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Effect of Sperm Vacuoles on the Outcomes of IMSI: A Meta-Analysis

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Abstract

Purposes: Sperm vacuoles play a key role in the shape formation and maturity of sperms. The relationship between sperm vacuoles and the outcomes of IMSI remains uncertain. We performed a meta-analysis based on 710 infertile couples from seven published case-control studies to draw a more precise conclusion.

Methods: PubMed, EMBASE and Web of Science were searched to identify all eligible studies that investigated such relationship. Involved sperm samples were divided into four grades according to presence and size of vacuoles. We defined grade I and grade II as the good quality sperm, grade I and grade II as the low-quality sperm. Odd ratios with 95% confidence intervals were used to assess the strength of association between the sperm vacuoles and outcomes of IMSI.

Results: Our results showed that using grade (I+II) sperm obviously generated better outcomes compared with grade (III+IV) sperm using IMSI (Fertilization rate: pooled OR=1.14, 95% CI=1.01 to 1.28; Good quality of day-3 embryos: pooled OR=1.27, 95% CI=0.95 to 1.70; Clinical pregnancy rate: pooled OR=2.06, 95% CI=1.42 to 2.99; Live-birth rate: pooled OR=1.77, 95% CI=1.08 to 2.89).

Conclusions: Sperm vacuoles can negatively affect sperm development and result in arrested fertilisation. Sperm with intact nucleus is suggested utilised to improve the outcomes of artificial insemination using IMSI.

Keywords: IMSI; Male Infertility; Meta-Analysis; Sperm Vacuoles

Introduction

Infertility is a multifactorial social problem affecting 10%-15% of couples, of which 50% are caused by male factors [1,2]. Various treatments for male infertility, such as conventional *In Vitro* Fertilisation (IVF), intra-uterine insemination, Intracytoplasmic Sperm Injection (ICSI) and Intracytoplasmic Morphologically Selected Sperm Injection (IMSI), have been utilised for this condition. Sperm morphology has been a significant factor in the outcome of Assisted Reproductive Technology (ART) since Benjamin, et al. introduced the Motile Sperm Organelle Morphology Examination (MSOME) in 2001 [3,4].

MSOME evaluates the sperm head, acrosome, midpiece and tail through a real-time morphological analysis at high magnification (×6000). Characteristics including the size, number and location of vacuoles are used to identify and select the spermatozoa with normal morphology [3,4]. Now, MSOME has been utilised in IMSI for eligible sperm selection, and this process can significantly enhance the pregnancy rate compared with conventional IVF-ICSI [3]. Sperm vacuoles, which are randomly distributed in the sperm head, are common sperm malformations [5,6]. Nuclear vacuoles were produced during the early stages of spermiogenesis and was suggested to be a risk factor of sperm maturation, which were not induced or modulated by routine laboratory environments [7]. In addition, vacuoles has also been reported to be associated with capacitation and acrosomal status, which suggested that vacuoles can be a positive factor for fertilization [8]. The specific fundamental mechanism of vacuoles in the spermatozoa nucleus is still not clear, and whether vacuoles is a positive or negative factor in fertilization remains controversial.

Many clinical studies have discussed the advantages of IMSI over conventional IVF or ICSI. However, the outcomes of IMSI between the sperms with or without vacuoles varied from each other.For instance, while Katja, et al. and Christophe, et al. have reported that large vacuoles can induce a lower fertilization rate[9,10]another study performed by Pierre, et al. found no significant association between them[11]. The outcomes of IMSI can be divided into two categories in more detail: the "Early" assisted outcomes (fertilization rates) and the "Late" outcomes (pregnancy rates, implantation rates, and live-birth rates)[12]. Many studies have discussed the relationship between sperm vacuoles and the "Late" or "Early" outcomes of IMSI respectively[9-15]. However, there is still no consensus reached up to now, whatever with "Early" outcomes or "Late" outcomes (Table 1).

	first author, publication year	Arie, 2006	Pierre, 2008	A. De, 2012	Katja, 2012	Ermanno 2013	Katja, 2013	Christophe, 2014
early outcomes	Fertilization rate	NS	NS	NM	P<0.01	NS	NS	P=0.01
	Good quality of day-3 embryos	NS	NS	P=0.362	P<0.01	NS	NS	NS
late outcomes	Clinical pregnancy rate	P<0.01	NM	NM	NM	P<0.05	NM	NS
	Live-birth rates	NM	NS	NM	NM	P<0.05	NM	NS
P-value is set at 0.05. NS = Not Statistically Significant. Arrow (↑) = Statistically significant. NM = Not mentioned.								

