Blastocyst development from supernumerary embryos after intracytoplasmic sperm injection: a paternal influence?

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The success of intracytoplasmic sperm injection (ICSI) warrants further study on the role of paternal factors in early human embryogenesis. To investigate whether poor sperm parameters can influence embryo development, we examined the development of ICSI-fertilized embryos to the blastocyst stage. We present results of blastocyst development from supernumerary ICSI embryos after co-culture on monkey kidney epithelial cells. In addition, we compare the development of supernumerary embryos to the blastocyst stage after ICSI and in-vitro fertilization (IVF). Of 168 supernumerary ICSI embryos, 45 (26.8%) developed to blastocysts. Sperm concentration and morphology did not influence blastocyst development. In contrast, blastocysts arose from spermatozoa that had a significantly higher ($P = 0.015$) forward progressive motility compared with spermatozoa from those patients who failed to produce blastocysts (42.7% versus 28.2%, respectively). Overall the rate of embryo development to the blastocyst stage after ICSI was lower (26.8%) than that after IVF (47.3%). When the rate of blastocyst development was calculated for patients with three or more supernumerary embryos, it remained significantly higher for the IVF patients than for the ICSI patients (45.6% versus 30.0%). There was no significant difference in the mean cell number and quality of the supernumerary embryos between the IVF and ICSI patients. This study confirms previous reports that have postulated that abnormal spermatozoa may manifest a negative paternal effect on preimplantation embryo development.
Key words: human blastocysts/ICSI/in-vitro fertilization/paternal influence

Introduction

The role of a paternal factor in early human embryogenesis is gaining more attention because of the development of new techniques for the treatment of men with poor spermatozoa, as indicated by the success of intracytoplasmic sperm injection (ICSI), and because of the improvement in our knowledge of sperm biology, for example through the characterization of the *DAZ* gene (Reijo *et al.*, 1995). It can be hypothesized that a paternal effect on embryonic development would occur only after the appearance of the first paternal transcriptional products in the embryo, i.e. after embryonic genome activation. In fact, a negative paternal effect need not necessarily be at the genetic level but can also be observed in components that are transmitted by the spermatozoa. For example, incorrect formation of the sperm aster due to a centrosome defect can lead to failure as early as fertilization or in subsequent cell cycles (Asch *et al.*, 1995; Simerly *et al.*, 1997). A negative paternal influence on embryo development would therefore be expected to be more prominent in men with poor semen quality. In a study examining patients undergoing routine in-vitro fertilization (IVF) treatment, Janny and Ménézo (1994) found that, when the usual parameters of sperm quality were good, there was a strict linear relationship between cleavage and blastocyst formation rates. Their results presented evidence of a strong paternal effect on human preimplantation embryo development and blastocyst formation.

ICSI bypasses many upstream events associated with classical sperm–egg interactions, including plasma membrane and cortical region interactions. It is unclear whether bypassing these steps in the pathway of sperm transformation after cytoplasmic penetration leads to inherent problems, arising in either nuclear decondensation, pronuclear migration and/or mitosis. Furthermore, it is expected that sperm defects in microtubule function or centrosomal reconstitution will not be resolved by ICSI-assisted fertilization.

Recently, a number of studies have reported the successful culture of human embryos to the blastocyst stage, with and without feeder cells, and improved pregnancy rates after the transfer of fresh (Olivennes *et al.*, 1994) and frozen blastocysts (Kaufmann *et al.*, 1995). Transfer of embryos at the blastocyst stage provides a better synchrony between the uterine endometrium and embryo. The other advantages of such a procedure include the possibility of the selection of better-quality embryos with a higher implantation potential (Nakayama *et al.*, 1995), supporting the concept that some embryos with chromosomal or genetic abnormalities may cleave, but fail to reach the blastocyst stage (Edwards and Hollands, 1988).

