



REVIEW ARTICLE

The Effect of Different Sperm Concentration in Semen on Clinical Outcomes of *In Vitro* Fertilization (IVF) and Intracytoplasmic Sperm Injection (ICSI): Retrospective Study

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Abstract

Objective: The objective of this retrospective study was to determine whether clinical outcomes (clinical pregnancy, implantation, live births and miscarriage) were affected by the application of IVF or ICSI insemination with different sperm concentrations in ejaculate in unselected population of infertility patients.

Materials and methods: Data of 786 *in vitro* fertilization (IVF) and 1268 intracytoplasmic sperm injection (ICSI) cycles were retrieved and analyzed according the stratification of the cycles as follows: Group with low (0-19.9), normal (20-99.9), above normal (100-150) and high (> 150 M/ml) spermatozoa concentration in ejaculate.

Results: The concentration of spermatozoa in ejaculate negatively affected clinical outcomes including clinical pregnancy ($P < 0.01$), implantation ($P < 0.03$), live births ($P < 0.02$) and miscarriage rate ($P < 0.01$) in ICSI treatment. In contrary, such an effect was not observed in the IVF cycles. However, comparing IVF and ICSI subgroups, the profound negative effect on clinical outcome was evident in ICSI patients when the concentration of spermatozoa in semen was > 100 M/ml and > 150 M/ml ($P < 0.05$ to $P < 0.001$ range).

Conclusions: Our results demonstrate the negative effects of ICSI on embryos implantation, clinical pregnancy, live births, and miscarriage rate when semen had above normal to very high concentrations of spermatozoa in ejaculate. However, such an effect was not observed in the IVF treated patients.

Keywords

Intracytoplasmic sperm injection, Sperm concentration, Clinical pregnancy, Live birth, Implantation rate

Introduction

Intracytoplasmic Sperm Injection (ICSI) was introduced in 1992, as a revolutionary step in improving fertilization rates in male factor infertility patients. Recently, this technique has become the treatment of choice in many clinical circumstances such as: Male factor infertility, unexplained infertility, poor ovarian reserve, advanced maternal age, prior fertilization failure with conventional insemination, preimplantation genetic testing (PGT), insemination of oocytes after *in vitro* maturation (IVM), and in patients with normal semen parameters and/or female factors [1-3]. As a result, 67% of assisted reproduction treatment (ART) cycles in the United States use ICSI, 65% in Europe and almost 100% of ART cycles in the Middle East [4-6]. Report prepared by the International Committee for Monitoring Assisted Reproductive Technologies stated that ICSI was performed 1.4 times more than conventional *in vitro* insemination in Asia, and twice as much in sub-Saharan Africa [6].

However, safety and efficacy of ICSI for non-male factor infertility has not been fully elucidated yet.



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Conventional insemination (IVF cycle) was usually performed with satisfactory results when semen samples have normal parameters or a very high concentration of spermatozoa. Moreover, it has been reported that only 10% of infertile and 15% of fertile men had more than 100×10^6 spermatozoa/ml in ejaculate [7]. Thus, it is a very small group of men who have not been scrutinized for ICSI suitability, possibly due to the lack of rationale for fertility problems and/or necessity for explicit testing. This is why the efficacy of ICSI especially with a very high concentration of spermatozoa in semen, has not been fully investigated yet.

Mild ovarian stimulation with fewer follicles on the ovaries has recently become the main stream of fertility treatment [8]. To guarantee fertilization and adequate number of embryos for transfer, ICSI is often performed regardless of semen parameters or etiology of infertility [9,10].

The above rationale encouraged us to analyze ICSI outcome where the semen with high concentration of spermatozoa in ejaculate was used. Thus, the objective of this retrospective study was to determine whether an application of ICSI as a method of insemination with semen containing different concentration of spermatozoa in ejaculate affects the clinical outcome (clinical pregnancy, implantation, live births and miscarriage) in an unselected population of infertility patients.