Table 1: Association between spermatozoa vacuoles and the outcomes of assisted reproductive technology.

Thus, we performed this meta-analysis to identify the specific association between sperm vacuoles and the early or late outcomes of IMSI respectively.

Materials and Methods

We used the keywords 'Sperm Vacuole', 'IMSI' or 'Intracytoplasmic morphologically selected sperm injection' to explore the PubMed, EMBASE and Web of Science. In addition, we also searched the Chinese literature to get a more comprehensive result. The inclusion criteria were listed below: (1)associated with sperm vacuoles and IMSI; (2) a human study; (3) a well-defined population and study design, i.e., all patients included in the study were in similar age, the number of previous failed ICSI attempts etc; (4) Odds Ratios (ORs) about the early outcomes or the late outcomes are available or can be calculated; and (5) the involved semen samples were divided into four groups according to presence or size of vacuoles: grade Ⅰ, no vacuole; grade Ⅱ, presence of at most two small vacuoles; grade Ⅲ, with more than two small vacuoles or at least one large vacuole; and grade Ⅳ, large vacuoles in conjunction with abnormal head shapes or other abnormalities [11,13,16].

We defined grade Ⅰ and grade Ⅱ as the good quality sperm, grade Ⅲ and grade Ⅳ as the low-quality sperm. Data were extracted from the "Early" and "Late" outcomes respectively, and the extracted-data elements included the following: (1) first author's name and publication year; (2) ethnicity and nationality of the included population; (3) the quantity of the injected MII oocytes and fertilized oocytes associated with spermatozoa in different grade; and (4) the quality of the 3-day embryos, the clinical pregnancy rate, the live-birth rate associated with spermatozoa in different grades. All these data are listed in (Table 2).

First author, publication year	Nationality	Dominant ethnicity	Study design	Included number		Injected MII oocytes		Fertilized oocytes	
				high	low	high	low	high	low
Arie,2006[13]	Israel	Asian	Retrospective	28	28	228	236	148	168
Pierre,2008[11]	Switzerland	European	Respective	34	8	209	69	185	55
A. De,2012[14]	Belgium	European	Prospective	176	148	NM	NM	125	139
Katja,2012[9]	Slovenia	European	Prospective	30	22	176	148	127	69
Katja,2013[15]	Slovenia	European	Prospective	25	19	33	30	25	19
Ermanno,2013[12]	Italy	European	Retrospective	63	38	464	251	342	168
Christophe,2014[10]	France	European	Prospective	39	52	242	426	187	286
Case: High quality spermatozoa; Control: Low quality spermatozoa; NM: Not Mentioned.									

Table 2: Main characteristics of studies included in the meta-analysis and associated fertilization rate in each study.

All statistics associated with an assessment index were evaluated by ORs with 95% Confidence Intervals (CIs). Heterogeneity was assessed usingHiggins I^2 statistic andCochran Q test. A random-effect model was applied if significant heterogeneity was observed ($p<0.10$ or $I^2>50\%$); otherwise, the fixed-effect model was utilised. An OR value greater than 1 indicated good outcome for the grade Ⅰ and Ⅱ spermatozoa and was considered statistically significant if the 95% CI did not include 1, with $P < 0.05$. Egger's linear regression test was applied to estimate the publication bias with a funnel plot. A p value less than 0.05 was considered statistically significant. The meta-analyses were carried out with Stata12 (StataCorp LP, College Station, TX, USA).

