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SPERM-ZONA ADHESION PROPERTIES ARE RELATED TO THE CONVENTIONAL SEMEN PARAMETERS

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Abstract: The conventional semen analysis, which includes sperm volume, pH, sperm count, concentration, motility and morphology measurements, does not provide enough data on the fertilization ability of the spermatozoa. Since the sperm zona binding properties were found to be highly predictive for the assisted reproduction outcome, our aim was to estimate how the sperm-zona adhesion score (SZAS) was related to the conventional semen parameters.

A total of 141 semen samples were collected from patients of Nadezhda Women's Health Hospital. Regular sperm analysis was performed according to WHO 5th edition (2010). Whole zonae from healthy donors' were acid solubilised and immobilised on petri dishes. 2.5 x105 motile spermatozoa from each patient were placed in the dishes and the adhered spermatozoa were counted. Sperm-zona adhesion score (SZAS) presents the sperm-zona adhesion rate evaluated by the number of adhered spermatozoa in 1 mm2 of zona pellucida coated area.

No relation was observed between the SZAS and the physico-chemical properties of the semen (volume and pH), and the sperm count and concentration ($p>0.05$). The percentage of progressively motile spermatozoa and the zona adhesion rate showed slight positive correlation while the percentage of immotile spermatozoa was negatively related to the SZAS ($r=0.25$; $P=0.03$ and $r=-0.16$; $p=0.05$, respectively). The morphological evaluation also related positively to the zona adhesion ability of the spermatozoa ($r=0.18$; $p=0.03$).

In conclusion, reduced sperm mobility and morphological abnormalities may indicate dysfunctional zona adhesion properties of the spermatozoa.

INTRODUCTION

Routine spermogram is a convenient analysis for initial medical examination during assisted reproduction treatment. However, the conventional semen analysis

does not provide enough information on the causes of male infertility (Wang and Swerdloff, 2014). About 15% of infertile men have normal semen analysis – a fact which complicates a definitive diagnosis (Evgeni et al., 2014). There is a need for the application of additional tests that can accurately determine the functional aetiology of the male infertility. Hence a series of tests have been introduced in routine laboratory practice, aiming to enrich the diagnostic information regarding sperm functional abilities (Evgeni et al., 2014).

The sperm adhesion to zona pellucida is a cellular interaction which represents the first direct physical contact between the male and female germ cells (Reid et al., 2013). Multiple studies have demonstrated that there is a strong correlation between the sperm fertilisation potential and its adherence properties (Vogiatzi et al., 2013). Only mature and functionally active spermatozoa are able to adhere to and penetrate the zona (Liu and Baker, 2000). The inability to cross the selection barrier causes infertility in many patients and imposes the need for an adequate assisted reproduction treatment.

The sperm-zona adhesion rate is evaluated by the number of adhered spermatozoa in 1 mm2 of zona pellucida and is presented by the sperm-zona adhesion score (SZAS). The aim of the present study was to estimate how the SZAS is related to the conventional semen parameters.

MATERIALS AND METHODS

Semen samples

Semen samples were obtained from 141 patients of Nadezhda Women's Health Hospital. Samples were allowed to liquefy for 30 min at 37°C. The semen volume and pH were measured and the sperm count, concentration and motility were assessed by computer assisted sperm analysis (MedeaLAB-CASA software, ver. 5.3). Sperm morphology was evaluated manually according to WHO (2010) criteria. Patients were classified by semen quality group as normozoospermic (N), teratozoospermic (T), oligoteratozoospermic (OT), asthenoteratozoospermic (AT) and oligoasthenoteratozoospermic (OAT).

Sperm-zona adhesion assessment

Sperm-zona adhesion test was performed as previously described (Ganeva et al., 2019a). Briefly zonae pellucidae from healthy donors' germinal vesicles (GVs) were acid solubilised and immobilised on petri dishes. 2.5 x10^5 motile spermatozoa from each patient were placed in the dishes and the adhered spermatozoa were counted. Results are presented as number of adhered spermatozoa in 1 mm2 of zona pellucida coated area (sperm-zona adhesion score, SZAS). The cut off value for classification of our cases was accepted to be 65 ("good" zona adhesion with SZAS≥65 and "bad" zona adhesion with SZAS<65) (Ganeva et al., 2019b).

Statistical analysis

Data were checked for normal distribution using Kolmogorov-Smirnov test. Normally distributed data were subsequently analysed using ANOVA test followed by LSD multiple comparison post hoc testing. Data that were not normally distributed were analysed by non-parametric Kruskal–Wallis followed by LSD multiple

comparisons post hoc testing. Spearman correlation test showed the relationship between the common semen parameters and the sperm-zona adhesion score. Statistical analysis was performed using IBM SPSS Statistics v.21 Software. A value of $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

Conventional semen parameters and SZAS were analysed and compared in a total of 141 men. The mean age of the included patients was 38±8 years. Sperm characteristics including SZAS are presented in Table 1.

