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The Influence of Sperm Concentration in the Ejaculate Used for ICSI on the Outcome of the ART Cycle

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Abstract

The sperm concentration in the ejaculate might be a crucial factor predicting the success of the ICSI procedure. Data in the literature is controversial and not recent in spite of many technical improvements added to the ART laboratory. To examine the influence of sperm concentration in the ejaculate used in the laboratory on the live-birth rate following ICSI and embryo transfer we conducted a retrospective study on all patients that were treated at our IVF unit during the period of January 2011 and July 2014. A total of 1145 ICSI cycles were divided into four groups according to the ejaculated sperm concentration (millions/ml) on the day of oocyte retrieval: group I: <1 (254 cycles); group II: 1-5 (89 cycles); group III: 5-9 (110 cycles); group IV: 10 or more (692 cycles). Groups I-III were the study groups and group IV served as the control. The demographic background, parameters of the ovarian response and parameters of the laboratory and clinical outcomes were compared between the four groups including 2PN fertilization rate; embryo implantation rate (IR); clinical pregnancy rate (CPR) and live birth rate (LBR) per cycle initiated and per embryo transfer (ET). Comparing the four groups of sperm concentration, ICSI in group 1 resulted in significantly decreased 2-PN fertilization rate (56.3 ± 25.7, 58.4 ± 25.2, 63.3 ± 28.7, 63.3 ± 30.7 for groups I, II, III, IV respectively, p=0.006). However, live-birth rates (23.2, 24.7, 23.6, 24.0, for groups I, II, III, IV respectively, p=0.35) as well as all the other clinical parameters examined were comparable between the groups. Logistic regression stepwise analysis, found that sperm concentration had no predictive value for live-birth achievement.

Keywords: Intracytoplasmic sperm injection; Fertilization rate; Sperm count; Oligospermia

Introduction

Since the revolutionary introduction of ICSI to the field of ART by Palermo et al. [1] many men with various degrees of testicular dysfunction of spermatogenesis achieved fatherhood [2]. The fertilization potential of a sperm sample is assessed by several parameters including concentration, motility and morphology. WHO published data on ejaculate parameters of recent fathers that may reflect natural fertilization and conception potential [3]. There is controversy regarding the influence of the semen sample parameters on the fertilization potential of these sperm cells *in vitro* and the exact threshold level below which ICSI should be performed [4]. Various studies emphasized the importance of sperm motility and morphology [5-10] on the outcome of ICSI. However when ICSI is performed, a single sperm cell is injected into each oocyte. The embryologist makes the decision to inject one which is alive and motile and has satisfactory morphology thus possibly neutralizing these parameters effect. Indeed Van der Westerlaken et al. [11] emphasized the limited value of the sperm sample parameters to predict ICSI outcome. In general sperm cell concentration is among the parameters influencing the ease of the embryologist to find suitable spermatozoon adequate for ICSI. Whereas several studies [5,6] found no correlation between spermatozoa concentration and ICSI outcome other investigators reported decreased fertilization rate after ICSI with sperm concentration below 1 million/ml [12,13] and a negative correlation with decreasing concentrations below 1 million/ml [7].

Presently, embryologists gained experience and expertise in the ART laboratory in performing ICSI, are using updated microscopes, new culture media for sperm and oocytes, have more efficient incubators for embryo culture, each factor playing a role in their contribution towards improvement in the treatments success rate. Therefore our aim was to estimate the current influence of sperm concentration in the initial ejaculate given on the day of oocyte retrieval on the outcome of ICSI, including 2PN fertilization rate as well as pregnancy outcome including live birth rates.

Materials and Methods

Patient population

This study was designed as a retrospective study on all patients that were treated at the IVF unit of Barzilai University Medical Center during the period of January 2011 and July 2014, who underwent ICSI using ejaculated sperm. The study was reviewed and approved by our IRB. To reduce female confounding factors we excluded cycles with patients aged 40 years or older or had endometriosis. A total of 1145 ICSI cycles were divided into four groups according to the ejaculated sperm concentration (millions/ml) on the day of oocyte retrieval: group I: <1 (254 cycles); group II: 1-5 (89 cycles); group III: 5-9 (110 cycles); group IV: 10 or more (692 cycles). Groups I-III were the study groups and group IV served as the control. In group IV ICSI was performed on part of the oocytes retrieved in normospermic cases on their first cycle or because of abnormal parameters in previous sperm analysis such as motility or morphology. Data was extracted from patients' files, tabulated and analyzed.