Results

Summary of the Enrolled Studies

Our search of vacuoles and IMSI associated literature yielded 103 manuscripts published before February 2017 using Pubmed, EMbase, and Web of Science we have also searched the literature in Chinese. However, none Chinese literature fulfil the inclusion criteria of us. Seven studies were considered finally in the meta-analysis (Figure 1).

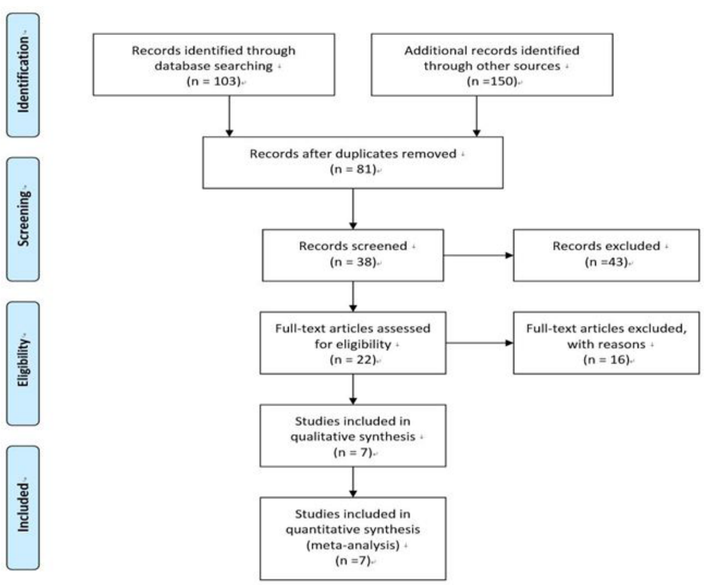

Figure 1: Article selection process for meta-analysis.

And the main characteristics of the involved studies were showed in (Table 2).It is notable that among these seven included article, Arie, et al. did not strictly divided the spermatozoa into four grades, and instead they defined the spermatozoa with a normal nuclear shape but large vacuoles as "Low quality sperm" while the spermatozoa with strictly defined morphologically normal nuclei as "Good quality sperm"[13]. We included this study because its classification criteria were similar to the other six articles. Among these 7 studies, a total of 710 participants were included; 1 study focused on Asians, and 5 evaluated Caucasians. The specific relationship between spermatozoa vacuoles and IMSI outcomes was shown in (Table 1).

All the seven-literature discussed the relationship between the "Early" outcomes of IMSI and sperm vacuoles. In terms of the fertilization rate, only 2 studies showed that sperm vacuoles are significantly associated with fertilization rate in IMSI [9,10], while the other four studies showed no significant association between fertilization rate and IMSI [11-13,15]. In addition, two studies found spermatozoa vacuoles are significantly related to the good quality of day-3 embryos [9,14], while the other five found no significant relationship [10-13,15]. Four studies have discussed the relationship between the "Late" outcomes of IMSI and sperm vacuoles. Two studies proved that sperm vacuoles were significantly related to clinical pregnancy rate [12,13]. One study suggested that sperm vacuoles were significantly associated with live-birth rate [12].

Result of early outcomes

Fertilization rate is significantly increased when grades I and II sperm were used. Seven studies referring to the fertilization rate were analysed by a random-effect model ($p=0.001$, $I^2=76.7\%$) (Figure 2(A)).

