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Andrology

The effects of sperm quality on embryo development after intracytoplasmic sperm injection

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***Purpose*:** To explore the possible relationship between sperm quality and embryo development, pregnancy and implantation rates, in patients undergoing intracytoplasmic sperm injection (ICSI).
***Methods*:** Fertilization and cleavage rates, quality of embryos, blastocyst development, pregnancy and implantation rates were analyzed in 1020 embryos from 219 couples undergoing first ICSI treatment cycle. The couples were allocated in five groups, according to semen parameters: Group 1: patients with normal semen parameters, Group 2: patients with mild oligo-astheno-teratozoospermia, Group 3: patients with severe oligo-astheno-teratozoospermia, Group 4: patients with obstructive azoospermia, Group 5: patients with non-obstructive azoospermia.
***Results*:** Fertilization and cleavage rates, quality of embryos as well as blastocyst development rates were significantly reduced, as semen quality decreased. However, no significant differences were observed in clinical pregnancy and implantation rates.
***Conclusion*:** Overall, a negative relationship was observed between semen quality and embryo development, even before activation of the embryonic genome, suggesting that sperm can affect embryogenesis from a very early stage.

KEY WORDS: Embryo morphology; ICSI; implantation rates; male factor infertility; semen quality.

INTRODUCTION

The introduction of intracytoplasmic sperm injection (ICSI) in 1992 (1), followed by reports of high success rates using this method (2), has opened a new era in the treatment of previously untreatable cases of male factor infertility. In broad terms, male infertility can be divided into oligo-astheno-teratozoospermia (OAT) and azoospermia. Even the most severe cases of OAT can nowadays be successfully treated with ICSI. Furthermore, ICSI can be used in cases of obstructive azoospermia (OA) or non-obstructive azoospermia (NOA) where sperm can be extracted from the epididymis or the testis.

The wide application of ICSI on severe male factor infertility and the better understanding of sperm biology, led to more detailed investigation of potential paternal effects on embryo quality and development. There is published evidence that poor semen parameters result in low blastocyst formation rates after in vitro fertilization (IVF) (3,4), suggesting that sperm can influence human pre-implantation embryo development. In addition, blastocyst formation rates were shown to be lower after ICSI than after IVF (5). Embryo development, pregnancy and implantation rates have been found to be affected by the source of sperm (ejaculated, epididymal, or testicular) and the type of male factor infertility (OA or NOA) (6). ICSI

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using testicular sperm has been found to produce lower fertilization and pregnancy rates than normal ejaculated spermatozoa (6). Finally, fertilization, embryo development, blastocyst formation, pregnancy and implantation rates after ICSI are significantly lower in NOA patients in comparison to OA patients (7–9).

However, there is still an ongoing debate as to whether sperm quality can influence embryo development, as other studies have shown that embryos from OAT patients have the same developmental and implantation potential with those from normospermic men (10).

The aim of this study was to explore the possible relationship between sperm quality and embryo development, pregnancy and implantation rates in patients undergoing ICSI.

MATERIALS AND METHODS

Patients

A total of 219 couples undergoing ICSI for the first time between the years 2003 and 2004 were included in this study. This cohort represents the total number of couples who underwent ICSI in our unit, with no additional exclusion criteria. The couples were classified in five groups according to sperm parameters as follows:

- Group 1: men with normal semen parameters (sperm concentration $\geq 20 \times 10^6$/mL, motility $\geq 50\%$ [types a and b, according to the WHO criteria] (11) and morphology $\geq 30\%$ normal forms), which served as the control group
- Group 2: men with mild OAT (sperm concentration $<20 \times 10^6$ /mL and motility $<50\%$ and morphology $<30\%$ normal forms)
- Group 3: men with severe OAT (sperm concentration $<1 \times 10^6$ /mL, motility $<25\%$ and morphology $<10\%$)
- Group 4: men with OA (sperm was recovered by fine needle aspiration—FNA)
- Group 5: men with NOA (sperm was recovered through testicular sperm extraction—TESE)

Sperm Preparation

Only cycles with fresh semen were included in the study. Semen was collected on the day of oocyte retrieval. Ejaculated specimens were left to liquefy for approximately 20–30 min prior to preparation. They were then mixed with equal volume of sperm preparation medium (Medicult, DK) and centrifuged at 1100 rpm for 5 min. The pellet was overlaid on Pure Sperm® (NIDACON International AB, Sweden) gradients of 95 and 47.5%, and centrifuged at 300 $\times$ *g* for 20 min. Subsequent washing was performed in 3 mL of sperm preparation medium at 1100 rpm for 5 min and the final solution was kept at 37°C until insemination or the ICSI procedure.