Sperm preparation and ICSI

Controlled Ovarian Hyper-stimulation (COH) was performed using either the routine long protocol of pituitary suppression followed by ovarian stimulation or the multiple dose antagonist protocol. Oocytes were retrieved by vaginal ultrasound-guided follicular puncture. After liquefaction of freshly ejaculated semen sperm concentration was

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assessed using a Makler chamber. Then the ejaculated sperm cells were processed by density gradient centrifugation (centrifuged for 5 minutes followed by three layer Percoll gradient (Irvine scientific, Santa Ana, CA), centrifuged at 300g for 20 minutes and the final pellet washed and centrifuged at 1800 g for 5 min). Routine ICSI was performed according to the methodology described by Van Stertegheim et al. [14]. 16-18 hours after microinjection fertilization was assessed under an inverted microscope and considered normal when two clearly distinct PN containing nucleoli were present. Cleavage was assessed 24 and 48 hrs later. Embryo transfer was performed on day 2 or 3 after oocyte retrieval. The best embryos were selected for transfer. Supernumerary top quality embryos were frozen and the remainders were followed for blastocyst formation. After ET, all patients received luteal support, including 800 mg of vaginally administered micronized P (Utrogestan; Laboratories Piette International S.A.) or IM injections of progesterone. Serum hCG levels were measured 14 days after embryo transfer. Implantation rate was determined by dividing the number of gestational sacs by the number of embryos transferred. Clinical pregnancy was defined as a visible sac and the presence of a fetal heartbeat on the 7th gestational week.

Statistical Analysis

The demographic background, parameters of the ovarian response and parameters of the laboratory and clinical outcomes were compared between the four groups. Our primary outcome was the 2PN fertilization rate and secondary outcomes were embryo Implantation Rate (IR); Clinical Pregnancy Rate (CPR) and Live Birth Rate (LBR) per cycle initiated and per Embryo Transfer (ET). Also information regarding the pregnancy outcome was gathered including chemical pregnancies, ectopic pregnancies, clinical pregnancies, Early Spontaneous Abortions (ESA) and deliveries.

Given a two-sided significance level of 0.05 and a difference of 5% in the fertilization rate between the groups, the group size needed for 80% statistical power was 80 cycles in each of the four groups.

Quantitative statistics gave descriptive parameters such as mean ± SD and frequencies, presented as percentage. Statistical analysis was performed using the χ^2 test for comparison of the group's outcome variables and other categorical variables, and Student's two-sided *t* test regarding continuous variables. ANOVA was used where appropriate to estimate differences of means between the groups. Linear regression analysis was used to examine the correlation of sperm concentration to live-birth. A p-value of <.05 was considered statistically significant. The data was analyzed using SPSS (version 21, SPSS, Inc.).

Results

The demographic background of the patients included in our study is presented in Table 1. Comparing the four groups, the major indication for ICSI in the study groups (groups I-III) was male factor due to the impaired sperm profile whereas the control (group IV) included patients who underwent ART due to various indications when a proportion of their oocytes was treated by ICSI. Patients in group I were significantly younger and had a significantly higher percentage of nulligravida compared to patients in group IV. Comparing the four groups, no significant differences were found regarding parity, cycle number, BMI, baseline FSH.

Parameters of the ovarian response in the four groups are presented in Table 2. Examining the ovarian response to COH, no significant differences were noted between the study groups and the control regarding the length of treatment, estradiol and progesterone levels on the day of hCG administration. Although a similar number of oocytes were retrieved between the groups, in the control group a significantly lower number of oocytes underwent ICSI, because in these patients some of the oocytes underwent insemination. The mean number of fertilized oocytes was similar between the groups and a similar mean number of embryos were transferred. No significant differences were found endometrial thickness between the groups.

