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Value Analysis of Sperm Spontaneous Acrosome Reaction in Male Fertility Evaluation

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Abstract

Objective: Using correlation analysis between the sperm spontaneous acrosome reaction rate (SARR) and routine semen parameters in male infertility patients, we explored the value of the sperm spontaneous acrosome reaction in the evaluation of sperm functions.

Methods: The participants comprised of 219 male infertility patients who had visited the Center for Reproductive Medicine, Shandong University from October 2016 to March 2017. According to the sperm SARR, we classified the patients with infertility into the control group and the case group. According to the WHO human semen examination and processing laboratory manual, fifth edition, recommendations and standards. We obtained routine semen parameters including semen volume, sperm concentration, total motility, and progressive sperm rate, and we measured sperm motility rate and the proportion of sperm with normal morphology.

Results: With regard to the total motility, progressive sperm rate, sperm survival rate and the proportion of sperm with normal morphology, there were statistically significant differences between the two groups ($p<0.01$), but the data concerning patients' age, semen volume, or sperm concentration showed no significant difference ($p>0.05$).

Conclusion: Sperm SARR in patients with infertility is closely related to routine semen parameters and plays a supplementary reference in male fertility evaluation.

Keywords: Acrosome reaction; Sperm function; Spontaneous acrosome reaction; Sperm motility; Progressive sperm rate

Introduction

The acrosome is characterized by the unique properties of sperm cells, and acrosomal enzyme contains various hydrolytic enzymes that allow sperm to break through the external barrier to the oocyte [1-4]. Sperm can release a variety of enzymes after the acrosome reaction to stimulate sperm cells to combine with egg cells.

Only sperm with an intact acrosome could combine with the egg zona pellucida to trigger an acrosome reaction [5]. If the sperm acrosome reaction happens before sperm combine with the egg zona pellucida, there is no stimulation of sperm and egg cells which would lead to combination; this kind of acrosome reaction is called a spontaneous acrosome reaction.

Recent studies of the acrosome reaction mainly concentrate on the acrosome reaction mechanism and molecular regulation mechanism [6-9], but there has been less research about the importance of the sperm spontaneous acrosome reaction and the relationship between the sperm spontaneous acrosome reaction and the sperm capability. In this paper, we will evaluate the significance of the sperm spontaneous acrosome reaction through analysis and study of SARR sperm and semen parameters.

Materials and Methods

Clinical data

This study was a retrospective analysis of 219 patients with male infertility from October 2016 to March 2017. Our study has been approved by the Institutional Ethic Review Board of Shandong University. We collected all male infertility patients' related data for statistical analysis and did not set other inclusion and exclusion criteria.

All results of patients' sperm analysis were extracted from the patient's medical records. According to the reference value of 10% of the sperm acrosome reaction rate, as being the critical value, patients with infertility were divided into the case group (109 cases) and the control group (110 cases). According to the differences in SARR, the control group and the case group was divided into two small groups respectively.

Semen sample collection

All semen samples were collected by patients themselves using the masturbation method in essence, in which they were instructed before sample collection. Patient guidance on sample collection included patients' abstinence days which were between 2 days and 7 days; the complete semen was to be collected in a one use sample tube, kept warm and delivered to the laboratory within 30 minutes. The semen volume was registered, and the sample was placed in a 37°C water bath while waiting for semen liquefaction.

Routine semen analysis

Semen was blended by a one-use dispette after liquefaction, and 5 µl placed on semen analysis slides, semen routine analysis was measured using computer-aided sperm analysis (CASA, sperm class analyzer, Spain) to obtain parameters including sperm concentration, sperm total motility, and sperm progressive sperm rate.

Sperm low permeability analysis

The liquefied semen (100 µl) in sperm hypotonic expansion reagent (Anhui Anke Biotechnology) was mixed and placed in the 37°C water bath for 30 minutes. Then, at least 200 sperm were counted using 40X magnification and the survival sperm rate was calculated.

