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The effect of intracytoplasmic sperm injection and semen parameters on blastocyst development *in vitro*

Jane E.Miller and T.Timothy Smith[1]

North Hudson IVF Center, 385 Sylvan Avenue, Englewood Cliffs, NJ 07632, USA

[1]To whom correspondence should be addressed. E-mail: twopn@optonline.net

The present study compares the development and quality of blastocysts derived from conventional oocyte insemination with those derived from intracytoplasmic sperm injection (ICSI). Oocytes were collected from patients undergoing ovarian stimulation with human menopausal gonadotrophins for IVF. Patients with normal semen were assigned to conventional oocyte insemination while those with progressive motility <20% and/or normal sperm morphology ≤4% were assigned to ICSI. Resulting embryos were cultured for up to 6 days. The mean number and percentage of embryos reaching the blastocyst stage and the mean number and percentage of blastocysts of high quality on days 5–6 were assessed for both treatment groups and compared. The influence of paternal factors (sperm concentration, motility, progressive motility, morphology) on blastocyst development and quality were assessed by regression analyses. Significantly more ICSI-derived embryos arrested at the 5- to 8-cell stage ($P = 0.024$) concomitant with the activation of the paternal genome than those derived from conventional oocyte insemination. Significantly fewer ICSI-derived embryos reached the blastocyst stage on days 5–6 ($P < 0.001$) and significantly fewer ICSI-derived embryos were of high quality ($P = 0.002$) compared with conventional oocyte insemination. When treatment groups were combined and evaluated by regression analysis, progressive motility and sperm morphology were significantly correlated with diminished blastocyst development and quality ($P < 0.05$). From these data, we conclude that paternal factors and/or performing ICSI in cases of severe male factor infertility may have a detrimental effect on blastocyst development and their quality.

Keywords: blastocyst/ICSI/paternal effects/spermatozoa

Introduction

Prior to the use of intracytoplasmic sperm injection (ICSI) for the treatment of male factor infertility, several authors reported that sub-optimal semen parameters such as poor morphology and/or poor motility led to decreased fertilization and pregnancy rates (Kruger *et al*., 1988; Ron-El *et al*., 1991; Enginsu *et al*., 1992; Thanki *et al*., 1992). In many cases, the decreased pregnancy rate was attributable to inadequate fertilization which, in turn, resulted in fewer embryos for transfer. Later studies revealed that even when higher fertilization rates were achieved, poor semen parameters continued to be associated with poor embryonic growth (Ron-El *et al*., 1991; Parinaud *et al*., 1993; Jones *et al*., 1998). When ICSI was introduced into clinical use for the treatment of male infertility (Palermo *et al*., 1992; Van Steirteghem *et al*., 1993), fertilization rates improved dramatically. However, implantation rates for ICSI-derived embryos continued to be lower than those generated through conventional oocyte insemination, suggesting diminished quality of ICSI-derived embryos (Palermo *et al*., 1993).

The diminished potential of ICSI embryos may be due to paternal influences or the ICSI procedure itself. There is a considerable body of evidence to suggest that sperm DNA from severe oligoasthenoteratozoospermic patients, the same patients who benefit the most from this procedure, possess anomalies such as loose packaging of the chromatin and DNA strand breaks (Evenson *et al*., 1980; Foresta *et al*., 1992; Sailer *et al*., 1995). When ICSI is employed, these abnormal spermatozoa are capable of achieving fertilization (Sakkas *et al*., 1998; Ahmadi and Ng, 1999), but DNA anomalies may still result in developmental arrest during the pre-implantation phase. On the other hand, the ICSI procedure itself is invasive and may lead to the physical disruption of nuclear or cytoplasmic components within the oocyte which are necessary for continued development.