Materials and Methods

Patients

This retrospective study was approved by the MH Institutional Review Board (Ref # 201304041J). The data of patients receiving assisted reproduction treatment (ART) between 2000 and 2015 in a private setting were retrieved from our electronic database according the specification approved by IRB. The study consisted of 786 cycles of IVF and 1268 cycles of ICSI. To create study groups, all cycles were stratified (not necessarily consistent with WHO recommendation) by sperm concentrations in the semen as follows: Low 0-19.9 (60 IVF and 556 ICSI cycles); normal 20-99.9 (613 IVF and 628 ICSI); above normal 100-150 (89 IVF and 65 ICSI) and very high > 150 M/ml (24 IVF and 19 ICSI cycles) spermatozoa concentration in ejaculate. Only freshly ejaculated spermatozoa of the partners or husbands were used for insemination in each study group. Excluded from this study were cycles with oocyte or sperm donors, epididymal and testicular spermatozoa, gestational carriers or pre-implantation genetic diagnosis/screening (PGD/PGS).

Controlled induction of ovarian ovulation

The common protocols of ovarian stimulation with both long lupron and GnRH antagonist (ganirelix and cetrotide) with pure gonadotropins/menotropins (Gonal F, Follistim, Repronex, Bravelle) were used in IVF and ICSI cycles. Ovarian stimulation started on day 3

with 75-150 IU gonadotropins daily with doses adjusted every 2-3 days based on follicle size and estradiol levels in the blood. Serum levels of estradiol, progesterone and FSH (FSH level only on Day 3 of menstrual cycle) were determined using an automated system - Immulite 1000 (Siemens Medical Solutions Diagnostic, Tarrytown, NY, USA). Follicular growth was monitored with periodic ultrasound scan (GE ultrasound machine, ProSeries Logix 400 BE) of the ovaries followed by determination of estradiol concentrations in the blood. Ovulation was triggered with 5,000-10,000 IU hCG (Profasi, Serono, USA) injection when at least one follicle was ≥ 17 mm in diameter, endometrial thickness > 8 mm and the estradiol level reached approximately 1200-1700 pg/ml.

Semen sample processing

On the day of egg retrieval, a semen specimen of a patient's husband/partner was collected by masturbation into a sterile container after 2-4 days of sexual abstinence. After complete liquefaction at room temperature, the semen was assessed for volume, sperm count, and motility using IVOS (Hamilton Thorn, Massachusetts, USA). The semen was processed using density gradient (90%/45%, PureSperm, Conception Technologies, CA, USA) with centrifugation for 20 minutes \times 300 G. The supernatant was discarded and the pellet was washed twice (8 minutes, 300 \times G centrifugation) with the sperm washing medium (Conception Technologies, CA, USA). The spermatozoa were suspended in a 1 ml medium and the concentration and motility of spermatozoa were again determined.

Insemination

Sequential culture media (fertilization, cleavage, Sage, Bedford, NJ) were used. Retrieved oocytes were inseminated with 100,000 sperm/oocyte (IVF) or single spermatozoa was injected (ICSI) into mature oocyte (M II). Fertilization and embryo development (number of cells, evenness of cells, percentage of fragmentation) were evaluated daily and selected embryos were then transferred on Day 3 using a transabdominal ultrasound approach.

Clinical outcome

The level of β hCG (quantitative) in serum was determined on day 10 and 12 after embryo transfer. Patients with positive pregnancy tests were evaluated 5-6 weeks later for the presence of gestational sac(s) and fetal heart activity using ultrasound. The luteal phase was supported with a vaginal gel (Crinone 8%, Serono, USA) or micronized progesterone (100 mg/day). The luteal support was maintained until the 10th weeks of gestation or was discontinued if the pregnancy test was negative. Information on all miscarriages, and live births were obtained from the patients. The outcome measures (clinical pregnancy, live birth and miscarriage) were expressed per embryo transfer.

Table 1: Clinical outcomes of IVF and ICSI cycles in relation to sperm concentration in semen.

Sperm Concentration M/ml	Clinical Pregnancy (%)			Implantation Rate (%)			Live Birth Rate (%)			Miscarriage Rate (%)		
	IVF	ICSI	P value	IVF	ICSI	P value	IVF	ICSI	P value	IVF	ICSI	P value
0-19.9	31.7	40.5	NS	12.5	20.7	NS	26.8	34.2	NS	31.3	25.30	NS
20-99.9	43.6	36.1	NS	21.0	18.6	NS	38.4	31.2	NS	21.53	25.69	NS
100-150	37.1	24.6	0.05	19.1	9.7	0.05	32.6	23.1	0.05	30.19	33.11	NS
> 150	41.7	15.8	0.01	24.3	8.7	0.01	41.7	5.3	0.001	33.33	75.00	0.001
Overall effect	P < 0.96	P < 0.01		P < 0.67	P < 0.03		P < 0.21	P < 0.02		P < 0.09	P < 0.01	

IVF: *In Vitro* Fertilization; ICSI: Intracytoplasmic Sperm Injection.

