Semen parameters and sperm morphology in men in unexplained recurrent spontaneous abortion, before and during a 3 year follow-up period

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To investigate the role of the 'male factor' in the pathogenesis of recurrent spontaneous abortion (RSA), especially sperm morphology abnormalities, 120 previously selected couples with unexplained RSA were studied for sperm parameters retrospectively and prospectively. The patients were subdivided into three subgroups, depending on their reproductive outcome during the 3 years of follow-up study: (i) 48 RSA couples who achieved a successful pregnancy; (ii) 39 RSA couples who experienced further abortions; and (iii) 33 RSA couples who experienced infertility during the follow-up period. A semen analysis was performed twice at the time of inclusion in the study, and twice again during the 3 year follow-up period. No significant differences in semen parameters were observed between the RSA males and fertile controls. Instead, significant differences were observed between the group of RSA couples who experienced infertility during the follow-up and the other two groups (RSA couples who achieved successful pregnancy and RSA couples who experienced miscarriages and no live birth during the follow-up) for sperm concentration ($P < 0.01$ and $P < 0.01$ respectively), sperm motility ($P < 0.01$ and $P < 0.01$ respectively) and sperm morphology abnormalities ($P < 0.01$ and $P < 0.01$ respectively). Sperm morphology abnormalities do not seem to be involved in determining RSA; instead, they are an aetiological factor in determining infertility in patients, along with the other semen parameters, in the RSA couple's subsequent reproductive life. Semen analysis is an important test in the clinical management of RSA couples.
Key words: infertility/male factor/recurrent spontaneous abortion/reproductive performance/sperm morphology

Introduction

Abnormal sperm morphology has been associated with an increased abortion rate in couples attending an in-vitro fertilization (IVF) programme in which the male had severe sperm head abnormalities compared with normal fertile couples (Kruger *et al.*, 1988). Furthermore, abnormal sperm morphology has been related to fertilization failure and poor embryo cleavage in IVF (Oehninger *et al.*, 1989). Even though the researchers used different criteria to assess the sperm morphology, these data may be correlated with the traditional assessment method (Davis and Gravance, 1994).

The relevance of the male factor in determining recurrent spontaneous abortion (RSA) still remains unclear. Except for cytogenetic abnormalities in peripheral blood karyotyping, no direct evidence has been reported of any 'male factor' involvement. Direct cytogenetic analyses of human spermatozoa have shown that chromosomal abnormalities are not infrequent in normal men (Brandiff *et al.*, 1985; Martin and Rademaker, 1987). However, no association between chromosomal abnormalities and abnormal sperm morphology has been described (Martin and Rademaker, 1988; Rosenbusch *et al.*, 1992). In the male partners of RSA women, even though chromosomal aneuploidy is not increased, a higher frequency of structural abnormality has been observed (Rosenbusch and Sterzik, 1991). However, sperm nucleus defects have been associated with infertility (Zamboni, 1987), and the association of teratozoospermia, chromosomal aberration and male infertility has been documented (Abramsson *et al.*, 1982).

In our study, the incidence of abnormal sperm morphology was evaluated in men whose partners experienced RSA of an unexplained aetiology to determine (i) whether the men are more likely to have an abnormal sperm morphology than those whose partners have no reproductive difficulty, and (ii) whether abnormal sperm morphology influences the reproductive performance in these couples during a 3 year follow-up study.

Materials and methods

A total of 120 couples with idiopathic RSA and three or more consecutive spontaneous abortions were evaluated and selected from a larger group of patients with RSA (~300 couples). The couples were monitored for at least 3 years in the Reproductive Endocrinology Clinic of the Obstetrics and Gynecology Department, University of Rome 'La Sapienza', Rome, Italy. The patients were observed from January 1988 to November 1994. Idiopathic RSA was defined in the absence of uterine abnormalities, diabetes, hypofibrinogenaemia or thrombocytosis, anti-nucleus antibodies, anti-phospholipid antibodies, anti-thyroid antibodies, midluteal progesterone and thyroid hormone abnormalities, and positive cervical cultures for mycoplasma, ureaplasma and *Chlamydia* in the women, or karyotyping abnormalities in both partners. The patients were followed for at least 3 consecutive years of regular sexual intercourse without any contraception, and their reproductive performances were recorded by oral interviews in clinical files. These patients were subdivided into three subgroups: (i) 48 couples who achieved a successful pregnancy during the follow-up, (ii) 39 couples who experienced abortive pregnancy without a live birth during the follow-up and (iii) 33 patients who experienced