Semen quality groups and the SZAS

According to the results of the semen analysis patients were classified as normozoospermic (n=33), teratozoospermic (n=86), oligoteratozoospermic (n=5), asthenoteratozoospermic (n=14) and oligoasthenoteratozoospermic (n=3). Comparison of the SZAS was done between normozoospermic, teratozoospermic, oligoteratozoospermic, asthenoteratozoospermic and oligoasthenoteratozoospermic infertile men. There were no significant differences in the mean SZAS between these groups ($p>0.1$), except for the OAT. Oligoteratozoospermic patients had significantly lower SZAS compared to the other four groups ($p<0.05$).

Table 1 Mean semen characteristics and SZAS of the studied patients.

Seminal parameters	Mean±SD	Ref
Volume, ml	3±2	≥1,5
pH	8±0,3	≥7,2
Sperm concentration, $x10^6$/ml	97±64	≥15
Sperm count, $x10^6$	272±194	≥39
Rapid progressive class A, %	24±9	≥25
Progressively motile (A+B), %	51±8	≥32
Immotile class D, %	38±13	≤60
Morphology, %	9±3	≥14
SZAS, sperm/mm^2	107±85	≥65

Table 2 Mean SZAS and standard deviation in the studied semen quality groups.

Semen quality group	n	Mean±SD	N, (%) cases <65 SZAS
All	141	107±85	52 (37%)
Normozoospermia	33	106±84	12 (36%)
Abnormal /T, OT, AT, OAT/ groups	108	88±32	39 (36%)
Teratozoospermia	86	112±88	29 (33%)
Oligoteratozoospermia	5	112±80	2 (36%)
Asthenoteratozoospermia	14	91±82	6 (40%)
Oligoasthenoteratozoospermia	3	37±32	3 (100%)

Our study revealed, that 37% of all the patients (n=52) have SZAS below the accepted cut off value for successful pregnancies (SZAS=65) (Ganeva et al., 2019b). In addition, between 30-40% of patients with teratozoospermia (T), oligoteratozoospermia (OT) and asthenoteratozoospermia (AT) have SZAS below 65 (Table 2). All patients with oligoasthenoteratozoospermia (100%) have poor zona adhesion score (SZAS<65). Interestingly, 36% of the normozoospermic patients also showed low SZAS.

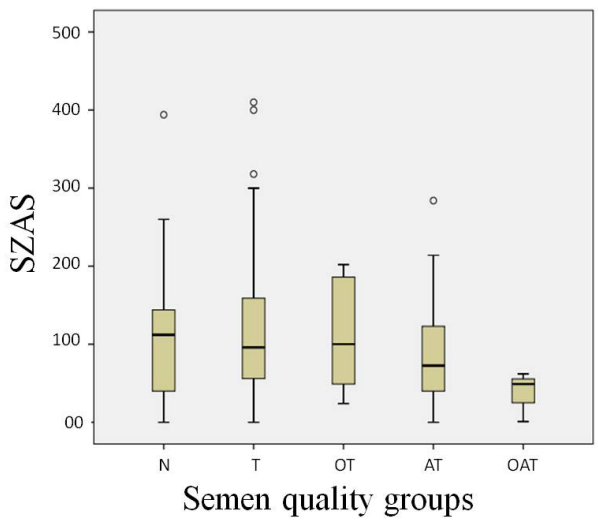

Fig. 1 Box plot, presenting the SZAS in the semen quality groups: normozoospermic (N), teratozoospermic (T), oligoteratozoospermic (OT), asthenoteratozoospermic (AT) and oligoasthenoteratozoospermic (OAT).

Conventional semen analysis parameters and SZAS

The conventional semen analysis parameters of all patients were analyzed in relation to their SZAS. No relation was found between the SZAS and the physico-chemical properties of the semen (volume and pH), as well as the sperm count and concentration ($p>0.05$).

However, significant correlation was observed between the SZAS and the sperm motility and morphology. The percentage of progressively motile spermatozoa (Class A) and the zona adhesion rate showed slight positive correlation while the percentage of immotile spermatozoa (Class D) was negatively related to the SZAS ($r=0.25$; $P=0.03$ and $r=-0.16$; $p=0.05$, respectively). Also the forward motility (Class A+B) showed significant correlation with SZAS. The morphological evaluation also related positively to the zona adhesion ability of the spermatozoa ($r=0.18$; $p=0.03$) (Table 3).

Forward motility rate (Class A+B) sperm was categorized in two groups: ≥32% and <32%, according to WHO 2010. A total of 123 patients with a motility

Table 3 Spearman correlation between sperm-zona adhesion score (SZAS) and conventional semen parameters.

		Volume	pH	Sperm conc.	Sperm count	A%	**A+B%**	B%	C%	D%	Sperm morph.
SZAS	r	-0,01	-0,03	0,09	0,09	0,25	0,18	0,03	-0,1	-0,16	0,18
	p	0,9	0,7	0,3	0,3	0,003*	0.03*	0,7	0,2	0,05*	0,03*
	N	141	141	141	141	141	141	141	141	141	141

rate ≥32% had a mean zona adhesion of 111 SZAS, while the patient group with a motility rate <32% showed a 78 SZAS ($p>0.5$) (Fig.2A). Class D (group of sperm immotile spermatozoa) was also subdivided into subgroups with a cut off value of 60% (immotile rate >60% and less than 60% respectively) Again no significant differences between the groups ($p>0.05$) was reported (Fig.2B). Similar lack of significance was observed according to the morphology of the sperm (Fig.2C).