When culturing of embryos to the blastocyst stage of development became feasible for routine clinical application through the use of co-culture (Ménézo *et al*., 1990, 1992) or chemically defined, sequential culture medium (Gardner, 1998; Gardner *et al*., 1998; Marek *et al*., 1999), it became clear that paternal influences could affect the development of surplus embryos to the blastocyst stage when ICSI was employed (Shoukir *et al*., 1998; Dumoulin *et al*., 2000). A recent study (Griffiths *et al*., 2000) where ICSI was performed in the absence of male infertility suggests that the ICSI procedure itself compromises embryo development. However, the results

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of these studies should be interpreted with caution, because these observations were based on the continued culture of embryos considered unsuitable for transfer or cryopreservation on day 3 of development. In addition, these studies predate recent improvements in culture media and may represent developmental ability under sub-optimal culture conditions. The present study was undertaken to address these problems by culturing all embryos through to the fifth or sixth day of development using a chemically defined, sequential culture system. We compare here the developmental capability of embryos generated through conventional oocyte insemination and ICSI and assess the results in terms of paternal influences.

Materials and methods

Patients

Couples undergoing IVF at a private infertility centre were assigned to one of two treatment groups: (i) ICSI ($n = 32$) or (ii) conventional oocyte insemination ($n = 31$) based on the presence or absence of a contributing male factor. Male factors were considered severe enough to warrant assignment to the ICSI group when sperm morphology was ≤4% normal forms as determined by Tygerberg strict criteria (Kruger *et al*., 1988) and/or when forward progressive motility after processing was ≤20%. Although maternal age and diagnosis were not considered in the assignment of patients to treatment groups, maternal age was recorded for later comparison.

Semen analysis and preparation

Semen specimens for IVF were produced on-site by masturbation, allowed to liquefy at 37°C and evaluated within 30 min of collection. Specimens were analysed for sperm concentration, percentage of motile spermatozoa and forward progressive motility (defined as velocity >25 µm/s and straightness >80%) using a computer-assisted semen analyser (CASA, Hamilton Thorn Master C v.10, Beverly, MA, USA) and sperm morphology using a modified Papinicoulou stain (Spermac®, Fertipro, Beernum, Belgium) and Tygerberg strict criteria. Following analysis, a concentrated population of motile spermatozoa was prepared by density gradient centrifugation through a commercially available, silane-coated silicon gradient material. Processed spermatozoa were washed twice in commercially available human tubal fluid culture medium (HTF) modified with 20 mmol/l HEPES (mHTF) and supplemented with 10% plasma protein fraction (PPF) (Plasmarc®; Baxter Healthcare Corp., Glendale, CA, USA). Processed spermatozoa were again evaluated by CASA and incubated at room temperature prior to use for insemination or ICSI.

Ovarian stimulation and oocyte collection

Patients underwent ovarian stimulation with human menopausal gonadotrophins (HMG) following pituitary down-regulation with a gonadotrophin-releasing hormone (GnRH) agonist. Final oocyte maturation was induced with human chorionic gonadotrophin (HCG) when the largest follicle(s) reached a mean diameter of 18 mm. Oocyte–cumulus complexes were recovered by transvaginal ultrasound-guided needle aspiration at 36–37 h after HCG administration. Follicle aspirates were collected in tubes containing pre-warmed (37°C), pre-gassed (5% CO_2) Earle's balanced salt solution supplemented with 2 IU/ml heparin. Oocyte collection and identification were carried out in a vertical laminar flow hood equipped with a warm (37°C) work area. When dishes were out of the incubator, correct media pH was maintained using a steady stream of charcoal-filtered tri-gas mixture (5%O_2/5%CO_2/90%N_2) under a glass cover. Aspirates were examined using a stereomicroscope. Once identified, oocyte–cumulus complexes were rinsed twice in warm HTF supplemented with 10% PPF and incubated for 4–6 h to facilitate final cytoplasmic and nuclear maturation prior to conventional oocyte insemination or ICSI. The number of oocytes collected per patient was recorded for future analysis.

