SPERM NUCLEAR VACUOLES RATIO AS A DETERMINANT FOR UNEXPLAINED INFERTILITY AND ITS RISK FACTORS

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Abstract

Objective: To evaluate the role of nuclear vacuole ratio as determinant of unexplained infertility and assessment of its risk factors.

Patients and method: This prospective comparative study included two groups of infertile couples: group A included 20 couples with unexplained infertility and group B included 20 couples with secondary infertility due to female factor and normal semen analysis. MSOME was used for further individual sperm retrieval. Vacuoles > 50% of nuclear area were considered large nuclear vacuoles (LNV).

Results:55% of males with unexplained infertility have spermatozoa with LNV versus only 10% of males with secondary infertility. It was estimated that odds ratio of presence of LNV among males with unexplained infertility is 11 (95% CI: 1.7 – 115.2). Older age, smoking habit and presence of chronic disease are significant risk factors for presence of spermatozoa with LNV with odd ratio 19.5, 26.7 and 30.3 respectively.

Conclusion: Spermatozoa with LNV are potential cause for infertility among couples with unexplained infertility and further research is highly recommended.

Keywords: Infertility, MSOME, Nuclear vacuoles, Large nuclear vacuoles.

Introduction

Infertility has been defined as one year unprotected intercourse without conception (1). In most of cases of infertility, the exact cause couldn't be identified using the standard infertility testing procedures and it is known as unexplained infertility (2). Although great advances have been achieved in the field of assisted reproductive techniques, live births remain relatively low (3). It has been well established now that sperm selection plays a crucial role to success and failure of implantation (4, 5). The integrity of sperm DNA is important determinant for successful human reproduction. One specific sperm alteration is the presence of large nuclear vacuoles. DNA damage has been linked to large nuclear vacuoles (6).

It has been found that motile sperm organellar morphology examination (MSOME) is the only method to precisely detect large nuclear vacuoles in human spermatozoa for ICSI (7). Berkovitz et al. (8) has reported that microinjection of spermatozoa (intracytoplasmic morphologically selected sperm injection; IMSI) with a normal nuclear shape but large vacuoles affects ICSI pregnancy outcome (reduces pregnancy rate and increases early abortion).

In the present study we aimed to evaluate the role of large nuclear vacuoles as a potential cause among couples with unexplained infertility and evaluate its risk factors.

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Subject and methods

After approval of ethics committee of Faculty of Medicine-Suez Canal University, this prospective comparative cross sectional study was conducted among two groups of infertile couples. Group A included 20 couples with unexplained infertility with female age ≥ 20 years, male partner aged < 40 years, normal semen analysis, normal results of all standard investigations to identify female factor for infertility (uterine, tubal or ovarian) and presence of unprotected sexual intercourse. Group B included 20 couples suffering from secondary infertility due to well identified female factor distributed as 3 cases with tubal abnormalities, 6 cases with ovulatory abnormalities, 9 cases with tubal abnormalities and 2 cases with pelvic adhesions. Group B males have normal semen analysis. The required sample size was calculated based on power of the study of 80% and α-error of 0.05 (9). All of the studied patients were subjected to full assessment via history taking, and clinical examination.

Motile Sperm Organellar Morphology Examination: Freshly ejaculated semen samples were used in the study. All samples were labeled and discarded after use in the present study the routine selection procedure of motile high density spermatozoa for IMSI will be performed on the basis of a two-layer Sil Select density gradient system (FertiPro N.V. Beernem, Belgium). The obtained motile high density sperm fraction was used for further individual sperm retrieval based on motile sperm organellar morphology examination (MSOME) (6). According to Bartoov, the spermatozoa were classified into 5 grades: **Grade I:** free of any morphological abnormality, 4.0-5.0 µm in length and 2.5-3.5µm in width with vacuoles ≤ 4% of nuclear area and no regional disorders. **Grade II:** Short, long, wide or narrow. **Grade III:** spermatozoa with regional disorders. **Grade IV:** Vacuoles 5-50% nuclear area. **Grade V:** vacuoles > 50%nuclear area.

Couples were asked to have continued unprotected sexual intercourse for 3 consecutive cycles. Couples were evaluated for pregnancy rates. Pregnancy was diagnosed with positive urine pregnancy test and confirmed by ultrasound examination.

Statistical analysis

Microsoft Excel 2003 (Microsoft Corporation NY, USA) and SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows were used to analyze data. Data were statistically described in terms of mean, standard deviation, frequencies (number of cases) and percentages. For quantitative variables Student t test and analysis of variance was used to test significance of difference and for categorical data Chi square test was performed. P-value < 0.05 was considered statistically significant.

Results:

Table (1) presents the sociodemographic characteristics of studied males and female partner. Males in both groups were matched as regarding age, residence, prevalence of chronic diseases and smoking. Duration of marriage was longer among couples with secondary infertility.

It was estimated that spermatozoa with large nuclear vacuoles (LNV) were more prevalent among males with unexplained infertility than males with secondary infertility (55% versus 10% respectively, OR: 11, 95% CI: 1.7 – 115.2) (Table 2). We have found that older age, smoking habit and presence of chronic diseases are significant risk factors for presence of LNV. 76.9% of males with spermatozoa having LNV are smokers versus only 11.1% of males with nuclear vacuoles ratio (NVR) ≤ 50% (p-value = 0.001, OR: 26.7) (Table 3).