In a previous study (Shoukir *et al.*, 1998), we have presented our results on the development of supernumerary embryos to the blastocyst stage after conventional IVF. We found that, out of 423 supernumerary embryos, 200 developed to the blastocyst stage (47.3%). Reports in the literature of blastocyst development from supernumerary embryos after ICSI are scant. Barnes *et al.* (1995) reported a case of blastocyst development and birth after in-vitro maturation of primary oocytes, ICSI and

assisted hatching. The aim of this study is to present the results of blastocyst development from supernumerary embryos after ICSI and to compare them with those after routine IVF, in order to determine whether there is a paternal effect on blastocyst development.

Materials and methods

The present study was performed on patients entering the IVF programme at the Clinic of Infertility and Gynaecologic Endocrinology, Department of Obstetrics and Gynaecology, University Hospital of Geneva, Geneva, Switzerland between April 1992 and August 1997. In total, 90 and 60 cycles were assessed where the patient underwent a routine IVF treatment or ICSI, respectively, with embryo transfer and development of supernumerary embryos. All patients without supernumerary embryos were excluded. The stimulation protocol and patient selection procedure adopted by our group have been previously described (Sakkas *et al.*, 1994; 1996).

Collected oocytes (day 0) were fertilized in our standard culture medium Whittingham's T6 (Quinn *et al.*, 1982) supplemented with 10% maternal serum. Serum was prepared from blood taken on the first day of stimulation. Oocyte retrieval took place between 0830 and 1030 h. For normal IVF, insemination was performed between 1530 and 1630 (~1600) h with 5×10^4 motile spermatozoa obtained by either swim-up or mini-Percoll gradient centrifugation. In cases where the normal sperm morphology of the patient was 15–20 or <15%, insemination was performed with 15×10^4 or 3×10^5 motile spermatozoa, respectively.

ICSI was used for couples in whom the male partner had very poor semen quality. Of the 60 ICSI cycles, 39 and 9 cycles were performed because patients had less than 10 and between 10 and 20×10^6 sperm per ml, respectively, while in the remaining 12 cycles the male had more than 20×10^6 sperm per ml but had poor motility or poor morphology and routine IVF was not considered as an option. No cases of repeated fertilization were included in this study.

ICSI was performed between 1300 and 1500 (~1400) h. For the ICSI procedure the injection pipette was prepared with an internal diameter of 5–7 µm with a 30° bevel and no spike was placed on the tip. The cumulus cells were removed ~2–3 h after oocyte aspiration by pipetting the oocytes in HEPES-buffered T6 medium containing 1 mg/ml type IV hyaluronidase (Sigma, Buchs, Switzerland). All manipulations were carried out on a warming stage and under oil. Oocytes were placed on a glass slide with a depression in HEPES-buffered T6 medium during the procedure. Spermatozoa were selected from a droplet of culture medium containing 5% polyvinylpyrrolidone (PVP-360; Sigma). Following immobilization by stroking the midpiece-tail region, the spermatozoa were injected into the cytoplasm of the oocyte, which was held with the polar body in the 12 o'clock or 6 o'clock position. After injection, the oocytes were examined for any damage, washed carefully and placed in culture medium.

The following morning (day 1) the oocytes were removed from the medium, washed and placed in 20-µl culture drops under oil (light white mineral oil; Sigma) in petri dishes, and the presence of two pronuclei (2PN) was assessed. Embryo transfer was routinely performed on day 2. However, when a patient had three or more previous implantation failures, the embryos were co-cultured on African green monkey kidney epithelial (Vero) cells from the 2PN stage and the transfer was performed on day 3. Routinely, a maximum of three embryos was transferred to the patient, normally between 1000 and 1500 h.

On the day of transfer, the number of cells was assessed to determine the cleavage rate, and each was assigned a quality score based on the presence of fragments and clarity of the cytoplasm of the blastomeres. The ratings given to embryos for cell number were 1 for 1-cell, 2 for 2-cells, 3 for 3-cells, 4 for 4-cells, 6 for between 4- and 8-cells and 8 for >8-cells. The ratings given to embryos for quality were the same as those used by Cummins *et al.* (1986) except that the values of 0 for poorest and 3 for best embryo were given. Patient serum human chorionic gonadotrophin (HCG) was measured 14 days after embryo transfer (Total hCG: IMX, Abbot, Abbot Park, USA). Patients in whom the intrauterine fetus or fetuses displayed a heart beat at ultrasound examination 4–5 weeks after transfer were considered to have achieved a clinical pregnancy.