ICSI and insemination

If ICSI was required, oocytes were stripped of surrounding cumulus cells by incubation for 30–60 s in pre-warmed mHTF containing hyaluronidase (80 IU/ml; Conception Technologies, San Diego, CA, USA). Oocyte stripping and evaluation was performed in a vertical laminar flow hood equipped with a warm work surface (37°C). Brief hyaluronidase treatment was followed by repeated pipetting through a 135 µm diameter polycarbonate pipette tip in pre-warmed mHTF supplemented with 10% PPF to remove the remaining cumulus and coronal cells. Stripped oocytes were washed twice in mHTF supplemented with 10% PPF and checked for maturity (presence of the first polar body) using a stereomicroscope. Mature oocytes were transferred to HTF supplemented with 10% PPF and incubated at 37°C in a humidified atmosphere of 5% CO_2 in air until the injection procedure (~30 min). The ICSI procedure was performed on mature (metaphase II) oocytes as described by Van Steirteghem *et al*. (1993). Micro-injection pipettes (5–7 µm, 50° bevel, spiked) were purchased from a commercial source (Humagen, Charlotte, NC, USA). ICSI was performed using Narashige hydraulic joystick manipulations mounted on a Nikon Diophot 200® inverted microscope equipped with Hoffman Modulation Contrast optics and a heated stage (37°C).

In preparation for injection, 1 µl of sperm suspension was introduced into a 5 µl drop of 10% polyvinylpyrrolidone (PVP) solution under mineral oil. Prior to injection, spermatozoa were immobilized by striking the principle piece of the tail with the injection pipette to induce damage to the plasma membrane. Care was taken whenever possible to select only morphologically normal spermatozoa for injection. Immobilized spermatozoa were individually injected head first into oocytes secured in place with the polar body at the 12 or 6 o'clock position to avoid disturbing the meiotic spindle. A small amount of aspiration pressure was applied to the injection pipette until the oolemma broke. Care was taken to aspirate a minimal amount of cytoplasm into the injection pipette and to introduce a minimal volume of PVP solution into the ooplasm during injection. Injections were carried out in 20 µl drops of mHTF medium supplemented with 10% PPF under mineral oil at 37°C. To minimize exposure to ambient conditions, no more than three oocytes were injected at a time. Following injection, oocytes were washed twice in pre-warmed HTF, rinsed through two 50 µl drops of G1.2 medium (IVF Sciences Scandinavia, Goteborg, Sweden) and incubated at 37°C in a third 50 µl drop of G1.2 medium under mineral oil in an atmosphere of charcoal-filtered, tri-gas mixture in a gas-tight desiccator.

When no male factor was present, up to three cumulus-intact oocytes were inseminated with 50×10^3 motile spermatozoa/oocyte in 50 µl droplets of G1.2 medium under mineral oil and incubated at 37°C in an atmosphere of charcoal-filtered, tri-gas mixture in a gas-tight dissector.

Embryo culture, development and assessment

At 17–19 h post-insemination or injection, all oocytes were checked for maturity and evidence of normal fertilization (two pronuclei) and transferred to a fresh 50 µl drop of G1.2 medium. The number of mature oocytes in both groups was noted for later comparison. Three to five embryos were incubated in each 50 µl drop. At 70–74 h post-insemination or injection (day 3), embryo development (cell number

and degree of fragmentation) was recorded. Embryo fragmentation was assessed using a semi-quantitative scale (1 = none, 1.5 = 1–10%, 2 = 11–20%, 3 = 21–30%, 4 > 30%). Following assessment on day 3, embryos were washed through two 50 µl drops of G2.2 medium (IVF Sciences Scandinavia) and incubated in a third 50 µl drop of G2.2 medium under mineral oil.

