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RELATION BETWEEN MORPHOLOGICAL DEFECTS IN HUMAN SPERMATOZOA AND SPONTANEOUS ABORTIONS IN PATIENTS UNDERGOING ICSI

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Abstract: To investigate the effect of the 'male factor' and especially the role of sperm morphological defects in the pathogenesis of spontaneous abortion, 107 men undergoing ICSI were studied for semen parameters retrospectively. The patients were divided into two groups, depending on their reproductive outcome: (1) 65 couples who achieved a successful pregnancy (live births); (2) 42 couples who experienced spontaneous abortions. Couples with anovulation, tubal disease, endometriosis, polycystic ovarian syndrome and severe male factor infertility were excluded. Only cases with good quality oocytes were included. Evaluation of morphology was performed according to Tygerberg-Kruger strict criteria. Due to the retrospective nature of the study, the approval of our institutional board was not required. Statistical analysis was performed by using statistical program SPSS (version 21.0). Independent- Sample t-test was performed to find out whether any significant mean difference exist between the studied groups. Results were reported as mean ± standard deviation. Significant differences were considered when *p*-value is less than 0.05.

Significant differences in the percentage of spermatozoa with multiple defects were observed between the two studied groups of patients. The mean value of multiple defects in the abortion group was higher compared to patient group with live births (40.26 ± 9.78 vs. 29.83 ± 7.24; P=0.047). We found a dramatic increase of spontaneous abortions above a cut-off level of 40%. Miscarriage rate among the couples with more multiple defects in males than the mentioned cut-off was significantly higher (74%).

Patients with higher frequency of spermatozoa with multiple defects have an increased chance of spontaneous abortion. Thus, it is concluded that multiple defects found in human spermatozoa may serve as an essential morphological biomarker in the abortion risk assessment models.

INTRODUCTION

The impact of human sperm morphology in assisted reproduction remains controversial (Kruger, 1986; Menkveld *et al.,* 1990; Lundin K. et al., 1997; Kruger T., 1999; Van Waart J. Et al., 2001). Its real value as a prognostic parameter in determining recurrent spontaneous abortion (RSA) after *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) still remains unclear. The poor prognosis could be due to genetically determined sperm pattern defects (short tail syndrome, globozoospermia etc.) or caused by environmental factors, including medications (Menkveld, R., 2010). The effect of sperm morphology on recurrent implantation failure has been studied by some researchers and specific cut-off points were proposed (Van Zyl J. et al. 1999; Van Zyl J. and Menkveld R., 2006;). Furthermore, abnormal sperm morphology has been associated with spontaneous abortion (Kruger *et al.,* 1988; Oehninger S., et al., 1989). However, other authors failed to observe such a relation (Joseph H. et al. 1994; Sbracia M. et al., 1996).

In our study in order to investigate the effect of the 'male factor' and especially the role of sperm morphological defects in the pathogenesis of spontaneous abortion, we compared the frequency of morphological abnormalities in couples who achieved a successful pregnancy and live birth and couples who experienced spontaneous abortions.

MATERIALS AND METHODS

Patients

A total of 107 men undergoing ICSI were studied for semen parameters retrospectively.

Study design

The patients were divided into two groups, depending on their reproductive outcome: (1) 65 couples who achieved a successful pregnancy (live births); (2) 42 couples who experienced spontaneous abortions. Couples with anovulation, tubal disease, endometriosis, polycystic ovarian syndrome and severe male factor infertility were excluded. Only cases with good quality oocytes were included.

Due to the retrospective nature of the study, the approval of our institutional board was not required.

Seminal preparation and evaluation of semen variables and sperm Morphology

Semen samples were obtained by masturbation on the morning of oocyte recovery after a recommended 72 h – 120h of sexual abstinence. Samples were allowed to liquefy and semen analysis was undertaken according to WHO criteria (WHO 5TH Edition, 2010). Semen volume, sperm concentration, sperm motility, and sperm morphology were included in the semen analysis. Semen concentration and motility were assessed by CASA (*Microptic*, S.L., Barcelona, Spain). Sperm morphology was evaluated in a single sample, counting one hundred cells, according to the strict criteria proposed by Kruger/Tygerberg and adopted by the World Health Organization (10), using Sperm Stain Kit (Microoptic S.L.). Finally, 107 slides were randomly distributed to three technicians who assessed them blindly. These technicians were chosen for their experience and accuracy in sperm morphology assessment. They all had worked in the IVF laboratory for at least four years and on a monthly basis assessed 100 to 180 smears made from semen samples. The number of spermatozoa with morphological abnormalities of the head, midpiece, and tail were recorded. Head defects included macrocephalic, microcephalic, elongated, pyriform, round, amorphous and double heads. Midpiece defects included bent, asymmetric, thin and thick midpiece. Tail defects included, short, coiled and double tails. The total number of head, midpiece and tail defects found in a sample was given as cumulative head, cumulative midpiece and cumulative tail defects, respectively. Spermatozoa that have more than one morphological defect were classified as having a multiple defect. Normal sperm and abnormal sperm categories were defined by strict criteria (WHO, 2010) and all abnormalities of each sperm were recorded which makes it possible to calculate the Multiple Anomalies Index, MAI (Jouannet et al., 1988; WHO, 2010) - the mean number of anomalies per abnormal sperm.