Supernumerary embryos were co-cultured on Vero cells using T6 supplemented with vitamins and amino acids until the blastocyst stage as previously described (Sakkas *et al.*, 1994). The blastocysts were then frozen using the protocol described by Ménézo *et al.* (1992) except that T6 medium was used in place of Ménézo's B2. Briefly, the blastocyst was immersed in a 5% glycerol solution (Sigma) in T6 medium + 10% serum for 10 min, followed by 10 min in a solution of 9% glycerol in T6 + 10% serum containing 0.2 M sucrose. The programmed freezing curve was –1°C/min from 23°C to 5.5°C. Automatic seeding was performed, and the embryo was cooled slowly from –5.5°C to –30°C at a rate of –0.3°C/min and then stored in liquid nitrogen.

At the time of transfer, the blastocysts were thawed rapidly at room temperature. The cryoprotectant was removed using seven steps, with decreasing concentrations of glycerol and each step lasting 5 min. In all steps, the blastocysts were processed at room temperature under a stream of gas (5% CO_2, 5% O_2 and 90% N_2). After thawing, the blastocysts were allowed to recover for 3 to 4 h in co-culture before transfer. In normally ovulating patients, the transfer was performed during a natural cycle monitored by ultrasound echography and hormone assay. Transfer was timed according to the urinary luteinizing hormone (LH) peak. In patients with anovulation, ovarian stimulation was performed using human menopausal gonadotrophin and ovulation was triggered by HCG. An HCG determination was conducted 7 days after transfer.

The statistical evaluations used were analysis of variance followed by Scheffé's *F*-test for comparisons of mean values, and Fisher's exact test or χ^2 analysis with continuity correction for comparison of pregnancy rates.

Results

The development of supernumerary embryos to the blastocyst stage after ICSI

The development of supernumerary embryos to the blastocyst stage was assessed in 60 cycles after ICSI. In fresh cycles, cleavage-stage embryos were transferred on either day 2 or 3, with only one cycle in which blastocyst transfer was performed on day 6. Of 168 supernumerary embryos, 45 developed to blastocysts (26.8%). Figure 1 represents the number of frozen expanded and early blastocysts in relation to their development in culture prior to cryopreservation. Of 42 frozen blastocysts, 52.4% had reached the blastocyst stage on day 6, and were frozen on that day, compared with 28.6% on day 7.

The clinical characteristics of patients grouped according to whether supernumerary embryos developed to blastocysts are presented in Table I. Owing to the small number of day-3 transfer embryos (5 cycles in patients with and 8 cycles in patients without blastocyst development), we combined these cycles with the corresponding day-2 transfers. There were no
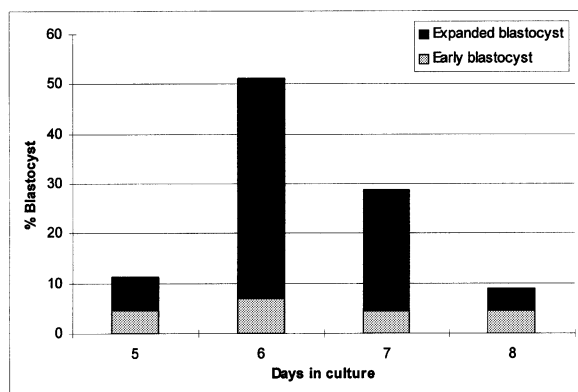
Figure 1. The percentage of expanded and early blastocysts following ICSI in relation to the number of days in culture. Embryos were frozen on the day they reached blastocyst stage. ICSI = intracytoplasmic sperm injection.

