Fertilization failures and abnormal fertilization after intracytoplasmic sperm injection

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This study addresses the incidence of failed (0%) and suboptimal (<50%) fertilization after intracytoplasmic sperm injection (ICSI), variation in the ICSI fertilization rate for specific couples, and the causes of fertilization failure and abnormal fertilization after ICSI. Failed fertilization occurred in only 37 of 1343 cycles (3%). The risk of failure was highest (37%) when only one oocyte was injected, and was lowest (0.8%) when five or more oocytes were collected. The incidence of suboptimal fertilization and the variation in the fertilization rate were studied in 87 couples who each had three cycles of ICSI in which four oocytes were injected with ejaculated spermatozoa. Approximately 74% of these couples achieved >50% fertilization in every cycle. Only 26% of the couples had <50% fertilization in one or more cycles, and most of these (17%) had only a single cycle with suboptimal fertilization. Only four of the 87 couples (5%) had suboptimal fertilization in all three cycles. The difference between the maximum and minimum fertilization rate for a couple was used as an index of variation of the fertilization rate. It was found that 47 couples (54%) had 0–25% variation, 33 couples (38%) had 26–50% fertilization and only seven couples (8%) had >50% variation. The causes of failed and abnormal fertilization were studied in unfertilized and abnormally fertilized oocytes after staining with Hoechst 33342. In total, 1005 unfertilized oocytes were studied, of which 828 (82%) were still at metaphase II and 177 (18%) were activated. Most of the oocytes (83%) contained a spermatozoon and, in the majority of these oocytes, the sperm head was partially or completely decondensed. Hence, failure of oocyte activation was the principal cause of fertilization failure. A similar pattern was observed in activated, unfertilized oocytes, although there was a higher incidence of intact spermatozoa in these oocytes compared with metaphase II, unfertilized oocytes. Interestingly, 56% of the activated oocytes contained a decondensed sperm head which was not processed into a male pronucleus. A total of 169 abnormally fertilized oocytes was also studied. Two anomalies were found: digyny due to retention of the second polar body and its subsequent transformation into a third pronucleus, and abnormal pronuclear size and number.

Key words: fertilization rate/ICSI/oocyte activation/sperm head decondensation

Introduction

Intracytoplasmic sperm injection (ICSI) has become the premier treatment modality for severe male factor infertility, yielding excellent pregnancy and implantation rates using ejaculated and surgically collected spermatozoa, and does not appear to increase the risk of congenital malformations (Palermo *et al.*, 1993, 1995; Van Steirteghem *et al.*, 1993; Devroey *et al.*, 1994; Payne *et al.*, 1994; Tournaye *et al.*, 1994, 1995; Payne and Matthews, 1995). Despite this revolution, however, there are several aspects of this assisted fertilization technique which are less than perfect. First, it may not overcome oocyte defects (Gabrielsen *et al.*, 1996). Second, the overall fertilization rate is only 40–70% per injected oocyte (Van Steirteghem *et al.*,

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1993; Payne and Matthews, 1995), which results in a significant wastage of potentially fertilizable oocytes. Finally, complete fertilization failure still occurs and some couples only ever achieve suboptimal (<50%) fertilization (Payne *et al.*, 1994; Flaherty *et al.*, 1995a,b). These last two aspects of ICSI have received scant attention to date.

Since the introduction of ICSI in our unit in 1993, we have been interested in the causes of fertilization failure and abnormal fertilization after ICSI. In this report, we outline studies on the incidence of failed and suboptimal fertilization, variation in the fertilization rate, and the cytological causes of failed and abnormal fertilization after ICSI. Some of the results have been published elsewhere (Flaherty *et al.*, 1995a,b).

Materials and methods

Incidence of failed fertilization

The incidence of failed fertilization (0%) was studied in 1343 consecutive ICSI cycles that were performed in the Reproductive Medicine Units at The Queen Elizabeth Hospital and Wakefield Clinic, Australia, between June 1993 and June 1996.

Variability in ICSI fertilization rates

A study was undertaken to determine the incidence of suboptimal fertilization (<50%) and variation in the fertilization rate for specific couples. Data were retrospectively analysed from June 1993 to May 1996, and 87 couples met the following selection criteria: (i) ⩾4 oocytes had been injected (and survived) in each of three consecutive ICSI cycles, and (ii) ejaculated spermatozoa had been used. The mean ± SD female age was 32.9 ± 4.6 years, and the median number of oocytes injected was 8 (range 4–30).

