization and normal embryonic development. The failure of sperm decondensation in the oocytes may be a consequence of a subtle sperm abnormality like structural or biochemical defect associated with chromatin packaging or organization during spermatogenesis. [18, 19] Chromatin damage precedes the loss of fertilization potential and poor embryo quality, resulting in pregnancy loss. The intactness of the plasma membrane was evaluated by HOS test. It is not only an indicator of the chemical integrity of the plasma membrane but also its physical integrity. Few studies have reported the low HOS test scores in couples undergoing IVF which are associated with the higher rate of spontaneous miscarriages. [20, 21] Acrosin and Hyaluronidase are the two main acrosomal enzymes (proteases), which plays an important role in penetration of spermatozoa through outer membrane of oocyte.

For all the three function tests we observed statistically significant difference in both RPL group when compare to control no significant difference were observed in between RPL groups. RPL2 individuals showed higher subnormal scores for all the function tests compare to RPL1. Absalan et al [22] tested the sperm DNA fragmentation by sperm chromatin depression (SCD) test and they observed significant difference between RPL and control group. Buckett et al [23] showed correlation with HOS test in recurrent miscarriage group and also showed the viability and quality of the sperm may have an impact on conception and miscarriage rates. Saxena et al [16] reported mean less scores for all the three function tests in RPL group when compared to controls. The pilot study carried out by Chaithra et al [24] reported significantly lower scores for semen profile and sperm function tests in the RPL group when compared to the control group. Our finding supports these previous reports.

CONCLUSION:

In conclusion routine semen analysis and sperm function tests may be one of the possible etiology of male leading to RPL. These tests are valuable and cost effective and can be performed in the basic andrology laboratories and should be included for the routine screening of male partner in RPL cases. It might also assist in selection of functionally superior sperm for the couples those who are opting for ART like IVF and ICSI which may increases the success rate.

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