Table I. Development of supernumerary embryos to the blastocyst stage following ICSI for patients grouped according to whether blastocysts were produced or not

	No blastocysts	Blastocysts	*P*-value
No. of cycles	38	22	–
No. of patients	28	18	–
Age of female	34.6 ± 4.6	32.9 ± 4.0	0.15
Age of male	37.9 ± 5.5	38.4 ± 6.1	0.99
No. of oocytes	429	255	
(per cycle)	(11.3 ± 5.2)	(11.6 ± 3.4)	0.81
No. of oocytes injected	400	236	–
No. of 2PN embryos	248	153	
(per cycle)	(6.5 ± 2.2)	(7.0 ± 2.1)	0.46
Fertilization rate	62.0	64.8	
No. of embryos on day 2	203	144	
(per cycle)	(5.3 ± 1.7)	(6.5 ± 1.8)	0.01
No. of embryos transferred	119	60	
(per cycle)	(3.1 ± 0.3)	(2.7 ± 0.3)	0.005
No. of supernumerary embryos	84	84	
(per cycle)	(2.2 ± 1.6)	(3.8 ± 2.0)	0.002
No. of blastocysts	0	45	
(per cycle)		(2.0 ± 1.3)	–

Results are expressed as mean ± SD.
ICSI = intracytoplasmic sperm injection; PN = pronuclear.

significant differences in the ages of the couples, number of oocytes and number of 2PN embryos between patients with and without blastocysts. However, there was a significant difference in the number of total and supernumerary embryos on day 2. When examining whether there was an influence of the husband's sperm parameters on blastocyst development, we found that there was no significant difference in sperm concentration and morphology between the groups. However, patients whose blastocysts developed after ICSI had spermatozoa that had a significantly higher ($P = 0.015$) forward progressive motility (mean ± SD) than those patients without blastocysts (42.7 ± 21.3 versus 28.2 ± 20.8, respectively).

The clinical pregnancy and implantation rates for both groups of patients is shown in Table II. Although the patients in the group who failed to produce blastocysts had a significantly higher number of embryos transferred, as shown in Table I, the clinical pregnancy rates were not significantly different between the groups. However, the implantation rates in the

Table II. Clinical pregnancy per transfer for patients with and without blastocysts after ICSI

	No blastocysts	Blastocysts
No. of transfers	38	21
Pregnancies following:		
Day-2 transfers	7/30[a]	7/16[a]
(%)	(23.3)	(43.7)
Day-3 transfers	1/8	1/4
(%)	(12.5)	(25.0)
Day-6 transfers	–	1/1
(%)		(100)
Total	8/38[a]	9/21[a]
(%)	(21.1)	(42.9)
Implantation rate per embryo transferred	9/119[b]	14/60[b]
(%)	(7.6)	(23.3)

[a]Not significantly different.
[b]Significantly different $P = 0.01$.
ICSI = intracytoplasmic sperm injection.

Table III. Mean cell number and quality of all embryos on day 2 of transferred embryos and of supernumerary embryos

	Day-2 transfer with no blastocyst development	Day-2 transfer with blastocyst development	*P*-value
Cell number of:			
All embryos on the day of transfer	3.2 ± 1.2 (157)	3.7 ± 1.2 (101)	0.002
Transferred embryos	3.5 ± 1.1 (93)	4.2 ± 1.0 (46)	0.0004
Supernumerary embryos	2.7 ± 1.2 (64)	3.2 ± 1.2 (55)	0.02
Quality of:			
All embryos on the day of transfer	2.3 ± 0.8 (157)	2.7 ±0.6 (101)	0.0001
Transferred embryos	2.5 ± 0.6 (93)	2.8 ± 0.4 (46)	0.002
Supernumerary embryos	1.8 ±0.9 (64)	2.6 ± 0.7 (55)	0.0001

The results are expressed as mean ± SD.
The number of embryos for each group is in the parentheses.

patients with blastocyst development were significantly higher than those in patients with no blastocysts. Only a limited number of transfers of thawed blastocysts have been obtained following ICSI. Of these, two clinical pregnancies in 9 cycles (22.2%) were established with a mean number of 1.8 blastocysts transferred per patient.

To evaluate whether the quality of embryos transferred can affect the development of the supernumerary embryos to the blastocyst stage, we calculated the mean cell number and quality for all embryos, transferred embryos and supernumerary embryos on the day of transfer (Table III). When comparing the supernumerary embryos of day-2 transfer patients, who did or did not have blastocysts, we found that there was a significant difference in the mean cell number and the mean quality/embryo.