Causes of failed and abnormal fertilization after ICSI

Unfertilized and abnormally fertilized oocytes were fixed ~17 h post-injection and then stained with Hoechst 33342 to determine the cytological consequences of ICSI. The study period was February 1994 to October 1995, during which a total of 854 ICSI cycles were completed. A total of 1005 unfertilized oocytes and 169 abnormally fertilized oocytes were examined from 469 of these cycles. In many cases, all of the unfertilized and abnormally fertilized oocytes from each of these cycles were examined; however, this was not possible in some instances due to logistical difficulties. Results for unfertilized oocytes were compared between ejaculated spermatozoa (412 cycles) and surgically recovered epididymal and testicular spermatozoa (57 cycles) using the Fisher's exact test. $P < 0.05$ was considered significant. Informed written consent was obtained from each couple and the study was approved by the Ethics of Human Research Committee at The Queen Elizabeth Hospital.

Patients, ovarian stimulation, sperm and oocyte preparation and ICSI procedure

Our protocols for patient selection, ovarian stimulation and the collection and preparation of spermatozoa and oocytes have been described by Payne *et al.* (1991, 1994). The manufacture of holding and injection pipettes and the injection procedure have been described by Payne (1995). Each oocyte was carefully checked 5–30 min post-injection to ensure that the spermatozoon was inside the oocyte, and injected oocytes were examined for evidence of fertilization ~17 h post-injection using differential interference contrast optics.

Preparation of oocytes for fluorescence microscopy

Procedures for staining oocytes with Hoechst 33342 have been described by Flaherty *et al.* (1995a,b). Briefly, oocytes were fixed in 0.1–0.5% glutaraldehyde for 2–3 h, rinsed in phosphate-buffered saline and then stained with 20 μg/ml Hoechst 33342 (Sigma Chemical Co., St. Louis, MO, USA) for 30–60 min. Stained oocytes were mounted individually on slides and gently compressed under 22×22 mm coverslips supported on all sides by petroleum jelly. Slides were coded and then stored at 4°C in a dark container.

Slides were examined by one observer (S.P.F.) who did not know either the origin of the oocytes or the fertilization rates for the cycles. Oocytes were carefully examined with a microscope equipped with phase-contrast optics and epifluorescence (UV excitation). The presence and loca-

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Table I. Incidence of failed fertilization (0%) in relation to the number of oocytes injected (and survived). Data are from 1343 intracytoplasmic sperm injection (ICSI) cycles performed in the Reproductive Medicine Units at The Queen Elizabeth Hospital and Wakefield Clinic, Australia, between June 1993 and June 1996

No. of oocytes injected (and survived)	Cycles (% of total)
1	19 (52)
2	7 (19)
3	2 (5)
4	2 (5)
5–10	5 (14)
≥11	2 (5)
Total	37

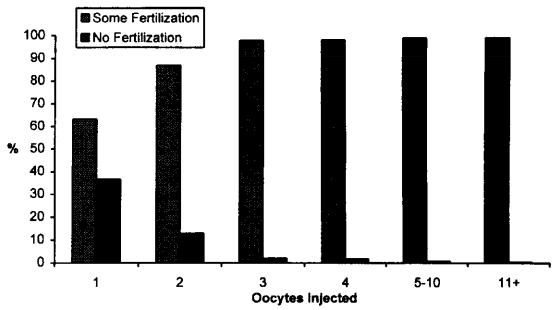

Figure 1. Frequency of successful cycles in which some fertilization occurred, and unsuccessful cycles with complete fertilization failure.

tion of polar bodies (PB), sperm heads, oocyte chromosomes and pronuclei (PN) were recorded for each oocyte. In some cases, the oocyte was rotated slightly under the coverslip to determine if the spermatozoon was in the oocyte or in the perivitelline space. Decisions about whether there was 1 PB, 2 PB or PB fragments were based on the appearance of the DNA in the various PB structures and other cytological findings, such as whether PN were present. DNA generally had a different appearance in the first PB and second PB, whereas fragments usually exhibited DNA with a similar appearance in each fragment.