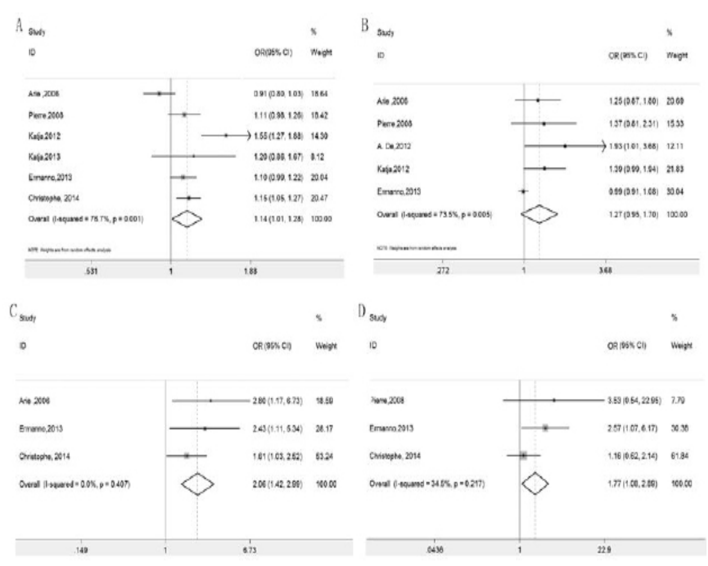

Figure 3(A-B): Sensitivity analysis of each included study. (A) Fertilisation rate analysis; (B) Good quality of 3-day embryos analysis.

After excluding this study, the heterogeneity reduces obviously ($p=0.727$, $I2=0.0\%$) and a significant combined OR is obtained (OR= 1.27; 95% CI= 0.95 to 1.70) (Figure 4).

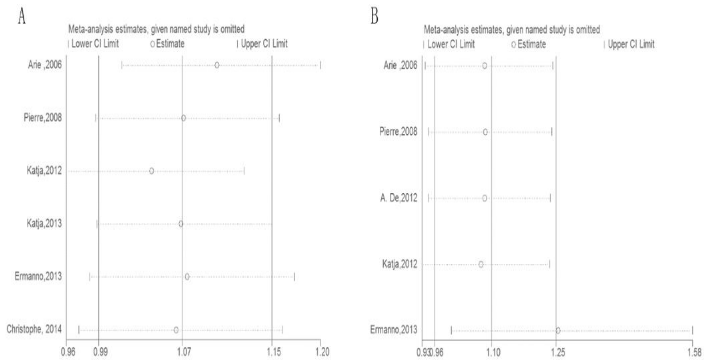

Figure 4: Forest plot of the sperm vacuoles associated with the quality of day-3 embryo of IMSI after excluding one study with high heterogeneity.

Result of Late Outcomes

In terms of the late outcomes of IMSI, no distinct heterogeneity existed among all studies, and all I^2 values are lower than 50%. Thus, fixed-effects model was used to evaluate all indices in our meta-analysis. The outcomes of the group with grades and Ⅱ sperm were evidently better than that of the group with grades Ⅲ and Ⅳ sperm. The ORs of clinical pregnancy and live-birth rates were 2.06 and 1.77, respectively, and corresponding 95% CI values 1.42 to 2.99 and 1.08 to 2.89 (Figure 2(C), (D)).All these statistical data showed that the fewer vacuoles the sperms have, the better the outcomes will be. The clinical rates in all studies were consistent, which indicates that IMSI may be the first therapeutic choice for infertility caused by male factors because it increases the clinical pregnancy and live-birth rates.

Discussion

Sperm vacuoles are pre-existing structures, and their number cannot be altered by in vivo conditions [7]. The size, number and location of the vacuoles in the spermatozoa are random and complex. The study of sperm vacuoles has become increasingly attractive since Benjamin et al. established the potential effect of sperm vacuoles on the outcomes of IMSI and introduced MSOME in 2001. To clarify the specific role of vacuoles in IMSI, we conducted a meta-analysis that included 710 cases, which were classified into two groups according to sperm vacuoles from seven case-control studies. We generated more precise conclusion on the relationship between vacuoles and outcomes. From the analysed data, we assume that the sperm vacuoles could be a marker for predicting the success of IVF/IMSI. The main distinct result between this meta-analysis and previous studies is that the sperm vacuoles evidently had a negative effect on the outcomes whether in the early or late period of zygote development.