The initial step in the diagnostic investigation of male infertility has been traditionally based on the conventional seminal profile (Khatun et al., 2018). Sperm-zona adhesion rate has emerged as a potential causative factor of reproductive failure and its assessment has been suggested as a useful adjunct to the laboratory methodology of male infertility evaluation, especially before the application of assisted reproduction technology (ART) (Liu et al., 1989). However, there are significant limitations regarding the ability of the conventional semen evaluation to determine the underlying mechanisms which cause men sterility.

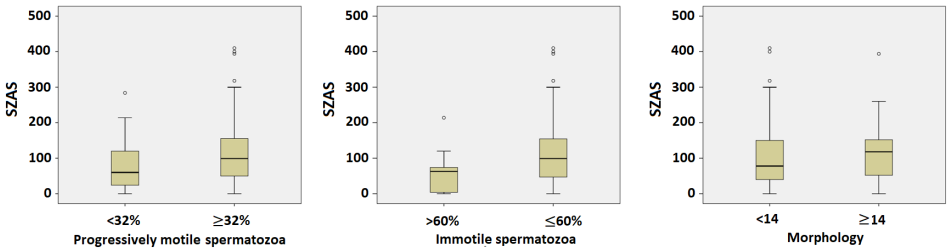

Fig. 2 Box plot presenting the SZAS, plotted by semen parameters dichotomized according to WHO reference values.
A) progressively motile; B) immotile; C) morphology.

That's why other parameters with potentially predictive value for the assisted reproduction outcomes have been applied. Sperm functionality towards the zona appears as distinct sperm characteristic apart from the semen analysis. It helps to identify the functional status of the sperm (Payan-Carreira et al., 2013). Some authors confirm that 31% of morphologically characterized as teratozoospermic (Liu and Baker, 2003) and 28% of oligozoospermic (Liu and Baker, 2004) patients have poor sperm zona binding measured by hemizona assay. In our study this percentage is concordant with those findings but the relevant groups show even higher values – 33% for teratozoospermic, 36% for oligoteratozoospermic. In addition, the share of "bad" zona pellucida properties is 40% in asthenoteratozoospermic and reaches maximum (100%) in oligoasthenoteratozoospermic patients (Table 2).

Interestingly, some studies show that half of the infertile normozoospermic men experienced poor zona-adhesion abilities (Liu and Baker, 2000). In our study, 36% of normozoospermic patients show SZAS lower than 65 (Table 2). It has been commented that infertility among those patients may be contributed to impaired zona-induced acrosome reaction (Liu et al., 2007).

The major limitation of the standard sperm evaluation remains the fact that many functional abnormalities that have negative effect on the clinical outcome could remain hidden (Payan-Carreira et al., 2013). There is a strong correlation between the sperm morphology and the sperm–ZP binding index. Grade A motility spermatozoa were found to be significantly associated with a successful fertilization (Sifer et al., 2005). According to our results, progressively motile spermatozoa (Class A) showed positive correlation to the zona adhesion rate while the immotile spermatozoa (Class D) were negatively related to the SZAS (Table 3). This correlation was confirmed by other authors using different methods of measuring the zona-connecting properties. Liu and Baker (Liu and Baker, 2004) found significant correlation between progressive motility and the number of sperm bound to zona pellucida. It has been documented that the impaired sperm-zona interaction is mainly caused by defects in sperm structure and function (Liu and Baker, 2000) like large symmetric anterior head region

(Garrett et al., 1997). Reduced sperm-zona adhesion can be also caused by missing or defect molecules on the spermatozoa membrane (Tardif et al., 2010). A number of receptor molecules have been investigated responsible for contact with the zona, such as zonadhesin (Tardif et al., 2010), ZP3R protein (Bleil et al., 1990), b-1,4-galactosyltransferase (GalT1) (Miller et al., 1992), etc. Other factors, including genetic ones, were also discussed in relation to the decreased ability of spermatozoa to adhere to ZP. They may be related to disturbances in spermatogenesis leading to sperm's inability to recognize and adhere to zona pellucida, for example high levels of H2O2 (Oehninger et al., 1995), genetic mutations in men ZPBP1 gene (Yatsenko et al., 2012) and many others.

CONCLUSION

In conclusion, the reduced sperm mobility and morphological abnormalities may indicate dysfunctional zona adhesion properties of the spermatozoa – a fact which subsequently affects their fertilisation ability.

AUTHOR CONTRIBUTIONS

G.S., R.G. and D.P. conceived the experiment; R.G., M.V., K.N. conducted the experiment; R.G. and D.P. analyzed the results. R.G. and D. N. wrote the main manuscript text and prepared tables and figures. G.S., R.G. and D.P. edited the manuscript and made its final revision. All authors critically reviewed and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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