IVF Protocols

Ovarian stimulation of multiple follicles was done with recombinant gonadotrophines and GnRH antagonists (Cetrotide) were used to suppress endogenous secretion of gonadotrophines. The ovaries were stimulated with the injectable FSH medication (Menopur) for about 7-12 days until multiple mature size follicles have developed. Patients who presented with at least two follicles with diameter equal or more than 15 mm after ovarian stimulation received injections of hCG. An aspiration was performed 36 hours after the injection of hCG.

Statistical Analysis

Statistical analysis was performed by using statistical program SPSS (version 21.0). Independent- Sample t-test was performed to find out whether any significant mean difference exist between the studied groups. Results were reported as mean ± standard deviation. Significant differences were considered when *p*-value is less than 0.05.

RESULTS AND DISCUSSION

Age of the women and men varied from 24 to 38 years (33.1 ± 6.4 years) and from 26 to 41 years (36.3 ± 6.2 years), respectively and they did not differ significantly between the investigated groups (P=0.79).

As can be seen in Table 1 and Figure 1 the average values of the standard semen variables were within the normal range in both groups. All of the studied specific sperm morphological abnormalities did not show significant differences between the investigated patient groups with the exception of the frequency of multiple defects. There was a higher rate of spermatozoa with multiple defects from men in the spontaneous abortion group in comparison with the group with successful pregnancy, which was statistically significant (40.26 ± 9.78 vs. 29.83 ± 7.24; P=0.047) (Fig. 1).

Table 1. Comparison of semen parameters in patients with spontaneous abortion and successful pregnancy.

	Semen parameter	Patients with spontaneous abortion (mean ± SD)	Patients with successful pregnancy (mean ± SD)	P value*
Standard semen variables	Sperm Volume (ml)	3.07	2,75	0.62
	Sperm concentration (cells x 10^6)	76.81	80.22	0.89
	Sperm motility (A %)	18.34 ± 8.21	20.52 ± 7.54	0.78
	Sperm motility (B %)	21.54 ± 9.58	22.32 ± 8.55	0.83
	Sperm morphology (Kruger) (%)	3.26 ± 2.03	3.67 ± 1.82	0.66
Sperm morphological defects (%)	Cumulative Tail defects	7.63 ± 4.09	6.50 ± 3.06	0.56
	Cumulative Midpiece defects	41.42 ±16.72	46.47 ±3.45	0.58
	Cumulative Head defects	74.42 ± 34.56	69.35 ± 43.22	0.91
	Multiple anomalies index (MAI) **	2.36 ± 0.30	2.30 ± 0.36	0.85
	Multiple defects	40.26 ± 15.36	29.83 ± 14.23	**0.04***
	Macrocephalic heads	14.32 ± 6.73	12.84 ± 8.22	0.66
	Microcephalic heads	17.68 ± 8.01	22.08 ± 10.69	0.21
	Elongated heads	10.16 ± 8.80	10.25± 10.48	0.97
	Pyriform heads	11.32± 6.62	7.75± 6.06	0.14
	Round heads	11.11± 7.70	14.58±11.54	0.32
	Amorphous heads	22.74±11.28	15.58±12.04	0.11
	Double heads	0.74±1.90	5.17±11.24	0.10
	Bent midpiece	6.89±6.98	6.92±4.61	0.99
	Asymetric midpiece	3.42±3.22	7.42±5.86	0.07
	Thin midpiece	0.95±1.35	3.08±3.91	0.08
	Thick midpiece	16.11±12.67	12.50±10.24	0.42
	Pinheads	5.11±2.60	4.58±3.47	0.64
	Short tails	7.84±5.88	9.00±10.52	0.70
	Coiled tails	6.63±5.61	5.08±4.54	0.49
	Double tails	1.05±1.68	1.92±2.37	0.35

* Significant difference between studied patient groups (P<.05).
** Multiple anomalies index (MAI) = Total number of isolated and associated anomalies in a sample / number of abnormal sperm.