The result of our meta-analysis suggests that both early and late outcomes of sperms without vacuoles or with one or two small vacuoles are better than the outcomes of sperms with higher grades of vacuoles (Figure 2(A, C, D), Figure 4). It is notable that after excluding one study performed by Ermanno, et al. in the quality of 3-day embryo analysis, the heterogeneity reduced remarkably[12]. In this article, only semen samples with severe impaired sperm morphology (percentage of normally confirmed spermatozoa ≤ 3% according to Kruger strict criteria[17]) were included, while other studies set an inclusion criteria with normally morphologic spermatozoa ≤ 10% or 14% [9,10]. Although the spermatozoa were divided according to the size and number of vacuoles in the same criteria as mentioned above in all these seven studies, initial percentage of teratospermatozoa might influence the selection process, subsequently increase the difficulty to search for grades Ⅰ and Ⅱ sperm. In addition, low percentage of the normal morphologic spermatozoa might result in a lower quality of the grades Ⅰ and Ⅱ sperm compared with other groups. We suppose that this inclusion criteria should be an important source of the heterogeneity in the quality of 3-day embryo analysis. However, we did not find any individual study with huge heterogeneity in the fertilization rate analysis (Figure 3(A)).

The outcomes of early stage via IMSI should be given particular attention because most studies showed that the vacuoles, especially in sperm grade (Ⅲ+Ⅳ), negatively affect embryo development in late period, which is corresponding to the findings in our study (Figure 2(A), 4). We propose two aspects to elucidate the early outcomes between our meta-analysis and the published data. First, sperm vacuoles are harmful to the early development of fertilised eggs. Katja et al. performed a time-lapse prospective study on the development of IMSI-derived embryos. They found that poor sperm morphology is one of the possible reasons for impaired embryo development [15]. The time for the developing embryos to enter the blastocyst stage was calculated to determine whether sperm morphology influence the developmental dynamics for embryos that develop to the blastocyst stage. If developmental processes, such as zygote formation, syngamy and cleavage are hindered, as well as subsequent events of genomic activation and blastulation, the embryo will arrest, and pregnancy will not be achieved [15,18]. Hence, based on our meta-analysis, we suggest that the sperm vacuoles harm embryo development. The optimal cut-off value for the percentage of Large-Nuclear Vacuole (LNV) sperm with maximised proper blastocyst formation was ≤24.5%, and the cut-off value with maximised blastocyst quality was ≤19.5% [19], which indicates the paternal influence on the early development from fertilisation to the blastocyst stage.

Moreover, it has reported that sperm vacuoles are associated with sperm DNA fragmentation. And sperm DNA damage increases the risk of IVF/ICSI outcomes, such as fertilisation, pregnancy and clinical pregnancy, in the patient with isolated teratozoospermia [20,21]. However, sperm DNA fragmentation is associated with abnormal quantitative parameters especially ellipticity, angularity and LNVs in the sperm head [22]. Therefore, the mechanism by which the sperm vacuoles contribute to the negative effect on the sperm DNA is indistinct. Some studies have reported that LNVs are indicative of abnormal chromatin packaging in human spermatozoa, and chromatin condensation is defined as one of the phenomena of sperm maturation[23,24].By using CMA 3-positive staining, we found that a higher degree of staining indicates a more deficient protamination, which may lead to abnormal chromatin packaging. That is, a greater percentage of spermatozoa with positive staining might be vacuolated if the inverse correlation could be determined, which may help elucidate why the use of grade (Ⅰ+Ⅱ) sperm in produce better outcomes than the use of grade (Ⅲ+Ⅳ). However, insufficient protamination is related only with the LNV sperm. No further evidence is found to support other types. Finally, our conclusions should be considered with caution because first, only seven articles were included in this meta-analysis, which is obsessed by potential existence of heterogeneity or publication bias, although it might not be statistically evident.

Conclusions

Early and late outcomes of sperms without vacuoles or with one or two small vacuoles were better than the outcomes of sperms with higher grades of vacuoles. This phenomenon indicates that sperm vacuoles negatively affect the sperm DNA, thereby arresting the development of blastocyst to form embryos. IMSI is a more advanced ART with better outcomes than those of conventional ART. IMSI using spermatozoa with none vacuoles can assist in selecting the adaptive sperm for infertile couples and improve embryo quality.

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