Comparison of the development of supernumerary embryos to the blastocyst stage after ICSI and IVF

The results of blastocyst development after routine IVF have previously been published by our group (Shoukir *et al.*, 1998).

Table IV. Comparison of the development of all supernumerary embryos to the blastocyst stage in patients who had transfer on day 2 following IVF and ICSI

	IVF	ICSI
No. of cycles	45	46
No. of patients	43	37
Age of female (years)	33.6 ± 3.3	33.6 ± 4.5
Age of male (years)	38.0 ± 8.2	37.4 ± 5.5
No. of oocytes collected	511	528
(per cycle)	11.4 ± 5.9	11.5 ± 5.1
No. of 2PN embryos	347	300
(per cycle)	7.7 ± 3.4	6.5 ± 2.2
No. of supernumerary embryos	173	120
(per cycle)	3.8 ± 2.7[a]	2.6 ± 1.9[a]
Cell number of supernumerary embryos	3.1 ±1.2	2.9 ± 1.2
Quality of supernumerary embryos	2.4 ± 0.7[b]	2.2 ± 0.9[b]
No. of cycles with blastocysts	35	16
No. of blastocysts	85	31
(% of embryos)	(49.1)[c]	(25.8)[c]
No. of blastocysts per cycle	1.9 ± 1.7[d]	0.7 ± 1.3[d]

[a]$P = 0.01$; [b]$P = 0.02$; [c,d]$P \leqslant 0.0001$.
Results are expressed as mean ± SD.
ICSI = intracytoplasmic sperm injection; IVF = in-vitro fertilization; PN = pronuclear.

The rate of blastocyst development of embryos after ICSI is lower (26.8%) than that after IVF (47.3%). The rate of blastocyst development was found to be significantly higher in IVF patients (49.1%) than that in ICSI patients (25.8%) (Table IV), if only patients who had transfer on day 2 were considered. The mean number of supernumerary embryos was however significantly higher for IVF patients. Of the supernumerary embryos, the mean cell number did not differ between the two groups. However, the supernumerary embryos obtained following IVF were of significantly better quality. It could be argued that IVF patients had more supernumerary embryos available to develop to the blastocyst stage. When patients with three or more supernumerary embryos in the IVF and ICSI groups were considered, the rate of blastocyst development remained higher for the IVF patients even though there was no significant difference in the number of supernumerary embryos between the groups (Table V). More importantly, in this sub-group of patients there was also no significant difference in the mean cell number and quality of the supernumerary day-2 embryos between the IVF and ICSI patients (Table V).

Discussion

The introduction of ICSI has revolutionized the treatment of severe forms of male infertility (Palermo *et al.*, 1992; Van Steirteghem *et al.*, 1993). However, following the successful treatment of these male factor patients, a number of questions have arisen. One relates to whether the arbitrary selection of spermatozoa from male factor patients to achieve fertilization by ICSI will lead to a negative paternal impact on embryogenesis.

In this study we have shown a lower overall rate of blastocyst development from embryos following ICSI than following IVF (26.8% versus 47.3%). This lower percentage of development might be due to numerous factors, ranging from the fact that

Table V. Comparison of blastocyst development following IVF or ICSI patients with three or more supernumerary embryos who underwent transfer on day 2

	IVF	ICSI
No. of cycles	28	18
No. of supernumerary embryos	149	80
(per cycle)	5.3 ± 2.4[a]	4.4 ± 1.9[a]
Cell number of supernumerary embryos	3.3 ± 1.2[a]	3.0 ± 1.2[a]
Quality of supernumerary embryos	2.4 ± 0.7[a]	2.4 ± 0.7[a]
No. of blastocysts	68	24
(%)	(45.6)[b]	(30.0)[b]
No. of blastocysts per cycle	2.4 ± 1.9[c]	1.3 ± 1.7[c]

Comparisons were made between IVF and ICSI data as follows: [a]not significantly different; [b]$P = 0.03$; [c]$P = 0.01$.
Results are expressed as mean ± SD.
ICSI = intracytoplasmic sperm injection; IVF = in-vitro fertilization.