Statistical Analysis

The results were analyzed using Statistica 10.0 software (Statsoft, Tulsa, OK, USA). Distribution of data was verified with Shapiro-Wilk test and Kolmogorov-Smirnov test with Lilliefors correction. The non-parametric U Mann-Whitney test was employed in view of a non-Gaussian distribution of data. The Kruskal-Wallis test was used to determine effect of sperm concentration on the clinical outcome. The Chi-squared test of independence with design 2 × 2 was used to test differences between IVF and ICSI groups in ART outcomes; clinical pregnancy, implantation, live birth, miscarriage, fertilization, and cleavage rates. Results of these analyses were reported as a percentage (Table 1), median and an interquartile range 25th-75th percentile (Table 2 and Table 3) and fertilization, cleavage rates as a percentage in (Table 3).

Results

The clinical pregnancy ($P < 0.01$), implantation ($P < 0.03$), live birth ($P < 0.02$), and miscarriage rate ($P < 0.01$) in the ICSI cycles were negatively affected by the concentration of spermatozoa in ejaculate (Table 1). In contrary, the clinical outcomes in the IVF cycles were not affected by the spermatozoa concentration. However, significant statistical differences ($P < 0.05$) were found in the clinical pregnancy, implantation, and live birth rates when stratified IVF and ICSI groups (concentration of spermatozoa in the range 100-150 M/ml) were compared. Also, high negative impact of the ICSI procedure on clinical pregnancy, implantation ($P < 0.01$), live births, and miscarriage rates ($P < 0.001$) were manifested when semen samples contained > 150 M/ml spermatozoa in ejaculate.

There were no statistical differences in the demographic parameters, distribution of different diagnoses, the length of male abstinence and number of days on stimulation between ICSI and IVF groups in our study (data not shown). Additionally, there were no statistical differences in the female age, endocrinological cycle characteristics (Table 2), fertilization, and cleavage rates (Table 3) between ICSI and IVF groups. The average number of embryos transferred (IVF group - 3.2/cycle; ICSI - 2.9/cycle) were comparable between the two treatment groups.

Discussion

The results of our retrospective review study show that routine application of ICSI insemination with a very high concentration of spermatozoa in ejaculate had a negative effect on clinical outcome in an unselected population of infertility patients. However, lack of such an effect in IVF cycles might suggest an involvement of cumulus cell and/or zona pellucida in selecting the best spermatozoa for fertilization and elimination defective one. The results of our study are in agreement with previously published findings [9,10] where clinical outcomes of ICSI were compared with traditional IVF treatment in couples with non-male factor infertility or patients who had low spermatozoa concentration. Bhattacharya, et al. [11] demonstrated also in their multicenter randomized controlled trial, that the use of ICSI in couples suitable for conventional IVF treatment does not offer any clinical benefits. The Practice Committee of the American Society for Reproductive Medicine (ASRM) and the Society for Assisted Reproductive Technology (SART) [3,4] stated in their 2012 critical review of literature that there is no data to support the routine use of ICSI for non-male factor infertility.

The least favorable clinical outcome in our study was in ICSI cycles when the spermatozoa concentration in the semen was more than 150 M/ml. However, published literature does not offer any information on spermatozoa characteristics and/or clinical outcome of ICSI when a very high concentration of sperm is present in ejaculate. Therefore, we can only speculate about the causes in our results. Firstly, it might be a result of pure chance as the sample size for that group was very small. However, the statistical analysis showed the differences in spite of low number of cycles in these groups. Secondly, the high concentration of sperm in semen might be a result of overproduction of spermatozoa during spermatogenesis and may lead to a defective maturation process of spermatozoa and/or trigger abnormal chromatin packaging and/or induce DNA fragmentation [12,13]. The DNA packaging is controlled by protamines and if deficiently deposited affects fertilization rates and embryo quality [14]. Furthermore, Avendano, et al. [15] showed that the presence of an

Table 2: Cycle characteristics for IVF and ICSI patients stratified by sperm concentration. Values are medians with interquartile 25-75% in parentheses.