FNA was performed under local anesthesia by inserting a 21-gauge butterfly needle into the two testicular poles from either testis. The aspirate was washed into a petri dish with a small volume of IVF medium (Medicult, DK). The presence of sperm was confirmed under an inverted microscope at $\times 200$ magnification. ICSI was performed immediately.

TESE was performed under general anesthesia. Several small testicular samples were extracted form the testes and placed on petri dishes containing equilibrated IVF medium. Seminiferous tubules were crushed and separated using glass slide edges and the presence of sperm was examined under an inverted microscope at $\times 200$ magnification. ICSI was performed immediately.

ICSI Procedure, Embryo Culture and Establishment of Pregnancy

Oocyte retrievals were performed after with GnRH agonist/recombinant FSH ovarian stimulation (long protocol), 36–38 h after the hCG injection, as previously described (12). The oocytes were stripped of the surrounding cumulus cells using hyaluronidase (80 IU/mL, Medicult, DK) and ICSI was performed between 38–42 h post the hCG injection according to Van Steirteghem *et al.* (2). Fertilization was check 16–20 h post ICSI by the presence of two pronuclei. Cleavage and embryo quality assessment was performed 42–49 h post ICSI. The embryos were graded, according to the shape, size and degree of fragmentation as follows:

- Grade 1.0: embryos with evenly sized blastomeres and no fragmentation
- Grade 2.0: embryos with uneven blastomeres and no fragmentation
- Grade 2.1: embryos with less than 10% fragmentation and uneven blastomeres
- Grade 2.2: embryos with 10–20% fragmentation and uneven blastomeres

Table I. Evaluation of Embryo Development After ICSI Between Normospermic (Group 1), Mild OAT (Group 2), Severe OAT (Group 3), OA (Group 4) and NOA (Group 5) Patients

	Group 1	Group 2	Group 3	Group 4	Group 5
Female age (years)	35.9 ± 0.5	33.7 ± 0.7	32.1 ± 0.8*	31.0 ± 0.7*	31.3 ± 0.9*
Number of oocyte retrievals (OR)	69	43	29	41	37
Number of MII oocytes injected	432	259	266	358	274
Number of MII oocyte fertilized (%)	331/432 (76.6)	184/259 (71.0)	184/266 (69.1)**	203/358 (56.4)**,***,†	147/274 (53.6)**,***,†
Number of embryos cleaved (%)	328/331 (99.0)	180/184 (97.8)	172/184 (93.4)**	194/203 (96.5)**	140/147 (95.2)**
Number of 4-cell, grade 1 and 2 embryos on day 2 (%)	64/328 (19.5)	20/180 (11.1)**	18/172 (10.5)**	20/194 (10.3)**	10/140 (7.1)**
Blastocyst formation rate on day 5 (%)	62/107 (58.0)	21/49 (42.8)	9/62 (40.6)**,***	3/60 (32.1)**,***	0/4 (0.0)**

Note. Data are given as mean ± SEM or as absolute values (percentages).
*$p < 0.05$ vs. group 1 (one-way ANOVA//Bonferroni).
**$p < 0.05$ vs. group 1 (Chi-square).
***$p < 0.05$ vs. group 2 (Chi-square).
†$p < 0.05$ vs. group 3 (Chi-square).

- Grade 3.1: embryos with 20–50% fragmentation and uneven or indistinct blastomeres
- Grade 3.2: embryos with more than 50% fragmentation and indistinct blastomeres

Embryo transfers were performed on days 2–5, depending on the number and the quality of the available embryos on day 2 (need of at least four good quality embryos to culture up to the blastocyst stage). For blastocyst culture, the embryos were transferred in G2 media (Vitolife, Sweden) on day 3 and embryo development was assessed every day. Luteal supplementation was administered in the form of progesterone vaginal tablets (Utrogestan 200 mg, tid) from the day of egg collection and until the 12th week of pregnancy, where appropriate.

Pregnancies were confirmed by serum β-hCG test, 15 days after the embryo transfer and clinical pregnancies were confirmed by vaginal ultrasound 30 days after embryo transfer showing the presence of an intrauterine gestational sac.