ICSI patients have poorer sperm parameters to technical problems associated with the ICSI procedure itself. The invasiveness of the ICSI procedure may cause subtle changes to the inherent structure of the oocyte and the resulting embryo. Indeed, in the monkey, Sutovsky *et al.* (1996) have shown that ICSI results in unusual chromatin, cytoskeletal and membrane events compared with those of normal fertilization, but eventually leads to pronuclear development. Motoishi *et al.* (1996) have however shown that bovine oocytes develop at the same rate to the blastocyst stage after ICSI as compared with that of control oocytes, indicating that the ICSI procedure is not detrimental to embryo development. ICSI embryos may also have a greater susceptiblity to certain in-vitro culture systems, notably co-culture which was used in this study. Recently, a number of sequential culture systems have been described using defined media which lead to improved blastocyst development (Gardner and Lane, 1997). The data presented in this study need to be verified by other groups performing ICSI and in different culture systems before we can obtain a final answer as to a whether there is a technical influence on the development of ICSI embryos. Although technical aspects may play a role in influencing ICSI embryo development, the main difference between the IVF and ICSI groups is the fact that ICSI patients have poorer sperm parameters. This would therefore point to a strong possibility of a paternal effect on embryo development.

The IVF and ICSI groups also had differences in the number of supernumerary embryos and in their cleavage rates and quality. The argument that the higher the number of supernumerary embryos in a cycle, the better the chance for the patient to have blastocyst development was however negated when the number of supernumerary embryos for IVF and ICSI patients was adjusted by omitting those cycles with two or fewer supernumerary embryos. However, there remains a significant difference in the rate of blastocyst development in favour of IVF embryos. Furthermore, when only patients with three or more supernumerary embryos were assessed in either group, the mean cell number and quality were not different between the supernumerary IVF and ICSI embryos. It has been suggested that ICSI produces a significantly higher proportion of morphologically superior embryos than conventional ones (Yang *et al.*, 1996) and high insemination concentra-

tion IVF (Oehninger *et al.*, 1996). In contrast, our results show that, whereas the mean cell number of day-2 supernumerary embryos did not differ, the quality of those embryos was better in IVF cycles than in ICSI cycles. Overall, the number of supernumerary embryos and the type (cell stage and quality) after IVF and ICSI did not appear to have a major influence on the outcome of blastocyst development.

Comparison of the groups of patients whose supernumerary embryos did or did not develop to the blastocyst stage after ICSI could therefore throw some light on the different factors which might affect blastocyst development. Since ICSI is used in patients with severe male factor infertility, a major influence by female factors would not be expected. This is supported by the finding that there was no difference in the female age, number of oocytes and number of MII stage-injected oocytes between the two groups. A female factor however cannot be completely excluded. This might be present when ICSI is used in cases where the sperm parameters are considered to be normal but a repeated failure of fertilization after routine IVF has occurred. The major differences between the ICSI patients with and without blastocysts were significantly more embryos on day 2 and more supernumerary embryos after embryo transfer, even though there was no difference in the number of 2PN embryos between both groups. Subsequently, the quality and cell number of all, transferred and supernumerary embryos were always superior in the group of patients that had blastocysts.

In examining the semen parameters of patients with and without blastocysts we found a significant difference only in sperm motility. Semen parameters, however, do not seem to correlate with the outcome of ICSI treatment (Palermo *et al.*, 1993; Nagy *et al.*, 1995). The motility of spermatozoa may be impaired by a number of factors, for example, free radicals, a wide variety of ultrastructural abnormalities in the sperm tail or defects in the pathways of ionic control (Ryder *et al.*, 1990; Aitken, 1995; Fraser, 1995). Although healthy births after ICSI using immotile testicular spermatozoa (Barros *et al.*, 1997; Kahraman *et al.*, 1997), and ejaculated and testicular spermatids (Antinori *et al.*, 1997; Fishel *et al.*, 1997) have been reported, the embryos tended to be of lower quality when totally immotile spermatozoa of any origin, i.e. ejaculate, epididymis or testis were used (Nijs *et al.*, 1996). In fact, Nijs *et al.* (1996) reported only one biochemical pregnancy (from totally immotile ejaculated sperm), the absence of any pregnancy from totally immotile epididymal spermatozoa and a relatively high miscarriage rate (50%) from initially immotile ejaculated spermatozoa. Interestingly, no supernumerary embryos could be cryopreserved for patients with totally immotile spermatozoa from ejaculate or epididymis in that study. This may suggest the importance of sperm motility in the prediction of ICSI outcome and blastocyst development from supernumerary embryos.