Table (1) :

Sociodemographic characteristics of the studied women:

		Unexplained infertility	2ndry infertility	p-value
Male partner				
Age	Mean ± SD Range	36.5 ± 4.5 23 – 41	32.9 ± 7.1 24 – 46	NS
Residence	Urban Rural	13 (65%) 7 (35%)	15 (75%) 5 (25%)	NS
Chronic disease	None DM Hypertension	14 (70%) 6 (30%) 0 (0%)	18 (90%) 1 (5%) 1 (5%)	NS
Smoker	Smokers Nonsmokers	9 (45%) 11 (55%)	4 (20%) 16 (80%)	NS
Duration of marriage (years)	Mean ± SD Range	1.2 ± 0.6 2–3.5	5.1 ± 2.4 3 - 10	0.001*
Female partner				
Age	Mean ± SD Range	24.5 ± 2.5 23 – 30	30.9 ± 4.1 26 – 37	0.001*
Age of menarche	Mean ± SD Range	13.5 ± 2.1 11 – 14	15.4 ± 2.9 13 - 16	0.02*
Rhythm of menstruation	Regular Irregular	20 (100%) 0 (0%)	14 (70%) 6 (30%)	0.02*

*Statistically significant difference
NS: no statistically significant difference

Table (2) :
MSOME results regarding nuclear vacuoles ratio:

		Unexplained infertility	2ndry infertility	OR (95% CI)	p-value
MSOME	NVR ≤ 50%	9 (45%)	18 (90%)	11 (1.7 – 115.2)	0.005*
	LNV (Nuclear vacuole ratio >50%)	11 (55%)	2 (10%)		
% of sperms with LNV (>50%)					
Mean ± SD		63.2 ± 15.2	29.7 ± 13.4		0.002*

*Statistically significant difference, NS: no statistically significant difference
LNV: large nuclear vacuoles, NVR: nuclear vacuoles ratio, OR: odds ratio,
CI: confidence interval

Table (3) :
Comparison between males with and without LNV:

	LNV (Nuclear vacuoles > 50%) (n=13)	Nuclear vacuoles ≤50% (n=27)	OR (95% CI)	p-value
Age	35.2 ± 6.5	26.9 ± 1.9	19.5 (5.8 – 183.7)	0.001*
Smokers	10 (76.9%)	3 (11.1%)	26.7 (3.6 – 223.7)	0.001*
Chronic disease	7 (53.8%)	1 (3.7%)	30.3 (2.7 – 1416.9)	0.001*

*Statistically significant difference,
LNV: large nuclear vacuoles, OR: odds ratio, CI: confidence interval

Discussion

The present study was concerned with a specific sperm nuclear content malformation that is the appearance of large vacuoles. According to Barth and Oko (10), only DIC microscopy could detect this defect. In the present study we used MSOME technique that involved an inverted light microscopy equipped with high power DIC (Nomarski/DIC) optics.

The present study has shown significant association between large nuclear vacuoles and presence of unexplained infertility. Multiple previous reports have shown findings supporting our results (6, 8 and 12).

Previous results have linked multiple DNA damages as DNA fragmentation with presence of large nuclear vacuoles (LNV) (6) and there is well established association between DNA impairment and male fertility (11).

In the study of Franco and colleagues (6), DNA fragmentation was significantly higher in spermatozoa with large nuclear vacuoles (LNV) (111/382; 29.1%) than in normal nucleus (NN) spermatozoa (65/410; 15.9%). Another form of DNA impairment was identified as the percentage of single-stranded denatured DNA (252/371; 67.9%) in spermatozoa with LNV was significantly higher than in NN spermatozoa (117/354; 33.1%). They have explained this high level of denatured DNA in LNV by precocious decondensation and disaggregation of sperm chromatin fibers (6).

Consistently, Vanderzwalmen and colleagues (12) have shown that selection of spermatozoa at high magnification is useful to identify more precisely the size and the number of nuclear vacuoles that greatly exert a negative effect on embryo development to the blastocyst stage. In accordance with the present findings, Berkovitz et al., (8) have found that LNV may affect the fertility via impaired embryo survival causing low pregnancy rates and high abortion rates meanwhile earlier stages (normal fertilization, development of top-quality embryos, and implantation)are not affected. This conclusion was strongly supported by studies performed much earlier with bovine sperm cells (10, 13 and 14).

The present study, in association with previous published evidences – has shown that large nuclear vacuoles can be considered as one of the causes of infertility couples with unexplained infertility. Older age, smoking habit and chronic disease are significant risk factors for large nuclear vacuoles spermatozo. Further studies are required to deeply investigate the role LNV among infertile couple with un-identified cause (unexplained infertility). Genetic and DNA studies are required to study the DNA impairment forms associated with LNV.

The main limitation of the present study is small sample size. Also the study was limited to analysis of spermatozoa using MSOME technique without DNA assessment. The study didn't advance for assessment of pregnancy rates among males with LNV.

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