Statistical Analysis

Data are given as mean ± standard error of the mean (SEM). Comparisons among the five study groups are made by means of ANOVA (Bonferroni as post-hoc test) in numerical parameters and by means of Chi-square test or Fisher's exact test in categorical parameters. Statistical difference was considered significant at a level of 0.05. Data analysis was made with the SPSS 11 software (SPSS, Chicago, Ill).

RESULTS

A total of 219 ICSI cycles were analyzed. The average female age in each one of the five study groups as well as the fertilization, cleavage, embryo quality and blastocyst formation rates are shown on Table I.

Increasing severity of male factor infertility was associated with decreased fertilization rates throughout the five groups. The difference was not statistically significant between groups 1 and 2 (76.6 and 71.0%, respectively) but was significant between groups 3 (69.1%), 4 (56.4%) and 5 (53.6%) compared to the controls (76.6%, $p < 0.05$). In addition, statistical significance was observed in the fertilization rate between group 4 and 5 as compared to group 2.

The same trend was observed in the cleavage rates. Although no significant difference was observed between groups 1 (99.0%) and 2 (97.8%), cleavage was significantly impaired in groups 3 (93.4%), 4 (96.5%) and 5 (95.2%) as compared to group 1 ($p < 0.05$).

Embryo quality, expressed as number of grade 1 and 2/4-cell embryos on day 2, was negatively affected, as semen quality reduced. This effect was significant in groups 2, 3, 4 and 5 as compared to group 1 (11.1, 10.5, 10.3 and 7.1% vs 19.5%, respectively; $p < 0.05$).

Finally, reduced blastocyst formation rates on day 5 were observed when semen quality was impaired. The difference was statistically significant for groups 3 and 4 compared to groups 1 and 2 (40.6 and 32.1% vs. 58 and 42.8%, respectively; $p < 0.05$). No blastocyst was obtained in group 5, due to the small number of embryos cultured up to that stage. No significant difference was found between group 2 (42.8%) and the control group.

Table II. Evaluation of Clinical Outcome After ICSI Between Normospermic (Group 1), Mild OAT (Group 2), Severe OAT (Group 3), OA (Group 4) and NOA (Group 5) Patients

	Group 1	Group 2	Group 3	Group 4	Group 5
Number of oocyte retrievals	69	43	29	41	37
Number of embryos transferred	2.7 ± 0.1	2.7 ± 0.2	2.6 ± 0.3	3.2 ± 0.2	3.3 ± 0.2
Clinical pregnancy rate/OR (%)	18/69 (26.0)	7/43 (16.2)	5/29 (17.2)	5/41 (12.1)	4/37 (10.8)
Implantation rate (%)	20/65 (30.7)	9/32 (28.1)	7/27 (25.9)	5/29 (17.2)	5/20 (25.0)

Note. Data are given as mean ± SEM or as absolute values (percentages).

Finally, no significant differences were observed in the clinical pregnancy and implantation rates between the five groups (Table II).

DISCUSSION

The role of male factor infertility on embryo development is gaining attention since the introduction of ICSI as a treatment option for patients with very poor sperm characteristics. The present study demonstrates reduced fertilization and cleavage rates associated with poor semen parameters, suggesting a very early onset of paternal effects on embryo development. This is an interesting finding considering the classical knowledge that the human embryo, during these early stages of development, is controlled by maternally inherited m-RNA, as the embryonic genome is not activated until after the 4-cell stage (13). Thus, if there is a paternal influence on embryo development, this should not be apparent until the 8-cell or morula stage. Nevertheless, our data seem to support the notion that paternal factors can influence embryo development at this early stage by instigating a weak transcriptional activity, necessary for nucleolar development, in the male pronucleus (14). Additionally, epigenetic factors related to early paternal effects on embryo development have been reported. These include factors such as phospholipase C–ζ (PLCζ), which are responsible for regulating calcium oscillations necessary for oocyte activation, but their mechanisms of action are not clearly understood (15). It has been shown that mouse oocyte calcium oscillations are promoted in the presence of PLCζ and are depleted in its absence (15). Furthermore, the sperm derived centrosome was shown to be the only functionally active centrosome in the early stages of embryogenesis. As this cellular organ is responsible for microtubule organization, any centrosomal dysfunction at fertilization can result in spindle disturbances and, subsequently, abnormal or arrested cleavage (5,14).