Results

Incidence of failed fertilization after ICSI

Over a 3 year period, there were 37 cycles (3%) out of a total of 1343 in which none of the injected oocytes became fertilized. These cycles had been undertaken by 33 couples, of whom three had more than one cycle of failed fertilization. Most of these failures (67%) occurred during a couple's first cycle of ICSI, and nine of these 22 couples (41%) did not pursue further treatment. The majority of the failed cycles occurred when only one or two oocytes were injected, although some couples did not achieve fertilization even when five or more oocytes were injected (Table I). The incidence of failed fertilization, compared to the incidence of some fertilization, was studied in cycles in which 1, 2, 3, 4, 5–10 or 11+ oocytes were injected (Figure 1). The risk of failed fertilization was highest when only one (37%) or two (13%) oocytes were injected, and when three oocytes were injected, the risk decreased to 2%. When five or more oocytes were injected, the risk was only 0.8%, and most of the failed cycles occurred in a few specific couples.

Variation in ICSI fertilization rates

Fertilization rates were analysed in 261 cycles undertaken by 87 couples. The mean ± SD fertilization rate was 71.5 ± 22.6% (range 0–100). Overall, 64 of the 87 couples (74%) had ≥50% fertilization in all three cycles, and 22 couples (25%) had ≥75% fertilization in every cycle. One couple achieved 100% fertilization in three consecutive cycles, while another couple had failed fertilization in every cycle.

Suboptimal fertilization (<50%) was experienced in one or more cycles by 26% of the couples; in most cases (18%), it occurred in only one of the three cycles. Four couples (5%) had suboptimal fertilization in two of the three cycles, and another four (5%) had poor fertilization in all three cycles. The probability of <50% fertilization in a given cycle was only 13%.

The difference between the maximum and minimum fertilization rate was used as an index of variation in the fertilization rate. The mean ± SD variation was 28.3 ± 16.2% (range 0–80). Three subgroups were used for further comparisons: 0–25% variation, 26–50% variation and >50% variation. It was found that 92% of couples had ≤50% variation during three consecutive ICSI

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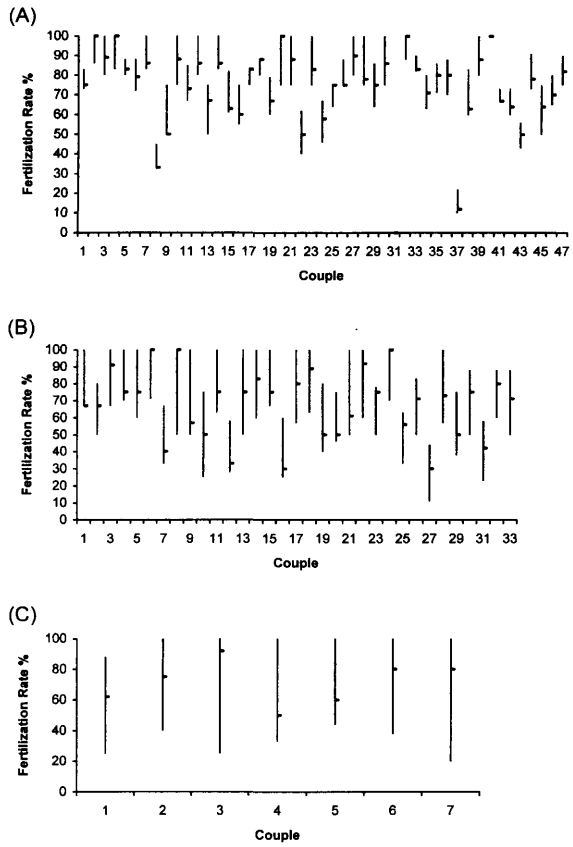

Figure 2. High–median–low charts showing fertilization rates for couples who had (**A**) <25% variation, (**B**) 26–50% variation or (**C**) >50% variation in fertilization rate during three consecutive intracytoplasmic sperm injection (ICSI) cycles.

cycles (0–25% variation, 54%; 26–50% variation, 38%; see Figure 2).

Causes of failed and abnormal fertilization after ICSI

Unfertilized oocytes

A total of 1005 unfertilized oocytes were examined, of which 828 (82%) were at metaphase II and 177 (18%) were activated. A spermatozoon was present in 83% of the oocytes, while it had been ejected in the remaining oocytes and was usually found in the perivitelline space.