Table I. Clinical characteristics of couples studied in the different groups: all recurrent spontaneous abortion (RSA) couples (A), RSA couples with successful pregnancy at the follow-up (B), RSA couples with abortive pregnancy and no live birth at the follow-up (C), RSA couples with infertility during the follow-up (D) and controls (E)

	A	B	C	D	E
n	120	48	39	33	30
Male age (years; ±SD)	36.7 ± 4.9	35.1 ± 3.6	37.4 ± 2.5	36.9 ± 3.9	35.8 ± 3.1
Female age (years; ±SD)	34.1 ± 3.6	33.8 ± 3.0	36.2 ± 3.3	35.6 ± 4.1	35.2 ± 2.8
No. of abortions	3.9 ± 2.8	3.6 ± 2.1	4.3 ± 2.6	3.8 ± 1.6	–
No. of previous live births	18	9	4	5	68

infertility during the follow-up period. Clinical data from all RSA patients and from the different subgroups are shown in Table I. Male partners of RSA couples provided semen samples when included in the study and during the follow-up period. The data from a semen analysis performed twice in each patient at the beginning of the study were considered for the comparison of all the RSA male partners with the controls. For the three subgroups of the follow-up, the data from the semen analysis performed twice in each patient during the follow-up period were considered. The mean values of the two semen samples analysed, before and during the follow-up, were used for the comparisons. A total of 500 semen analyses were performed in the male partners of the RSA couples. Semen samples from 30 healthy male partners of couples experiencing no reproductive difficulties (infertility or recurrent miscarriage) were also analysed and used as controls. Clinical data from these patients are shown in Table I.

Semen was obtained by masturbation into a sterile container after a minimum 48 h sexual abstinence. Samples were kept at room temperature and sent to the laboratory, where they were processed for sperm concentration, motility and morphology within 4 h of collection. Preparations were assessed microscopically by a single trained sperm morphologist, who was blind to the clinical status of the samples. Semen volume was measured in a calibrated test tube. Sperm concentration, percentage of motility (evaluated using a Makler chamber; Sefi Medical Instruments, Haifa, Israel) and morphology were determined according to the procedures described by the World Health Organization (WHO, 1987). For the morphological evaluation, spermatozoa were applied to microscope slides, dried and fixed in 100% methanol. Slides were stained with eosin and thiazine, and a minimum of 100 spermatozoa from each sample were evaluated. Spermatozoa with oval-shaped heads, intact acrosomes comprising 40–70% of the head area and flagella that were straight and regular in outline, and in alignment with the longitudinal axis of the head, were considered 'normal'. In addition, defects of the head (including the acrosome), midpiece and tail, were recorded. Head defects included amorphous, large, small, tapering, duplicate and pin (tails without heads) heads. Acrosomal defects included a deficient, large and small acrosome. Tail defects included coiled and duplicate tails. The intra-observer coefficients of variation was 3.6 for sperm concentration, 4.7 for sperm motility and 5.8 for sperm morphology.

A statistical analysis was performed using unpaired t-tests and a linear regression analysis. Results are reported as means ± SD.

Results

As shown in Table II, the mean values in terms of ejaculate volume, sperm concentration, total number of spermatozoa, sperm motility and sperm morphology of RSA men and controls were normal according to WHO criteria. There was also no significant difference in the ejaculate volume, sperm concentration, total number of spermatozoa and sperm motility between the groups. Similarly, there was no significant differ-

Table II. Seminal parameters in recurrent spontaneous abortion (RSA) male partners and controls