Sperm Concentration M/ml	FSH (Day 3) (IU/ml)		E ₂ at HCG injection (pg/ml)		P ₄ at HCG injection (ng/ml)		Endometrium thickness (mm)		Female Age (years)	
	IVF	ICSI	IVF	ICSI	IVF	ICSI	IVF	ICSI	IVF	ICSI
0-20	9.0 (7.0-10.6)	9.0 (8.0-11.0)	2549.0 (1792-3344)	1987.0 (1334-2556)	1.1 (1.0-1.4)	1.1 (0.8-1.6)	10.0 (10.0-13.0)	11.0 (9.0-12.0)	34.0 (31.0-38.0)	34.0 (31.0-36.0)
20-99.9	9.0 (7.8-12.0)	10.0 (8.0-12.0)	1952.0 (1427-2588)	1839.5 (1210-2454)	1.1 (0.7-1.4)	1.0 (0.6-1.4)	10.0 (9.0-12.0)	11.0 (9.0-12.0)	34.0 (32.0-38.0)	34.0 (31.0-37.0)
100-150	9.8 (8.0-12.0)	10.0 (8.9-13.1)	1832.0 (1297-2465)	1675.5 (1337-2380)	1.3 (1.1-1.6)	1.3 (1.0-1.6)	11.0 (10.0-13.5)	10.0 (9.0-11.0)	35.0 (32.0-38.0)	36.0 (33.0-39.0)
> 150	9.0 (7.0-11.5)	8.5 (8.0-11.0)	2321.0 (1174-3.027)	1682.0 (1110-3115)	0.7 (0.7-1.3)	1.4 (1.3-1.6)	11.0 (9.0-12.0)	10.0 (10.0-11.0)	34.0 (32.5-37.5)	35.0 (33.0-38.0)

FSH: Follicle Stimulating Hormone; E₂: Estradiol 17β; HCG: Human Chorionic Gonadotropin; P₄: Progesterone; IVF: *In Vitro* Fertilization; ICSI: Intracytoplasmic Sperm Injection.

Table 3: Semen parameters, fertilization, and embryo cleavage rates stratified by sperm concentration. Values are presented as medians with interquartile 25-75% in parentheses or percentage for fertilization and embryo cleavage rate.

Sperm Concentration (M/ml)	Volume of ejaculate (ml)		Sperm count (M/ml)		Sperm motility (%)		Fertilization rate (%)		Cleavage rate (%)	
	IVF	ICSI	IVF	ICSI	IVF	ICSI	IVF	ICSI	IVF	ICSI
0-19.9	2.5 (0.5-7.0)	2.5 (0.6-12.0)	14.7 (2.5-19.9)	6.1 (0.1-19.9)	66.0 (29.4-93.0)	35.0 (0.1-100.0)	61.84 (63.57)	53.36 (52.77)	94.10 (96.48)	96.50 (96.97)
20-99.90	3.0 (0.5-8.0)	2.5 (0.1-8.0)	50.0 (20.0-99.9)	40.6 (20.0-98.7)	69.0 (24.0-97.2)	61.0 (1.0-92.0)	63.57 (64.18)	52.77 (56.62)	96.48 (95.02)	96.97 (95.65)
100-150	2.3 (0.2-6.0)	2.0 (0.3-9.0)	119.8 (100.0-147.0)	114.2 (100.0-149.0)	82.0 (32.0-92.0)	77.0 (2.0-95.0)	64.18 (61.17)	56.62 (53.05)	95.02 (93.68)	95.65 (95.40)
> 150	2.3 (1.0-5.0)	2.0 (1.0-6.0)	168.3 (150.0-198.5)	162.0 (151.8-197.5)	84.0 (60.0-97.3)	68.0 (12.0-92.0)	61.17 (61.17)	53.05 (53.05)	93.68 (93.68)	95.40 (95.40)

IVF: *In Vitro* Fertilization group; ICSI: Intracytoplasmic Sperm Injection group.

increased proportion of normal spermatozoa with damaged DNA was negatively associated with embryo quality and pregnancy outcome after ICSI. Tesarik, et al. [16] suggested that abnormal DNA integrity may be causing impairment in the later embryo development but does not appear to be associated with poor fertilization. This statement remains compatible with our results showing a very high miscarriage rate without affecting fertilization.