The majority of the metaphase II oocytes contained a decondensed sperm head. The other oocytes either contained an intact spermatozoon which had not undergone decondensation, or the

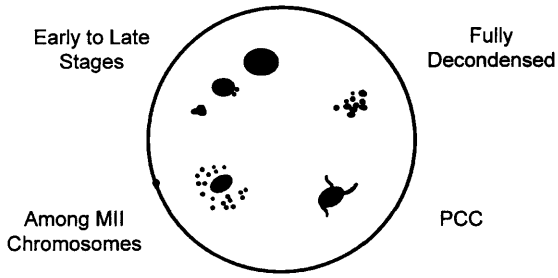

Figure 3. In unfertilized metaphase II oocytes, decondensed sperm heads were found at early to late stages of decondensation or were fully decondensed. Some sperm nuclei showed evidence of premature chromosome condensation (PCC), while others were located among the metaphase II (MII) chromosomes of the oocyte.

spermatozoon had been ejected from the oocyte (Table II). A comparison of ejaculated and surgically recovered spermatozoa showed only a significant difference in the frequency of oocytes containing intact spermatozoa (9 versus 22% respectively, $P < 0.001$). Decondensed sperm heads of different sizes and at different stages of decondensation (early to late) were observed. In some oocytes which contained a decondensed sperm head, the sperm head had either completely decondensed into clumps of chromatin (14%), showed evidence of premature chromosome condensation (PCC; 7%) or was associated with the metaphase oocyte chromosomes (10%). This is shown diagrammatically in Figure 3. Photomicrographs of these phenomena were presented in Flaherty *et al.* (1995a,b).

A similar pattern was seen in 177 activated but unfertilized oocytes, although the frequencies of decondensed sperm heads, intact spermatozoa and ejected spermatozoa were different from those in unfertilized, metaphase II oocytes (Table II). There were no significant differences between oocytes injected with ejaculated or surgically recovered spermatozoa. The most common pattern (77%) in these activated oocytes was 1 PN (female) and 2 PB (Figure 4). Digyny was the next most common condition (14%) and manifested as either 2 PN and 1 PB or 1 very large PN and 1 PB. Another group of oocytes (8%) were activated but had arrested at anaphase or telophase of the second meiotic division. One oocyte contained an unformed female PN, a decondensed sperm head and 2 PB (Figure 4).

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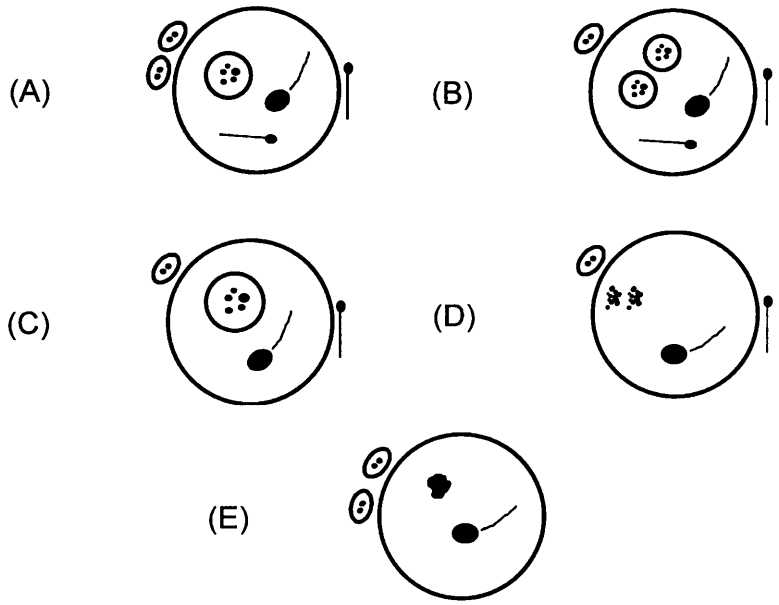

Figure 4. Activated oocytes usually had one pronucleus (PN) and two polar bodies (PB) (**A**), although some were digynic and had 2 PN and 1 PB (**B**), or 1 large PN and 1 PB (**C**). Some activated oocytes were at arrested anaphase or telophase (**D**), or the female PN had failed to form properly after ejection of the second PB (**E**). Decondensed, intact and ejected spermatozoa were found in these oocytes, although each of the three types was not necessarily found in all of these oocytes.

Table II. Observations on unfertilized oocytes

Presence/condition of spermatozoon in oocyte	Metaphase II oocytes (n = 828)	Activated oocytes (n = 177)	Fisher's test
Decondensed sperm head	616 (74%)	99 (56%)	$P < 0.001$
Intact spermatozoon	87 (11%)	35 (20%)	$P = 0.0014$
Spermatozoon ejected	125 (15%)	43 (24%)	NS

NS = not significant.