Sperm papanicolaou staining and morphology analysis

The blended liquefied semen (5 µl) was used to prepare an air-dried semen smear. The sperm smear was stained using a modified papanicolaou staining method. According to The WHO human semen examination and processing laboratory manual (5th), standard, we counted at least 200 sperm under oil immersion and recorded the number of different kinds of deformed sperm using strict criteria; we then calculated the percentage of sperm with normal morphology [10].

Sperm SARR determination

An agglutinating reagent with fluorescein isothiocyanate (FITC-PSA, sperm acrosome staining kits, Anhui Anke Biotechnology) was used to stain the acrosome immediately after processing of the semen sample. The liquefied semen (200 µl) was placed in a 1.5 ml microcentrifuge tube, and washed twice with 1 ml physiological saline and centrifugation.

The supernatant was removed, the precipitate blended and the sperm smear prepared. The smear was fixed in 95% alcohol after air drying for 30 minutes, meanwhile, the FITC-PSA working solution was prepared. The FITC-PSA working solution (100 µl) was added dropwise to the smear and followed by a cover glass. The smear was the placed at 4°C in the dark for 16 hours. Then the slides were washed by running water and air dried.

The slides were examined immediately at 1000X magnification with a NIKO biological microscope (NIKO, Japan) with a BP 450-490 nm excitation filter. For each sample, 200 sperms were observed and the percentage of acrosome-reacted sperm was determined. When there was a lack of fluorescence on the head or only a thin fluorescence was shown in the equator part of the sperm, the sperm was considered as having undergone spontaneous acrosome reactivity; when the sperm showed fluorescence on any part of the entire head, the sperm was counted as an unreacted sperm.

Statistical analysis

The statistical analyses were carried out by the Statistical Package for Social Science for Windows, for Social Sciences (SPSS, version 20.0, IBM, Chicago, IL, USA), and the data were presented using mean ± standard deviation (SD). All data conformed to standard normal distribution.

We used Student's t test to obtain the difference between the case group and control group for various semen parameters and the morphological index, and we used one-way analysis of variance in appropriate time to evaluate the difference of various parameters among different SARR groups. Pearson's correlation was used to perform the correlation analysis. $P<0.05$ was considered statistically significant.

Results

Sperm spontaneous acrosome reacted morphology

The reference standard is derived from Perry [11].The unreacted sperm showed bright green fluorescence surrounding the head. Sperm with spontaneous acrosome reactivity had no fluorescence on the head or showed only a very thin green fluorescence (Figure 1).

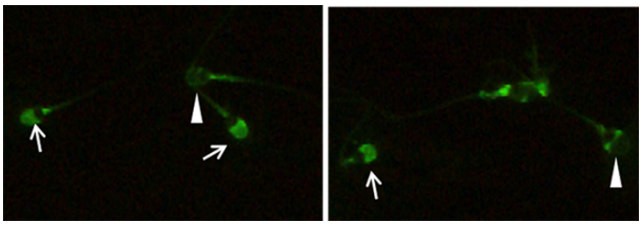

Figure 1: Sperm with spontaneous acrosome reactivity [Note: → indicates unreacted sperm; ▲indicates sperm with acrosome reactivity (1000X under a fluorescence microscope)].

Relationship of sperm SARR, routine semen parameters, and sperm morphological indexes

There were significant differences in the percentage of progressively motile sperm, total motility, sperm survival rate, and the proportion of sperm with normal morphology between the case group and control group ($p<0.01$). No statistical difference was observed in patients' age, semen volume, or sperm concentration in the 2 groups ($p>0.05$) (Table 1).

Comparison of various parameters among four small groups

When the sperm SARR was observed to be different, the semen routine parameters of the control group were also different from the case group. As the sperm SARR increased, the sperm parameters including the percentage of progressively motile sperm, total motility, sperm survival rate, and the percentage of sperm with normal morphology declined gradually, and those 4 parameters had a significantly negative correlation with SARR (Table 2).

In the 4 groups, patients' age, semen volume, and sperm concentration had no statistical difference, these parameters and SARR had no correlation (Table 2).