At 120–122 h and again at 140–144 h post-insemination, embryo development was assessed to determine the number of embryos reaching the blastocyst stage of development. Blastocysts were graded with regard to blastocoel cavity expansion (1 = 10–50% of embryo volume, 2 = 50–80% of embryo volume, 3 = >80% embryo volume, 4 = blastocyst has expanded beyond the size of the original embryo, 5 = fully expanded with trophectoderm herniating through a breach in the zona pellucida, 6 = completely hatched blastocyst) and the quality of the inner cell mass (A = many tightly packed cells, B = several loosely packed cells, C = very few cells) and quality of the trophectoderm (A = many cells forming cohesive epithelium, B = few cells forming loose epithelium, C = very few cells) (Gardner, 1999). Good quality blastocysts were defined as having a grade of 3AA–6AA on days 5–6.

Statistical methods

Treatment groups were compared to evaluate the effect of fertilization technique on the number and percentage of blastocysts formed and the number and percentage of good quality blastocysts by days 5–6 of development. When data were normally distributed, differences between treatment groups were assessed by one-way analysis of variance (ANOVA). Post-hoc analysis was performed using the Tukey test for all pair-wise comparisons. Data expressed as percentages were normalized prior to analysis by applying the arcsine square root transformation. When data were not normally distributed, differences between treatments were analysed by Kruskal–Wallis ANOVA on Ranks. In this case, post hoc analysis was performed using Dunn's test for all pair-wise comparisons. Simple linear regression analysis was used to determine correlations between semen parameters and outcome measures. All statistical operations were performed using SigmaStat® v2.03 (Jandel Scientific, San Rafael, CA, USA).

Table I. Comparison of maternal age, oocyte, fertilization and embryo characteristics between treatment groups

Parameter	ICSI (n = 32)	Insemin (n = 31)	P value
Maternal age	35.8 ± 4.11	33.6 ± 5.56	NS
No. oocytes retrieved	12.4 ± 6.02	13.7 ± 6.52	NS
No. mature oocytes	9.59 ± 4.73	12.4 ± 6.11	NS
Percent mature oocytes fertilized	82.6 ± 13.1	80.2 ± 16.9	NS
No. embryos >4 cell on day 3	6.06 ± 2.98	7.68 ± 3.77	NS
Embryo grade on day 3	1.16 (1.0–1.7)	1.42 (1.0–2.0)	0.05

Data are expressed mean ± the standard deviation with the exception of embryo grade on day 3, which is expressed as the median and range. Differences between means were tested for significance by ANOVA. NS = not significant.

Table II. Comparison between treatment groups of the stage of embryonic arrest for embryos failing to reach the blastocyst stage by day 6

Stage of arrest	ICSI (n = 32)	Insemin (n = 31)	P value
1- to 4-cell	15.6 ± 15.3%	15.9 ± 15.7%	NS
5- to 8-cell	27.2 ± 23.1%	9.8 ± 13.4%	0.024
9- to 16-cell	13.8 ± 19.2%	10.0 ± 15.6%	NS
Morula	12.9 ± 19.8%	13.1 ± 17.5%	NS

Data are expressed as mean ± the standard deviation of the mean. Differences between means were tested for significance by one-way repeated measures (ANOVA) following arcsine square root transformation and post-hoc analysis by the Tukey test. NS = not significant.

Table III. Comparison between treatment groups of blastocyst development and quality

Parameter	ICSI (n = 32)	Insemin (n = 31)	P value
No. blastocyst stage embryos	2.16 ± 1.97	5.29 ± 3.64	<0.001
Percent blastocyst stage embryos	30.3 ± 21.5	51.9 ± 27.9	0.003
No. high quality blastocysts	1.09 ± 1.82	3.33 ± 3.21	0.002
Percent high quality blastocysts	13.6 ± 21.3	28.2 ± 25.7	0.011

Data are expressed as mean ± SD. Differences between means were tested for significance by Kruskal–Wallis one way ANOVA on Ranks. High quality blastocysts were defined as expanded blastocyst stage embryos with a well-defined trophectoderm layer and adequate inner cell mass (see text for details).

Results

There were no significant differences between treatment groups (ICSI and conventional oocyte insemination) with respect to maternal age, number of oocytes retrieved, number of mature oocytes obtained, percentage of mature oocytes fertilized, number of embryos >4-cell on day 3 of development and a borderline significant difference in embryo grade on day 3 (Table I).