Abnormally fertilized oocytes

A total of 169 abnormally fertilized oocytes were examined (Table III). Two principal aetiologies were identified: digynic oocytes which had retained the second PB (Figure 5) and fertilized oocytes which contained abnormal or putatively fragmented PN (Figure 6). A variety of patterns was evident in each category, although the most common condition in digynic oocytes was 3 PN and 1 PB. Among oocytes with abnormal pronuclei, the most common finding was one fully formed PN of unknown origin, an accumulation of DNA which represented a second, unformed PN, and 2 PB. It was not possible to determine whether the male or female PN had failed to develop.

Table III. Aetiology of 169 abnormally fertilized oocytes

Digynic (n = 135)	3 PN, 1 PB	122
	3 PN (different sizes)[a], 1 PB	7
	⩾4 PN, 1 PB	2
	3 PN, 2 micro PN, 1 PB	1
	1 large PN, 1 small PN, 1 PB	3
Abnormal PN (n = 34)	2 PN (different sizes), 2 PB	7
	1 PN, unformed PN, 2 PB	20
	3 PN, 2 PB	3
	2 PN, 1 micro PN, 2 PB	3
	1 large PN, 2 small PN, 2 PB	1

PN = pronucleus, PB = polar body.
[a]Two large PN and one small PN, or one large PN and two small PN.

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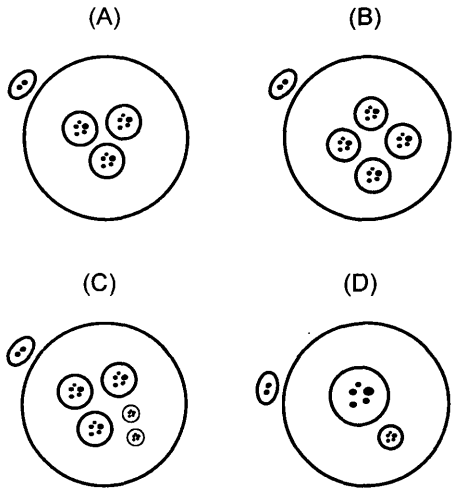

Figure 5. Most abnormally fertilized oocytes were digynic and had either three pronuclei (PN) and one polar body (PB) (**A**), ≥4 PN and 1 PB (**B**), 3 PN, micro PN and 1 PB (**C**), or 2 PN (one large, one small) and 1 PB (**D**). None of these oocytes contained spermatozoa in the oocyte or in the perivitelline space.

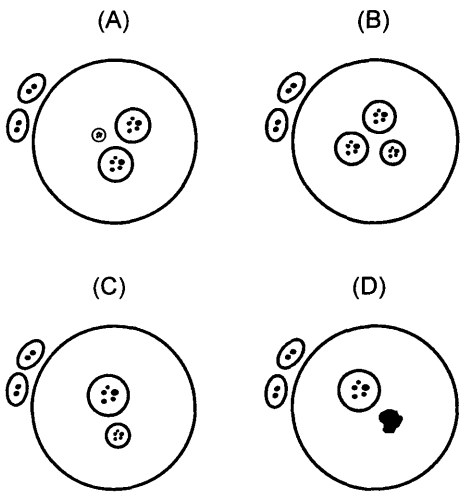

Figure 6. Fertilized oocytes were also classified as abnormal if they contained abnormal pronuclei (PN) or fragmented PN. These included oocytes with 2 PN, micro PN and 2 PB (**A**), 3 PN and 2 PB (**B**), 2 PN of different sizes (**C**) and 1 PN and an unformed PN (male or female) (**D**).

Results for individual couples

The results presented above are combined results from 469 treatment cycles, and they do not show the results and patterns for individual couples. Table IV presents results for six couples who underwent ICSI and achieved fertilization rates of 12–74% per injected oocyte. These couples illustrate the variable patterns obtained in individual cycles. Some couples, such as no. 1, had a consistent problem with oocyte activation which was manifested by a large number of unfertilized, metaphase II oocytes containing decondensed sperm heads. This couple had three cycles with only 12–22% fertilization, yet they conceived on the third cycle after the transfer of only one embryo. Other couples (nos. 3–5) showed a more heterogeneous aetiology, with a variety of patterns among a cohort of oocytes. Couple no. 6 is interesting in that six of the 20 fertilized oocytes were fertilized but were digynic (3 PN, 1 PB).