We found a dramatic increase of spontaneous abortions above a cut-off level of 40%. Miscarriage rate among the couples with more multiple defects in males than the mentioned cut-off was significantly higher (74%) (Fig. 1).

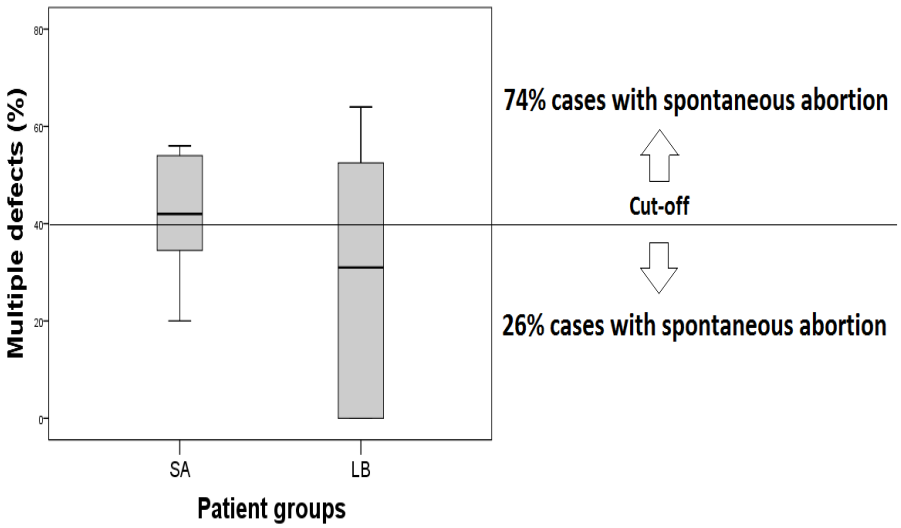

Fig. 1. Multiple defects in Group 1 (Patients with spontaneous abortion - SA) and Group 2 (Patients with live births (LB). The box plots show the distribution of multiple defects in 107 patients assigned to groups 1 (65 samples) vs. 2 (32 samples).

Miscarriage is a surprisingly common occurrence (15% of all clinically recognized pregnancies) and is definitely a problematic area in reproductive medicine (Ford H. et al., 2009). This is most often due to an unknown etiology and a lack of proven treatment algorithms. Moreover, the contribution of male factor and sperm morphology to spontaneous abortion is strongly neglected (Zidi-Jrah I. et al., 2016).

In the present study we did not find a significant difference in the standard semen parameters and most of the sperm morphological abnormalities in the patient group with spontaneous abortion compared to the patients with successful pregnancy (Table 1). However, a significant difference was observed in the percentage of spermatozoa with multiple defects (P=0.04). A similar tendency of increase of multiple defects in the patients with spontaneous abortion has been shown by other researchers, although they did not obtain such a statistically significant results (Grow *et al.,* 1994; Joseph H. et al. 1994; Sbracia M. et al., 1996).

It was previously reported for ICSI using human spermatozoa that there was a higher rate of early spontaneous abortion in patients with low frequency of morphologically normal cells (<4%) (Payne et al., 1994; Soderlund et al., 1994). The detailed assessment of the incidence of sperm morphological abnormalities and multiple anomalies index (MAI) are considered as more useful than a simple evaluation of the percentage of normal spermatozoa to study and predict the effect of specific morphological abnormalities on important pregnancy events (Auger J. et al., 2001). These findings indicated that the main problem with morphologically abnormal spermatozoa was that they may have resulted in a higher percentage of abnormal embryos which aborted early in gestation (Janny and Menezo, 1994).

We should notice that during ICSI, only morphologically normal human spermatozoa are typically used to fertilize the oocyte. However, the presence of specific sperm morphological defects in the sperm population could be considered as a key indicator of the "hidden" physiological problems occurring during spermatogenesis that persist in all spermatozoa in the particular semen sample.

CONCLUSIONS

Patients with higher frequency of spermatozoa with multiple defects have an increased probability of spontaneous abortion. Thus, it is concluded that multiple defects found in human spermatozoa may serve as an essential morphological biomarker in the abortion risk assessment models.

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REFERENCES

1. Auger J., Eustache F. Andersen A.G., Irvine D.S., Jorgensen N., Skakkebaek N.E., Suominen J., Toppari J., Vierula M. and Jouannet. P,2001. Sperm morphogical defects related to environment, lifestyle and medical history of 1001 male partners of pregnant women from four European cities, Human Reproduction vol.16 no.12 pp. 2710-2717.
2. Ford H.B.,MD, Schust D.J.,MD, 2009.Recurrent pregnancy loss: etiology, diagnosis, and therapy. Rev Obstet Gynecol. 2(2):76-83.
3. Grow, D.R., Oehninger, S., Seltman, H. et al. .1994. Sperm morphology as diagnosed by strict criteria: probing the impact of teratozoospermia on fertilization rate and pregnancy outcome in a large in vitro fertilization population. Fertil. Steril., 62, 559–567.