A significantly higher proportion of ICSI derived embryos arrested at the 5- to 8-cell stage of development concomitant with the activation of the paternal genome (Table II). We observed a significant decrease in number and percentage of embryos reaching the blastocyst stage of development following ICSI compared with embryos obtained through conventional oocyte insemination (Table III). In addition, there was a concomitant decrease in the number and percentage of good quality blastocysts following ICSI as assessed by morphological criteria.

When treatment groups (ICSI and conventional oocyte insemination) were combined, linear regression analysis revealed a significant correlation between three semen parameters (sperm concentration, progressive motility and morphology) and the percentage of embryos developing to the blastocyst stage and blastocyst quality. The correlation coefficients and statistical significance of these correlations are presented in Table IV.

Discussion

Several oocyte-associated factors can adversely affect the rate of embryo cleavage and embryo quality during the first 3 days of development and hence impair subsequent

Table IV. Correlation between various semen parameters and blastocyst development and quality

Regression analysis	r^2	*P* value
Concentration versus % blastocyst development	0.0845	0.042
Percent motility versus % blastocyst development	0.0345	NS
Percent progressive motility versus % blastocyst development	0.1130	0.039
Percent normal morphology versus % blastocyst development	0.1390	0.006
Concentration versus % high quality blastocysts	0.0217	NS
Percent motility versus % high quality blastocysts	0.0188	NS
Percent progressive motility versus % high quality blastocysts	0.0807	0.041
Percent normal morphology versus % high quality blastocysts	0.0748	0.048

Correlations were tested for significance by ANOVA. High quality blastocysts were defined as expanded blastocyst stage embryos with a well-defined trophectoderm layer and adequate inner cell mass (see text for details). NS = not significant.

development to the blastocyst stage. For example, the degree of nuclear and cytoplasmic maturation of the oocyte will be reflected in the oocyte's ability to fertilize and initiate development. Advanced maternal (oocyte) age is associated with early cleavage arrest and a reduction in embryonic developmental competence (Pantos, 1999). We found no significant difference between treatment groups for factors such as maternal (oocyte) age, fertilization rate and early embryo development and borderline significance in quality up to day 3 of culture (Table I). This is in agreement with a previous report showing no difference in the cell number or quality of day 3 embryos obtained through conventional oocyte insemination and ICSI (Shoukir *et al*., 1998). These data support the contention that any oocyte factors that may adversely affect embryo development during the first 3 days of development were equivalent in both treatment groups. It then follows that any differences observed between treatment groups in the rate of blastocyst development or blastocyst quality were expressed after the third day of development and subsequent to the activation of the embryonic genome at the 4- to 8-cell stage of development (Braude *et al*., 1988).

A significantly higher number of ICSI-derived embryos arrested at the 5- to 8-cell stage concomitant with paternal genomic activation (Table II), suggesting that genetic abnormalities of paternal origin may be responsible. The percentage of embryos arresting at the 5- to 8-cell stage in the present study (27.2 and 9.8% for ICSI and conventional oocyte insemination respectively) was considerably less than that reported by Van Blerkom (Van Blerkom, 1993) (45%), using Earle's balanced salt solution supplemented with maternal serum for embryo culture, and may reflect improved culture conditions using a complex sequential culture system.