Oocytes were examined from two couples who had failed fertilization in two and three consecutive cycles respectively, in which good numbers of oocytes (3–24) had been injected. In couple A (female age 34 years), none of eight oocytes fertilized after ICSI in their first cycle. Six of the eight unfertilized oocytes were fixed and stained with Hoechst 33342, and it was found that all six oocytes were at metaphase II and contained a decondensed sperm head. The sperm head was fully decondensed in two of the oocytes, while it exhibited premature chromosome condensation in another oocyte. In their second ICSI cycle, none of 16 oocytes fertilized, and 10 of the unfertilized oocytes were examined after staining with Hoechst 33342. Nine oocytes were at metaphase II and contained a decondensed sperm head, while the other oocyte was activated but had arrested at anaphase/telophase of the second meiotic division; it also contained a decondensed sperm head. Clearly, their problem lay in complete failure of oocyte activation. In couple B (female age 35 years), none of 24 oocytes fertilized in their first cycle, and 20 of these unfertilized oocytes were stained with Hoechst 33342. They were all still at metaphase II, and 15 oocytes contained a decondensed sperm head, in four the spermatozoon had been ejected, and one contained an intact spermatozoon. Once more, the problem lay with a complete failure of oocyte activation after ICSI. This couple later underwent a second cycle in which none of three oocytes fertilized.

Discussion

Since its introduction (Palermo *et al.*, 1992), ICSI has revolutionized the treatment of male factor

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Table IV. Unfertilized and abnormally fertilized oocytes from six representative couples[a]

Couple no.	1	2	3	4	5	6
No. of oocytes injected	17	11	21	24	20	27
Fertilization rate (%)	12	36	48	50	55	74
No. of metaphase II oocytes containing:						
Decondensed sperm head	11	2	6	4	2	2
Intact spermatozoon		1	2	4	2	
Spermatozoon ejected	2	3	1	3	3	
No. of activated oocytes containing:						
Decondensed sperm head					2	
Intact spermatozoon				1	3	2
Spermatozoon ejected			1		2	
No. of abnormally fertilized oocytes:						
Digynic (3+ pronuclei)			1	1		6
Abnormal pronuclei			1			

[a]Not all of the unfertilized and abnormally fertilized oocytes were studied in some of these cases.

infertility, and excellent pregnancy rates and implantation rates are now routinely achieved in couples who previously would have had to accept donor spermatozoa or remain childless. In published studies, however, overall fertilization rates are usually given, expressed as the fertilization rate per injected oocyte (Van Steirteghem *et al.*, 1993; Payne and Matthews, 1995). The overall fertilization rate gives an indication of the overall effectiveness of ICSI in overcoming defects which prevent fertilization, but it does not provide information on the results for individual couples or the variation in the fertilization rate in repeated cycles. This information is required to counsel couples properly and effectively manage their treatment. Hence, in this study, we have examined the incidence of failed and suboptimal fertilization after ICSI, the variation in the fertilization rate in consecutive cycles of ICSI, and the aetiology of failed and abnormal fertilization.

We have shown that failed fertilization is a rare occurrence after ICSI (3%) and is most frequent in cycles in which only one or two oocytes are injected. Our results are in general agreement with those of Liu *et al.* (1995), who also reported a 3% fertilization failure rate in 2732 ICSI cycles. In the present study, the risk of failed fertilization reduced from 37% when one oocyte was injected to only 0.8% when five or more oocytes were injected. This affected patient treatment, since most of the failed fertilization cycles occurred during first attempts at ICSI and many of these couples did not pursue further treatment. The risk of failed fertilization most seriously compromises treatment for poor ovarian responders who only produce one or two oocytes as well as patients undergoing ICSI in natural cycles. Both groups face the possible prospect of no fertilization as well as reduced conception rates due to the transfer of only one embryo. Nevertheless, it is possible to achieve pregnancies using ICSI in combination with natural cycles (Norman *et al.*, 1995). The risk of repeated cycles of failed fertilization was very low, although we identified a small subgroup of patients whose oocytes consistently failed to fertilize due to as yet undefined gamete defects which inhibited oocyte activation. These failures were not caused by technical error. Staining oocytes with Hoechst 33342 provided a clear diagnosis of activation failure in these cases.