Sperm characteristics	RSA (±SD)	Controls (±SD)
Ejaculate volume (ml)	3.4 ± 1.3	3.1 ± 1.0
Concentration (10^6/ml)	66.5 ± 29.1	65.2 ± 18.3
Motility (%)	46.8 ± 20.6	51.4 ± 18.3
Morphological alterations		
Head defects (%)	9.0 ± 7.1	7.3 ± 6.5
Acrosome defects (%)	3.1 ± 1.7	2.8 ± 1.4
Midpiece defects (%)	4.4 ± 2.9	3.6 ± 2.4
Neck defects (%)	3.1 ± 1.8	2.6 ± 1.1
Tail defects (%)	1.8 ± 1.3	1.4 ± 0.9
Multiple defects (%)	4.2 ± 2.1	3.9 ± 1.7
Total no. of alterations (%)	25.3 ± 12.2	21.1 ± 10.7

There were no significant differences.

Table III. Seminal characteristics in recurrent spontaneous abortion (RSA) male partners of couples with (A) successful pregnancy followed for 3 years, (B) further abortion and (C) sterility

Sperm characteristics	A	B	C
Ejaculate volume (ml)	3.6 ± 1.7	4.6 ± 0.9	3.7 ± 2.1
Concentration (10^6/ml)	67.5 ± 35.6	72.0 ± 22.7	52.8 ± 24.8[a]
Motility (%)	48.2 ± 23.0	56.3 ± 18.7	36.2 ± 16.7[b]
Morphologic alterations			
Head defects (%)	8.9 ± 7.0	8.6 ± 7.5	12.3 ± 8.4
Acrosome defects (%)	2.9 ± 2.1	3.0 ± 2.0	3.9 ± 3.0
Neck defects (%)	2.9 ± 2.2	2.6 ± 1.9	3.6 ± 2.4
Midpiece defects (%)	4.5 ± 3.9	4.4 ± 3.8	6.8 ± 6.1
Tail defects (%)	1.4 ± 1.0	1.5 ± 0.9	2.1 ± 2.0
Multiple defects (%)	4.8 ± 3.7	4.9 ± 3.4	7.1 ± 5.9
Total no. of alterations (%)	25.4 ± 13.3	25.1 ± 10.8	35.9 ± 11.7[c]

[a]C versus A, $P < 0.04$; C versus B, $P < 0.01$.
[b]C versus A, $P < 0.01$; C versus B, $P < 0.001$.
[c]C versus A, $P < 0.001$; C versus B, $P < 0.001$.

ence in either the percentage of total abnormal spermatozoa or the percentage of individual sperm defects between controls and RSA men.

In Table III, the semen analysis data for the follow-up period in the three subgroups are shown. The groups of patients who experienced live births or abortions had normal mean values according to WHO criteria for the ejaculate volume, the concentration of spermatozoa, the total number of spermatozoa, sperm motility and sperm morphology, and there was no significant difference between the two groups. Instead, the subgroup of patients who experienced infertility had mean values below the limits for sperm motility and total abnormal sperm morphology, and there was a significant difference in
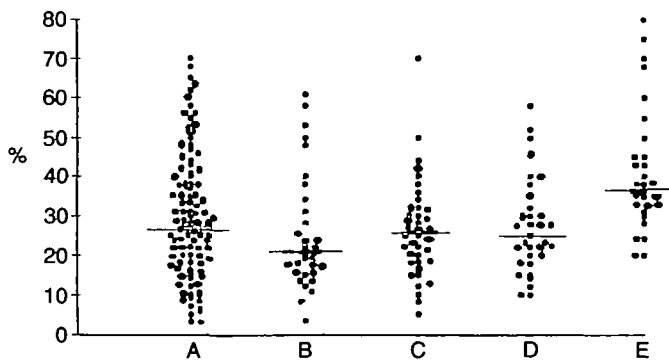
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Figure 1. Individual data of sperm morphology abnormalities in (A) 120 male partners of recurrent spontaneous abortion (RSA) couples, (B) 30 male partners of control couples, (C) 48 male partners of RSA couples with successful pregnancy, (D) 39 male partners of RSA couples with abortive pregnancies and (E) 33 male partners of RSA couples who experienced infertility.

sperm concentration, motility and morphology between this group and the other two groups. No significant differences were observed in the percentage of individual sperm defects between the group with infertility and the other two groups.