In agreement with previous authors using either Vero cell co-culture (Shoukir *et al*, 1998) or defined media (Dumoulin *et al*., 2000) to achieve blastocyst development *in vitro*, a significant decrease was observed in the rate of blastocyst formation following ICSI. However, these authors transferred or cryopreserved the most advanced and morphologically normal embryos on days 2–3 and cultured only supernumerary embryos to the blastocyst stage. The present study is the first to report a diminished rate of blastocyst formation following ICSI by evaluating the development of all embryos generated in a given cycle. Interestingly, in these studies the relative percentages of supernumerary embryos reaching the blastocyst stage following conventional oocyte insemination and ICSI [42.7 and 28.2 (Shoukir *et al*., 1998); 31.8 and 23.0% (Dumoulin *et al*., 2000) respectively] were comparable with the percentage of embryos reaching the blastocyst stage in the present study (51.9 and 30.3% respectively). As suggested previously (Rijinders and Jansen, 1998), these data illustrate that the criteria used on days 2–3 to select embryos capable of attaining the blastocyst stage is of limited value. Indeed, in the present study there was no significant difference between ICSI and conventional oocyte insemination groups with respect to cleavage rate on day 3, but a significant increase in developmental arrest was observed for ICSI embryos on days 3–4. This may explain why Palermo *et al*. (1998) reported that the pregnancy rate improved when a higher number of day 3 embryos were transferred following ICSI (Palermo *et al*., 1998).

In addition to having a diminished rate of blastocyst development, ICSI-derived blastocyst stage embryos were also of poorer quality than embryos generated by conventional oocyte insemination as judged by morphological criteria. Dumoulin and co-workers (Dumoulin *et al*., 2000) did not assess blastocyst stage embryos in terms of trophectoderm and inner cell mass development, but did report no significant difference in cell number between blastocysts derived from ICSI and those from conventional oocyte insemination. Although informative, cell number alone does not reflect blastocyst quality since the embryonic cells must be appropriately allocated between the inner cell mass and the trophectoderm. Of these, the number of cells in the inner cell mass is probably the most predictive of continued developmental potential (Hardy *et al*., 1989). A significant decrease in the number and percentage of ICSI-derived blastocyst stage embryos with well defined inner cell masses was found.

Regardless of the method of insemination, the combined data in this study suggest a paternal influence on blastocyst development. It was observed that sperm concentration, progressive motility and sperm morphology were significantly correlated with blastocyst development (Table IV). This is in agreement with Janny and Ménézo (1994), who reported that when sperm quality was good, there was a linear relationship between cleavage rate and blastocyst formation following conventional oocyte insemination. However, when spermatozoa with both poor motility and poor morphology were used for insemination, the predictive value of the embryo cleavage rate on blastocyst development was lost. These authors did not observe that embryos derived from poor quality spermatozoa arrested more frequently at any specific stage, but rather concluded that embryos derived

from poor quality spermatozoa simply had a higher likelihood of arresting prior to the blastocyst stage. Since in the present study ICSI was only employed when progressive motility or morphology was poor, this may explain why ICSI-derived embryos had diminished developmental potential.

Using ICSI to achieve fertilization, Shoukir and co-workers (Shoukir *et al*., 1998) did not find a correlation between the sperm concentration or sperm morphology and blastocyst development, but did report that oocytes injected with spermatozoa from ejaculates with high progressive motility had a significantly higher chance of reaching the blastocyst stage. This study also found a significant correlation between progressive motility and blastocyst development (Table IV). Strong progressive motility may be an indicator of adequate metabolic activity (viability) of the spermatozoon and its appropriateness for use in assisted fertilization. This may explain why when viable, but immotile, spermatozoa were used for ICSI, the resulting embryos were of diminished quality and had poor developmental potential (Nijs *et al*., 1996). Spermatozoa from asthenozoospermic patients also have a higher incidence of nuclear abnormalities leading to slow or partial chromatin decondensation in *Xenopus laevis* oocyte extracts (Griveau *et al*., 1992) which, in turn, could lead to early embryonic arrest. In addition, spermatozoa exhibiting poor progressive motility have a higher incidence of severe axonemal and centriolar defects (Hancock and de Krester, 1992; Sathananthan *et al*., 1996; Sathananthan, 1998). A defective proximal centriole inherited by the embryo at fertilization could lead to chromosomal and nuclear aberrations and abnormal development. However, progressive motility had a relatively small effect on blastocyst development (~11%) and quality (~8%), suggesting that other factors may play a more important role.