Laboratory and clinical outcome parameters in the four groups are presented in Table 3. Regarding our primary outcome parameter, 2PN-fertilization rate was significantly lower in group I compared with the control group (IV) (p=0.006). The percentage of the cycles without embryo transfer was comparable between the groups. Regarding our secondary outcome parameters, the clinical outcome measures such as embryo implantation rate, CPR and LBR per cycle or per Embryo Transfer (ET) were comparable between the groups. Similarly the percentage of pregnancies diagnosed as chemical, ectopic or missed were comparable between the groups (Table 4). Logistic regression stepwise analysis, found that sperm concentration had no predictive value for live-birth achievement. Parameters such as age, number of

P value	IV n=692	III n=110	II n=89	I n=254	Sperm Concentration
0.000	33.6 ± 4.2	33.1 ± 4.0	32.1 ± 4.5	31.9 ± 4.5	AGE (mean ± S.D.)
0.001	36.5	38.5	47.2	51.4	Nulligravida %
0.83	62	58.7	65.2	61.7	Nullipara %
0.473	3.21 ± 2.7	3.26 ± 2.5-	3.46 ± 2.9	2.94 ± 2.1	Cycle Number (mean ± S.D.)
0.326	29 ± 18.5	32.6 ± 32.5	31.2 ± 21.9	30.2 ± 12.3	BMI (mean ± S.D.)
0.271	6.69 ± 2.6	7.08 ± 2.9	7.14 ± 2.3	6.71 ± 2.5	Base Line FSH (IU ± S.D.)
					Major IVF indication
	48%	100%	100%	100%	Male factor
	24.3%				Mechanical
	15.9%				Unexplained
	12 %				Anovulation

Table 1: Patient's demographic characteristics.

P value	IV n=692	III n=110	II n=89	I n=254	Sperm Concentration
0.718	11.57 ± 9.8	11.1 ± 8.7	10.78 ± 5.7	10.9 ± 8.2	Length of treatment (days) (mean ± S.D.)
0.518	1701 ± 1091	1839 ± 1066	1725 ± 894	1650 ± 1232	Serun Estradiol on hCG day (pg/ml) (mean ± S.D.)
0.287	1.65 ± 9.8	2.45 ± 13.2	0.66 ± 0.48	2.91 ± 14.9	Serun Progesterone on hCG day (ng/ml) (mean ± S.D.)
0.56	9.3 ± 6.2	9.9 ± 6.3	8.8 ± 6.2	9.7 ± 6.2	Number of oocytes retrieved (mean ± S.D.)
0.000	6.92 ± 5.2	8.9 ± 5.68	8.48 ± 5.9	9.69 ± 6.25	Number of oocytes with ICSI (mean ± S.D.)
0.533	6233 (4.12 ± 3.5)	1128 (5.33 ± 3.6)	1041 (5 ± 4.9)	2200 (5.15 ± 3.6)	Number of oocytes with 2PN fertilization (total) (mean ± S.D.)
0.719	1.80 ± 0.28	1.83 ± 0.8	1.71 ± 0.8	1.77 ± 0.8	# Embryos transferred (mean ± S.D.)
0.930	11.4 ± 8.9	11.7 ± 8.7	10.9 ± 2.0	8.66 ± 17.7	Endometrial thickness (mm) (mean ± S.D.)

Table 2: Patient's ovarian response.

P value	Total n=1145	IV n=692	III n=110	II n=89	I n=254	Sperm Concentration
0.006	61.4 ± 29.2	63.3 ± 30.7	63.3 ± 28.7	58.4 ± 25.2	56.3 ± 25.7	Fertilization rate (mean % ± S.D.)
0.979	81 (7.0)	44 (6.3)	6 (5.4)	10 (11.2)	21 (8.2)	Number of cycles with no transfer (%)
0.85	(400/2049) 19.5	(246/1246) 19.74	(37/201) 18.41	(31/152) 20.4	(86/450) 19.11	Implantation rate (sacs/ ET'd) / (%)
	1064	648	104	79	233	Number of embryo transfers
	379	225	32	27	95	Number of pregnancies
	43 (3.8%)	25 (3.6%)	2 (1.8%)	3 (3.4%)	13 (5.1%)	Chemical pregnancies
	14 (1.2%)	7 (1%)	0	0	7 (2.8%)	Ectopic pregnancies
0.90	(322/1145) 28.1	(193/692) 27.8	(30/110) 27.2	(24/89) 26.96	(75/254) 29.5	CPR/Cycle (%)
0.128	(322/1064) 30.2	(193/648) 29.7	(30/104) 28.8	(24/79) 30.3	(75/233) 32.1	CPR/ET (%)
	49 (4.3%)	27 (3.9%)	4 (3.6%)	2 (2.25%)	16 (6.3%)	Number of ESA's
0.35	(273/1145) 23.8	(166/692) 24.0	(26/110) 23.6	(22/89) 24.7	(59/254) 23.2	LBR/Cycle (%)
0.25	(273/1064) 25.7	(166/692) 23.9	(26/104) 25.0	(22/79) 27.8	(59/233) 25.3	LBR/ET (%)