In Figure 1, a scatter plot is displayed showing the abnormal sperm morphologies in RSA patients, controls and the three different subgroups after the follow-up period.

No significant correlation was observed between male age and sperm concentration, male age and motility, and male age and abnormal sperm morphology. Instead, a positive linear correlation was observed between normal sperm morphology and sperm concentration ($r = 0.3$, $P < 0.026$).

Discussion

The role of the male factor in determining RSA is controversial. Few data are available in the literature and they are not definitive. Our data are concordant with a recent paper by Hill *et al.* (1994a) which states that abnormal sperm morphology and sperm motility concentrations are not significantly different in male partners of RSA women with respect to controls. Furthermore, the RSA couples who experienced miscarriages (or reproductive failure) during the 3 year follow-up period did not show differences in these sperm parameters compared with patients having live births or ongoing pregnancies. In addition, when the mean values from all four semen analyses performed in each patient were used for comparisons with controls, the results did not change (data not shown). These data suggest that no relationship exists between any major abnormality of sperm morphology and spontaneous abortion, as studied both retrospectively and prospectively. The RSA couples who experienced infertility (no clinically recognizable pregnancy in the absence of contraception) during the 3 year follow-up period showed a lower sperm concentration and sperm motility, and a higher abnormal sperm morphology, with respect to the other couples with RSA who achieved abortive or successful pregnancies. In these couples, like the infertile couples that did not experience RSA, the male factor plays a primary role in the aetiology of infertility (Mcload and Gold, 1951; Mortimer *et al.*, 1982; Rogers *et al.*, 1983). On

the other hand, the higher frequency of sperm abnormalities in RSA couples with infertility during the follow-up period also suggests that infertility may be caused by early embryo losses in a preclinical pregnancy stage. This may be in accordance with the data obtained from IVF studies, in which a high abnormal sperm morphology rate was associated with embryo failure at an early cleavage stage (4- to 16-cell stage; Marsh *et al.*, 1987; Kruger *et al.*, 1988; Oehninger *et al.*, 1989). Conventional sperm morphology studies using light microscopy do not resolve the problem of the role of the 'male factor' in determining abortion. To verify this hypothesis, in-depth studies are required in which a large number of RSA couples must be assembled and followed for a long period to record their reproductive performance. The possibility of a 'chemical pregnancy' and early embryo failure should also be evaluated, specific acrosome markers should be assessed with electron microscopy, and biochemical and genetic analyses should be used to determine possible spermatozoa abnormalities that may influence the incidence of embryo failure and spontaneous abortion. Recently, it has been reported that leukocytospermia may influence embryo survival by interfering with the maternal immune system (Hill *et al.*, 1994b).

The relevance of male gametes in the determination of genetic defects in offspring has been reported previously. Chromosomal abnormalities, both numerical and structural, in spermatozoa are not rare. Different studies have shown a rate of 4–5% aneuploidy in human spermatozoa. These abnormalities, especially the structural ones, seem to increase with paternal age, reaching 13% in males >44 years old (Martin and Rademaker, 1987; Rosenbusch *et al.*, 1992). Paternal age seems to be a significant risk factor for Down's syndrome when the man is >55 years old (Stene *et al.*, 1977). Furthermore, genetic mutations occur in the DNA of the sperm nucleus during spermatogenesis (Vogel and Rathenburg, 1975), with a rate that increases with paternal age. Men aged >40 years have a 20% greater chance of parenting a child born with serious defects (Lian *et al.*, 1986). However, in male partners of RSA couples, chromosomal aneuploidy in spermatozoa does not seem to be increased, even though an increased rate of structural anomalies has been reported (Rosenbusch and Sterzik, 1991).

In our series of patients, morphological abnormalities of spermatozoa are not associated with abortion. Furthermore, they are not correlated with paternal age, whereas they seem to be correlated inversely with sperm concentration.

In conclusion, semen abnormality is not a significant cause of RSA. Instead, abnormal sperm morphology and other semen characteristics seem to be involved in determining infertility in RSA couples. Semen analysis is not essential as a diagnostic test in RSA couples, but it is useful during their clinical management, especially for the diagnosis of possible successive infertility.

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