The alternative explanation might be, that some males with a very high concentration of spermatozoa in ejaculate (> 150 M/ml) might have a dysfunctional apoptotic controlling system that causes overproduction of spermatozoa. Thus, the excess sperm possibly with damaged DNA and/or poor DNA integrity and/or abnormal chromatin packaging [14,17] is not effectively eliminated [18,19]. Apoptosis occurs during normal spermatogenesis in the male reproductive system and plays an important role in controlling the spermatozoa population [20]. Failure to eliminate excess spermatozoa during spermatogenesis is a result of altered apoptotic process in the testes [18,19] which is associated with male infertility [21]. A significant inverse correlation between sperm concentration and morphology and the apoptotic index, have been found in patients with normal semen parameters by Ricci, et al. [22]. However, they were unable to show a similar correlation in patients with abnormal semen counts. Additionally, Chen, et al. [23] analyzed semen samples of 23 males and found that seven males had very high sperm concentration (more than 100 M/ml) with apoptosis in the range of 1.9-3.3% as assessed by the DNA diffusion assay. It is possible that some semen samples of patients with very high concentration of spermatozoa in semen in our study could contain more apoptotic spermatozoa than others (apoptosis was not determined in our semen samples) and this might possibly justify the lowest clinical pregnancy, implantation, and live birth rates and the highest miscarriage rate in the ICSI cycles of our study.

Generally, the spermatozoa for ICSI insemination is selected under 100x to 200x magnification with taking into consideration motility and morphological characteristics of spermatozoa. Under such conditions, morphological defects of spermatozoa (head, neck or tail) could be detected but not the nuclear vacuoles. Bartoov, et al. [24] demonstrated significantly higher pregnancy rates by injecting spermatozoa with a morphologically normal nucleus, compare to conventional selection of spermatozoa during ICSI. Additionally, Wirrflow, et al. [25] observed a significant improvement in the fertilization rate and embryo quality as well as a reduced number of miscarriages when ICSI was performed with hyaluronan bounded spermatozoa (HA-ICSI) compared to conventional ICSI (PVP-ICSI). Later Wirrflow, et al. [26] demonstrated significant reduction in pregnancy loss when hyaluronan - bounded

sperm was used for ICSI. Similarly, Parmegiani, et al. [27] showed improvement in the embryo quality and implantation rate when spermatozoa were selected for injection by a hyaluronan bounding procedure (HA-ICSI). In contrary, the study by Van den Bergh, et al. [28] showed no differences in fertilization, pregnancy, and implantation rates when comparing HA-ICSI to PVP-ICSI sperm selection. The assessment of sperm nuclear morphology was not performed in our study since this technology is usually not applied when semen quality is within normal parameters or when the sample has a very high concentration of sperm. Additionally, in our study there was lack of morphology results in the semen samples used for insemination. Morphology was only assessed during initial semen analysis. Based on the results of our study, an identification of functionally competent and genetically normal spermatozoa is becoming a necessity if ICSI will be used routinely for insemination. Perhaps an additional sperm functionality test(s) should be established particularly for males with a very high concentration of sperm in semen.

The lack of negative influence of sperm concentrations on the outcome of IVF, in our study, may stem from the ability of cumulus cells, zona pellucida, or oolemma to perform natural selection for the most functionally competent sperm for oocyte fertilization in order to maximize the chances of successful reproduction [29].

Additionally, the possible influence of diagnosis and the number of embryos transferred and/or length of male abstinence on the outcome of clinical pregnancy, implantation, and live birth rates in the ICSI group of our study may be excluded as a contributing factor.

In conclusion, to our knowledge, the results of our study are the first to show that high sperm concentrations in semen have a strong negative impact on the clinical outcomes when ICSI insemination is performed. Improvement of existing technologies in semen preparation or development of a better method of semen preparation and spermatozoa selection for routine use ICSI insemination is necessary to enhance clinical outcome. However, larger prospective studies or form of randomized controlled trials are required to confirm the link between high sperm concentrations and poor ICSI outcomes and results of our retrospective review study.

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