4. Hamamah S., A.Fignon and J.Lansac. 1997. The effect of male factors in repeated spontaneous abortion: lesson from in-vitro fertilization and intracytoplasmic sperm injection, Human Reproduction Update, Vol. 3, No. 4 pp. 393–400.
5. Janny, L. and Menezo, Y.J.R. .1994. Evidence for a strong paternal effect on human preimplantation embryo development and blastocyst formation. *Mol. Reprod. Dev.*, **38,** 36–42.
6. Joseph A. Hill, M.D.:j:Amy F. Abbott, B.S, Joseph A. Politch, Ph.D.1994. Sperm morphology and recurrent abortion FERTILITY AND STERILITY Vol. 61, No.4.
7. Jouannet, P., Ducot, B., Feneux, D. and Spira, A. (1988) Male factors and the likelihood of pregnancy in infertile couples. I. Study of sperm characteristics. Int. J. Androl., 11, 379-384.
8. Kruger, T., Coetzee, K. 1999. The role of sperm morphology in assisted reproduction. Hum Reprod Updat 5:172–178.
9. Kruger TF, Menkveld R, Stander FSH, Lombard CJ . 1986. Sperm morphological features as a prognostic factor in IVF. Fertil Steril 46: 1 1 18- 1 123.
10. Kruger, T.F., Acosta, A.A., Simmons, K.F., Swanson, RJ., Malta, J.F. and Oehninger, S. 1988. Predictive value of abnormal sperm morphology in in vitro fertilization. Fertil Sterii, 49, 112-117.
11. Lundin, K., So¨derlund, B. and Hamberger, L. 1997. The relationship between sperm morphology and rates of fertilization, pregnancy and spontaneous abortion in an in-vitro fertilization/intracytoplasmic sperm injection programme, Human Reproduction vol.12 no.12, pp.2676 2681.
12. Menkveld, R. .2010. Clinical significance of the low normal sperm morphology value as proposed in the fifth edition of the WHO Laboratory Manual for the Examination and Processing of Human Semen, Asian Journal of Andrology, 12: 47–58
13. Menkveld R, Stander FSH, Kotze TJW, Kruger TF, van Zyl JA .1990. The evaluation of morphological characteristics of human spermatozoa according to stricter criteria. Hum Reprod 5:586-592.
14. Oehninger S, Acosta AA, Morshedi M, Veeck L, Swanson RJ, Simmons K, et al. 1989. Corrective measures and pregnancy outcome in in vitro fertilization in patients with severe sperm morphology abnormalities. Fertil SteriI;50:283-7.
15. Sbracia M., Cozza G.,.Grasso J.A, Mastrone M. And Scarpellini F. .1996. Semen parameters and sperm morphology in men in unexplained recurrent spontaneous abortion, before and during a 3 year follow-up period, Human Reproduction vol.11 no.l pp. 117-120.
16. Soderlund, B., Lundin, K. and Hamberger, L. .1994. Results from IVF and ICSI correlated to sperm morphology. *Hum. Reprod.*, **9** (Suppl.4), 46.
17. Van Waart, J, Kruger T., Lombard, C., Ombelet, W. 2001. Predictive value of normal sperm morphology in intrauterine insemination (IUI): a structured literature review. Hum Reprod Updat 7:495–500.
18. Van Zyl JA, Menkveld R. 2006. Oligozoospermia: recent prognosis and outcome of 73 pregnancies in oligozoospermia couples. Andrologia; 38: 87–91.
19. Van Zyl JA, Kotze TJ, Menkveld R. 1990. Predictive value of spermatozoa morphology in natural fertilization. In: Acosta AA, Swanson RJ, Ackerman SB, Kruger TF, van Zyl JA, et al, editors. Human Spermatozoa in Assisted Reproduction, Baltimore: Williams and Wilkins;, pp. 319–24.

20. Zidi-Jrah I., Hajlaoui A., Mougou-Zerelli S., Kammoun M., Meniaoui I., Sallem A., Brahem S., Fekih M., Bibi M., Saad A., and Ibala-Romdhane S. 2016. Relationship between sperm aneuploidy, sperm DNA integrity, chromatin packaging, traditional semen parameters, and recurrent pregnancy loss, Fertility & Sterility, VOL. 105 NO. 1, 58-64.
21. WHO. 2010. WHO Laboratory Manual for the Examination and Processing of Human Semen. 5th ed. Geneva: WHO Press;.pp. 271.