Table 3: Outcome parameters.

P value	Total n=1145	IV n=692	III n=110	II n=89	I n=254	Sperm Concentration
ns	379/1145 37.4%	225/692 30.3%	32/110 29.1%	27/89 32.5%	95/254 33.1%	Number of pregnancies
ns	43 (4%)	25 (3.6%)	2 (2%)	3 (3.4%)	13 (5.2%)	Chemical pregnancies
ns	14 (1.2%)	7 (1%)	0	0	7 (3%)	Ectopic pregnancies
ns	322 (28.1%)	193 (27.3)	30 (27.3%)	24 (27%)	75 (29.5%)	Number of clinical pregnancies
ns	49 (4.3%)	27 (3.9%)	4 (3.6%)	2 (2.25%)	16 (6.3%)	Number of ESA's
ns	273 (23.8%)	166 (24%)	26 (23.6%)	22 (24.7%)	59 (23%)	Number of Deliveries

Table 4: Outcome of pregnancy.

oocytes retrieved, fertilized and number of embryos transferred, showed no significant correlation to live-birth (R square 1.011, p=0.867).

Discussion

The introduction of ICSI 23 years ago revolutionized the field of andrology enabling to offer fatherhood to men with severe testicular dysfunction. Sperm analysis gives indication for assessment of the fertilizing potential of the patient and *in vitro* fertilization is offered when the chances for *in vivo* fertilization are assessed as unsatisfactory. The exact threshold below which ICSI should be performed is controversial. Sperm profile of the ejaculate given to the IVF laboratory to be used actually on the very date of oocyte pick up may be more accurate to predict outcome of ICSI. The various parameters examined in the sperm analysis such as concentration, motility and morphology, represent the general picture and may not reflect the exact status of the individual sperm cells selected for ICSI. Therefore we performed a retrospective study to examine the impact of sperm concentration, a parameter that may express the ease of the embryologist to find a specific sperm cell eligible for injection, on the clinical outcome of the procedure. From the IVF laboratory perspective the primary outcome of ICSI may be the fertilization rate but from the patients perspective the main outcome parameter is live birth rate. Indeed our results indicate that fertilization rate in the group of <1 million sperm cell/ml was significantly inferior compared to those with >10 million sperm cell/ml. However, as in all patients a similar number of embryos were transferred, the clinical outcome of IR, CPR and LBR were comparable between the groups. The results in our study corroborate those by Hashimoto et al., who reported significant reduction of fertilization rates in the group of <1 million sperm cell/ml but no influence of sperm concentration on clinical pregnancy rates per ET in 908 ICSI cycles evaluated [12]. Also Arikan et al. [13,14] reported similar findings 560 ICSI cycles evaluated.

Our study is the largest study in the literature examining the influence of sperm concentration on the outcome of ICSI and we reported also data on LBR, a parameter not reported in previous studies. The limitations of our study was its retrospective nature, group I including also patients with cryptozoospermia having extremely low number of spermatozoa. Still our results may have a role in the consultation of patients showing that the LBR after ICSI is not affected significantly by the sperm concentration in the ejaculate used.

To conclude, we found that although sperm cells concentration of <1 million/ml in the ejaculate used for ICSI affected the fertilization rate; sperm concentration had no influence on the live-birth rate following ET.

Author Contributions

The work presented here was carried out through collaboration among the authors. SF, CO and SM defined the research concept, theme and methods. SF, GL, BSR and SM were responsible for data collection and carrying out the experimental work. SF and CO were responsible for data processing, analysis and interpretation of results. SF, CO, GL and SM drafted the article and all authors read and approved the final version

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