It was found that sperm morphology was significantly correlated to blastocyst development and quality (Table IV). Robinson and co-workers (Robinson *et al*., 1994) reported that isolated teratozoospermia did not have an effect on the percentage of patients with one or more supernumerary embryos reaching the blastocyst stage. However, fertilization was achieved by conventional oocyte insemination in their study and the zona pellucida provides a selective barrier to abnormally shaped spermatozoa (Liu and Baker, 1992) suggesting that the morphology of the fertilizing spermatozoon was normal or near normal. When ICSI is employed, the selective barrier of the zona pellucida is eliminated and replaced with the judgement of the embryologist performing the ICSI procedure. Although care was taken in the present study to inject preferentially spermatozoa with morphologically normal heads, in some cases morphologically normal spermatozoa were unavailable, necessitating the use of spermatozoa with approximately normal shaped heads. Aberrant sperm head morphology is correlated with a significant increase in numerical and structural chromosomal abnormalities (Lee *et al*., 1996), but spermatozoa with abnormal chromatin can form normal-appearing pronuclei following ICSI (Twigg *et al*., 1998), underscoring the usefulness of extended culture to the blastocyst stage to avoid the inheritance of an abnormal paternal genome after ICSI (Sakkas, 1999). As was the case with progressive motility, sperm morphology alone appears to play a relatively minor role in affecting blastocyst development (~14%) and quality (~7%). The ability of embryos to develop to the blastocyst stage does not appear to be solely dependent on paternal factors, since only ~50% of embryos inseminated by conventional means with 'good' spermatozoa were capable of reaching this stage.

ICSI and its associated procedures may be detrimental to embryo development (Griffiths *et al*., 2000). The manipulation of the oocyte prior to injection, including hyaluronidase treatment and repeated pipetting to remove cumulus and coronal cells, has been linked to parthenogenic activation (Fishel *et al*., 1992; Palermo *et al*., 1993; Van de Velde *et al*., 1997). Asynchrony between the timing of oocyte activation and sperm injection could lead to cleavage abnormalities and early embryonic arrest. The use of PVP to immobilize spermatozoa during injection has long been a cause for concern (Jean *et al*., 1996) and may reduce fertilization rate and diminish embryo quality (Tsai *et al*., 2000). This has prompted investigators to look into alternate methods of sperm immobilization (Jean *et al*., 1996; Montag *et al*., 2000). The actual sperm injection procedure may also impact embryo development. Recent studies have revealed significant differences in embryo quality and developmental capacity associated with subtle differences in the site of sperm injection (Van der Westerlaken *et al*., 1999; Garello *et al*., 1999; Blake *et al*., 2000). Although no functional polarity of the plasma membrane is present in the human oocyte and sperm fusion can occur anywhere over the surface of the oocyte (Santella *et al*., 1992), there does appear to be a functional polarity to cytoplasmic components which is maintained through the blastocyst stage (Antczak and Van Blerkom, 1997; Edwards and Beard, 1997). The injection procedure itself may disrupt cytoplasmic polarity which could impair downstream cleavage and blastulation events. Lastly, in the clinical laboratory, it is common practice to inject an acrosome-intact spermatozoon into the oocyte. Although no information is currently available in humans, when ICSI was performed in the Rhesus monkey, the acrosome of injected spermatozoa remained intact and delayed the decondensation of sperm chromatin (Sutovsky *et al*., 1996). This delay could lead to improper alignment and disruption of paternal chromosomes. These effects would be manifested at the time of paternal genome activation and could result in early embryonic arrest.

In this study, ICSI was performed for severe male infertility when failed fertilization was likely. As a result, the relative contributions of ICSI and paternal factors on blastocyst development cannot be discerned. However, it is clear from our data that when performing ICSI for severe male factor, one must expect a lower rate of blastocyst development and counsel patients appropriately. On the other hand, the significantly higher rate of blastocyst development following conventional oocyte insemination would suggest that ICSI should be used judiciously and specifically avoided when semen parameters are within the range where one would expect a reasonable fertilization rate.

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