Group	SARR (%)	n	Age (years)	Volume (ml)	Sperm concentration (×10^6/ml)	Progressively motile sperm (%)	Total sperm motility (%)	Survival rate (%)	Morphologically normal sperm (%)
Control	≤ 5	61	34.90 ± 6.24	3.28 ± 1.28	56.65 ± 56.10	40.74 ± 17.15	53.47 ± 22.46	68.61 ± 14.60	4.63 ± 2.77
	5<SARR ≤10	49	34.39 ± 5.02	3.47 ± 1.47	54.46 ± 58.43	40.78 ± 17.61	53.20 ± 21.55	65.82±14.84	4.27 ± 2.61
Case	10<SARR ≤ 15	91	35.65 ± 6.74	3.75 ± 1.52	50.58 ± 43.57	35.09 ± 18.28	41.98 ± 21.84	59.49±16.49	2.99 ± 1.94
	>15	18	34.00 ± 7.81	3.83 ± 1.99	34.47 ± 24.11	26.28 ± 23.07	33.62 ± 28.96	50.11 ± 23.73	2.43 ± 1.66
P^a	-	-	0.41	0.47	0.25	<0.01	<0.01	<0.01	<0.01
P^b	-	-	0.6	0.22	0.4	<0.01	<0.01	<0.01	<0.01
Relationship of sperm SARR, routine semen parameters and sperm morphological indexes are significantly differs at (P<0.01).									

Table 1: Relationship of sperm SARR, routine semen parameters and sperm morphological indexes (x ± s) [Note: p^a: VS case group, t test. p^b: one-way analysis of variance test in four small groups].

Parameters	r	P
Age (years)	-0.02	0.81
Volume (ml)	-0.04	0.59
Sperm concentration (×10^6/ml)	-0.09	0.17
Progressively motile sperm (%)	-0.27	<0.01
Total sperm motility (%)	-0.31	<0.01
Survival rate (%)	-0.41	<0.01
Morphologically normal sperm (%)	-0.23	<0.01
The sperm parameters including progressively motile sperm, total sperm motility, survival rate and morphologically normal sperm had a significantly negative correlation with SARR (P<0.01).		

Table 2: Correlation analysis between SARR and various semen parameters (n=219) [Note: SARR and semen parameters were analyzed with Pearson correlation analysis].

Discussion

The acrosome is a cystic structure covering two-thirds of sperm head before the sperm nuclei and lies between the cell membrane and nuclear membrane. It consists of 3 parts including the acrosome inner membrane, outer acrosome membrane, and acrosome antrum. When an acrosome reaction occurs, acrosome enzymes are released to promote acrosome inner membrane exposure, and then the specific molecules on the acrosome inner membrane participate in zone pellucida identification of egg cells [12].

According to the difference in the start-up factors of the acrosome reaction, the reaction is divided into an induced acrosome reaction or a spontaneous acrosome reaction. The induced sperm acrosome reaction occurs when some inducers such as progesterone, calciumions, and follicular fluid stimulate the acrosome reaction [13-16]. The present study focuses on the related mechanism of sperm and egg combination and the method of triggering the acrosome reaction by the way of experiment induced by this application agent. Although the spontaneous acrosome reaction affects sperm and egg combination, related research on the specific mechanism and clinical application is lacking. The aim of this research is to explore the value of the spontaneous acrosome reaction for evaluating sperm function.

Abu and co-workers think that sperm capacitation is an essential process before the acrosome reaction and process of fertilization [17]. In our study, we used fresh semen sample to check the spontaneous acrosome reaction without any special capacity experiment. The agglutinating substance could specifically bind to the acrosome enzyme glycoprotein sugar chain, and agglutinin labeled by fluorescein isothiocyanate was observed under the fluorescence microscope.