In addition to low fertilization and cleavage rates, significantly impaired embryo quality and blastocyst formation were also observed, especially when testicular sperm from OA and NOA patients was used for ICSI. A possible explanation for this may be that testicular spermatozoa are less mature and subsequently less competent to fertilize than the ejaculated ones, since the final steps of sperm maturation take place in the epididymis (6,7). Moreover, studies have shown a higher incidence of chromosomal aneuploidies in testicular spermatozoa from NOA patients as compared to OA patients (7), which may explain, at least partly, the lower embryo development rates observed in this group of patients, in the present study.

Embryo development can also be influenced by the quality of DNA in the sperm head. It has been reported that ejaculated sperm can exhibit DNA strand breaks (fragmentation) due to underprotamination during spermiogenesis (16,17). Incomplete protamine deposition or complete absence of protamines during spermiogenesis may result in abnormal chromatin packing in the sperm head and subsequently to abnormal chromatin decondensation at fertilization (16). The incidence of DNA fragmentation has been found to be higher in samples with low sperm concentration (18) and a significant negative correlation between DNA fragmentation and blastocyst development rates after IVF or ICSI has been reported (16,19–23). Evenson *et al.* (24) showed that even samples with normal semen parameters express some level of DNA fragmentation. It is speculated that oocytes have the ability to correct small scale DNA damage upon fertilization, but if that goes above a certain level, it might be difficult for the oocyte to cope and can possibly lead to fertilization failure or impaired embryo development (16). Benchaib *et al.* (21) illustrated that fertilization and pregnancy rates remain unaffected when DNA fragmentation does not exceed a 10% threshold value.

DNA fragmentation may also be an indication of a defective apoptotic mechanism. Apoptosis occurs

naturally in the testis, aiming possibly to limit the number of germ cells to numbers that can be supported by the Sertoli cells or to naturally deplete abnormal sperm populations. It is evident that abnormal spermatozoa express high levels of apoptotic markers such as Fas, Bcl-x and p53, suggesting that even though programmed cell death has been initiated, some sperm escape and go through spermiogenesis (16,17,22).

Additionally, the presence of reactive oxygen species (ROS) and ROS-related DNA oxidative products, such as 8-hydroxy-deoxyguanosine (8-OH-dG) have also been associated with sperm DNA fragmentation (17,25). It has been shown that as sperm concentration decreases, DNA fragmentation index and 8-OH-dG increase, suggesting that ROS and the relevant products may have a major impact on sperm DNA integrity (25).

Low fertilization, cleavage and embryo quality rates after ICSI have also been found by other groups (4,6–8), who compared the same parameters utilizing sperm from different sources: normal or abnormal ejaculated sperm, sperm from OA patients and sperm from NOA patients. Slower and lower blastocyst formation rates, after extended in vitro culture of embryos produced by ICSI, have been demonstrated in cases of epididymal sperm extracted from OA patients or testicular sperm extracted from NOA patients, as compared to the control group (5,9). Furthermore, other studies (6–8) have shown significantly lower pregnancy and implantation rates than the control group in oocytes fertilized with poor quality sperm. However, no such significant difference was found in the present study, although the number of cases may not be sufficient to demonstrate that. On the other hand, it has been demonstrated that testicular sperm recovered from patients with OA and NOA can be equally effective with ejaculated sperm in the fertilization, pregnancy and implantation rates after ICSI (26,27). Oehninger *et al.* (10) found that OAT patients achieve similar embryo implantation and developmental potential rates to men with normozoospermia treated by IVF. Greco *et al*. (28) investigated the percentage of DNA fragmentation between testicular and ejaculated sperm and compared the ICSI outcomes in two sequential attempts. Interestingly, testicular sperm had a lower incidence of DNA fragmentation than ejaculated sperm. Consequently, no significant difference in fertilization, cleavage and embryo quality rates were observed, when ejaculated or testicular sperm were used for ICSI, while pregnancy and implantation rates after ICSI with testicular sperm were significantly higher than with ejaculated sperm (28).

In conclusion, significantly lower oocyte fertilization as well as embryo cleavage and development rates were observed in our study when severely abnormal ejaculated or surgically retrieved sperm were used for ICSI. This, however, does not seem to affect pregnancy and implantation rates to a significant extent, suggesting the implication of maternally derived factors. Overall, a negative relationship was observed between semen quality and embryo development, even before activation of the embryonic genome, suggesting that sperm can affect embryogenesis from a very early stage.

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