The effect of a defect in sperm motility on early human embryogenesis and blastocyst development is not clearly known. The first epigenetic contribution of the spermatozoon is the centrosome, the microtubule organizing centre of the cell. It is evident that the sperm centrosome is the functional active centrosome in the human, while the female is inactive but may contribute some centrosomal material to the zygote centrosome (Sathananthan *et al.*, 1996). It is very likely that the paternal centriole is the ancestor of the centrioles in fetal and adult somatic cells. Preliminary studies on washed sperm pellets from men with immotile spermatozoa with no forward progression, poorly motile and highly motile spermatozoa show that there are more centriolar defects in immotile spermatozoa than in motile samples (Sathananthan, 1994). If a defective sperm centriole/centrosome is inherited by an embryo at fertilization, it may lead to abnormal cleavage and produce aberrant embryos (Sathananthan *et al.*, 1996). If embryo development is retarded or arrested, or if embryo cleavage is irregular, this might be a good indicator of mitotic spindle disturbances, very likely involving centriole/centrosomal dysfunction. The block in the development of supernumerary embryos in the group of patients who produced no blastocysts could therefore be related to a sperm motility defect reflected in mitotic spindle disturbances.

More importantly, the role of paternal DNA in early human embryogenesis cannot be ignored. Development of the human embryo after the 4-cell stage is controlled by the embryonic genome, in contrast to earlier stages, which are controlled by maternally inherited mRNA (Braude *et al.*, 1988). When comparing embryos at the day-2 stage, it appears that the overall morphology and cell number following IVF does not greatly exceed those following ICSI (current study; Staessen *et al.*, 1995). In this study the differences between IVF and ICSI supernumerary embryos manifested themselves between the day-2 stage and the time of blastocyst formation; ICSI embryos had a significantly lower potential to form blastocysts. This inherent deficiency may be linked to a post-embryonic genome activation event where the influence of the paternal genome appears. Spermatozoa that have the ability to fertilize may not necessarily be able to contribute to further embryonic development.

The influence of poor quality spermatozoa on the increased developmental arrest between embryonic genome activation and the blastocyst stage has also been discussed by Ménézo and Janny (1997). Apart from conditions including oligo-, astheno- and/or teratozoospermia, numerous other anomalies are evident in the spermatozoa of male factor patients. For example, the conventionally used semen parameters do not provide information on the quality of the DNA which is present in the sperm head. Numerous studies have indicated that mature spermatozoa of ICSI patients possess anomalies in their DNA (Sakkas *et al.*, 1997). Ageing of spermatozoa can result in DNA strand breaks and, although fertilization and first cleavage stages can occur, the genome of the spermatozoon may not be capable of completing embryogenesis and blastocyst development (Nijs *et al.*, 1996). Defective protamine packaging in sperm DNA and their incorrect replacement by histones during fertilization may also create problems such as asynchronous cleavage and delays in the cell cycle after fertilization. The lower rates of development to the blastocyst stage shown by embryos after ICSI compared with those after routine IVF indicate that a negative selection may occur during the preimplantation stage of embryos fertilized by abnormal sperm. The abnormalities in certain sperm that result in an

inability of embryos to reach the blastocyst stage remain largely unknown. Blastocyst culture following IVF may be a potential diagnostic tool.

This study has confirmed previous reports that have postulated that abnormal spermatozoa may manifest a negative paternal effect on preimplantation embryo development. A greater understanding of the molecular basis of male infertility is therefore needed to broaden our knowledge concerning the effect of abnormal spermatozoa on fertilization and embryo development and to avoid the inappropriate use of ICSI.

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