We observed particular fluorescent area on the sperm head without any special capacity experiment, and the particular fluorescent area was not seen in the spontaneously reacted acrosome head. We determined the sperm SARR for fresh semen without any capacity experiment procedure, and we found different degrees of spontaneous acrosome reaction of the sperm head in these semen samples. The results showed that whether or not sperm capacitation is necessary for sperm acrosome reactions, fresh sperm without any capacity experiment handling can also undergo the process of sperm acrosome reaction.

This study found that there was a significant difference in the semen total motility and progressive sperm rate between the case group and control group. This may be due to the incomplete sperm acrosome after acrosomal enzyme release, while sperm acrosome have acrosome enzyme complex systems related to sperm activity and sperm motility. The intact sperm acrosome indirectly leads to sperm total motility and progressive sperm rate decline. Reports have confirmed that the sperm motility rate and progressive sperm rate is closely related to the function of sperm fertilization [18,19].

This study also found that there exist significant differences in sperm motility rate and the proportion of sperm with normal morphology between the case group and control group, and these 2 function parameters exist a significant negative correlation compared

with sperm SARR, this also verifies that the SARR has a certain correlation with sperm function.

Work by Wiser has found that the sperm after spontaneous acrosome reaction lost fertilization ability. When the SARR is more than 10%, the sperm shows poor sperm quality [20], and it is consistent with our results. Although the total motility and progressive sperm rate in the case group is significantly lower than normal group, the value of the two semen parameters is higher than that stipulated in the WHO limit.

That is to say, according to the standard in The WHO human semen examination and processing laboratory manual, fifth edition, these semen parameters including sperm concentration, sperm motility and progressively motile sperm in the 2 groups are all normal. As sperm SARR gradually increased, the total motility and progressively motile sperm gradually decreased. When SARR is greater than or equal to 10% but less than 15%, the two indexes are still in the normal reference range, but the proportion of sperm with normal morphology is lower than the reference value 4%.

The proportion of sperm with normal morphology is fertilized "gold standard" of sperm quality assessment. This shows that although the semen samples were regard as a normal semen sample according to the semen routine parameters, the sperms were not always have high fertilization ability. So only through simple semen routine parameters to assess male fertility is not comprehensive. Spontaneous sperm acrosome reaction as indices of sperm function test can make up for the inadequacy of semen routine parameters assessment of male fertility, and spontaneous sperm acrosome reaction plays a supplementary reference for sperm function assessment.

The global incidence of infertility has been estimated as being as high as 10%–15%, and the male infertility incidence accounted for about 50% [21]. In recent years the number of male infertility patients has increased gradually due to the influence of internal and external factors. Accurate assessment of male fertility is the current problem that needs to be solved. The sperm acrosome contains more than 20 enzymes related to fertilization and is the key to start the investigation into the combining of the sperm and egg, and the structure, normal morphology and function of the acrosome is an essential part of the fertilization ability of sperm [22]. If sperm SARR increase, the number of sperm with complete acrosome will reduce, and thus lead to male infertility, so we speculated that sperm SARR can be used to assess the fertilization ability of sperm.

The problem of infertility is mainly treated by reproduction technology including *in-vitro* fertilization (IVF) or follicle intracytoplasmic sperm injection (ICSI). Although the ICSI technique can be done without the acrosome reaction by means of artificial fertilization, it is an invasive treatment, and the results of follow-up studies are not clear. Therefore IVF treatment is preferred for this kind of patient. Before choosing an appropriate assisted reproduction technology, we should comprehensive analysis of the indexes including semen routine parameters and SARR results. Technically it can reduce the failure rate of assisted reproduction technology for infertility patients, and it also can reduce the patient's pain and spending from the perspective of patients.

SARR as a complementary parameter for male fertility evaluation can help clinicians to choose a more appropriate way of assisted reproduction. Semen parameters including the percentage of normal sperm morphology and sperm spontaneous acrosome reaction rate were detected by microscopic observation. The parameters were acquired by the way of manual counting and the method itself has certain subjectivity. In addition, we did not set the inclusion and exclusion criteria, this is also the limitations of this study. The exact role of the sperm spontaneous acrosome reaction rate (SARR) and routine semen parameters in male infertility patients' needs to be further explored.

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