We studied the incidence of suboptimal fertilization (<50%) and variation in the fertilization rate in a subgroup of 87 couples who underwent three consecutive cycles in which oocytes were injected with ejaculated spermatozoa. The incidence of suboptimal fertilization was low and sporadic, and most couples consistently achieved ⩾50% fertilization in each cycle. Hence, provided adequate numbers of oocytes are available for injection, most couples can expect to obtain fertil-

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ization and transfer of embryos in each cycle. The fertilization rate was consistent from cycle to cycle for most couples, and while many couples had high fertilization rates, some consistently only had intermediate or low rates of fertilization.

The aetiology of failed and abnormal fertilization after ICSI was studied by staining oocytes with Hoechst 33342 so as to localize oocyte chromosomes, sperm heads, pronuclei and polar bodies. Some of these data have already been published as part of other studies (Flaherty *et al.*, 1995a,b). In all, 1005 unfertilized oocytes were studied. Most of these oocytes (82%) were unactivated and remained at metaphase II, while the remainder (18%) were activated but had failed to complete the events leading to fertilization. These unfertilized oocytes either contained decondensed sperm heads, intact spermatozoa or the spermatozoon had been ejected from the oocyte. The majority (56–74%) contained a decondensed sperm head, indicating that failure of oocyte activation, and not technical error, was the principal cause of fertilization failure. Clearly, in each instance the spermatozoon had been correctly injected into the oocyte. In a similar study, Dozortsev *et al.* (1994a) reported decondensed sperm heads in 50% of oocytes which were still at metaphase II after ICSI. Cytogenetic studies have also shown that this occurs in unfertilized oocytes resulting from routine IVF, albeit at a very reduced frequency (Schmiady and Kentenich, 1989; Balakier and Casper, 1991; Selva *et al.*, 1991; Plachot and Crozet, 1992).

There were several other interesting aspects of these unfertilized oocytes. First, ejection of spermatozoa from oocytes only accounted for ~12% of the unfertilized oocytes. Ejection of spermatozoa occurred some time after injection, and the reasons for this phenomenon remain unknown. Dozortsev *et al.* (1994a) recorded an even lower incidence of sperm ejection in their study, whereas Schmiady *et al.* (1996) reported that 20.3% of their unfertilized ICSI oocytes did not contain spermatozoa. Second, a proportion (11–20%) of the unfertilized oocytes contained an intact spermatozoon. This was lower than that (38%) reported by Dozortsev *et al.* (1994a). We feel that this aetiology may result from incomplete sperm immobilization prior to injection which causes insufficient damage to the sperm membrane (Dozortsev *et al.*, 1994b), so that the glutathione activity of the ooplasm (Perreault, 1992) cannot access the sperm nucleus. Third, despite alignment of the first PB at 12 o'clock prior to injection, decondensed sperm heads were found among the metaphase chromosomes in ~10% of the oocytes. This was also reported by Dozortsev *et al.* (1994a), and suggests that the PB is not always in close proximity to the metaphase spindle. Fourth, in ~7% of the metaphase II oocytes which contained a decondensed sperm head, the sperm DNA had the appearance of premature chromosome condensation. This was originally described in unfertilized IVF oocytes and probably indicates oocyte immaturity (Schmiady *et al.*, 1986; Calafell *et al.*, 1991). It has been described in other studies of unfertilized ICSI oocytes, although the frequency has varied from 2.5% (Bergère *et al.*, 1995), to 8% (Dozortsev *et al.*, 1994a), to as high as 28.6% (Schmiady *et al.*, 1996).

Approximately 5% of the injected oocytes were activated at 17 h post-injection, which is similar to the incidence (2–6%) of 1 PN oocytes obtained by routine IVF (Plachot and Crozet, 1992; Staessen *et al.*, 1993). Approximately 56% of these activated oocytes contained a decondensed sperm head, while a further 20% contained an intact spermatozoon. This demonstrates that these activated oocytes were not the result of asynchronous pronuclear development (Munné *et al.*, 1993; Staessen *et al.*, 1993), but were unfertilized or arrested at an early stage of fertilization. Furthermore, it indicates that there can be asynchrony between those processes which control oocyte activation and those which are responsible for sperm nuclear decondensation and PN formation. Since ooplasmic factors regulate sperm head decondensation, protamine–histone exchange and pronucleus formation (Perreault, 1992), the inability of these activated oocytes to process decondensed sperm heads fully may indicate oocyte immaturity or specific oocyte defects. Nevertheless, the spermatozoon also contributes to pronuclear formation by supplying the centrosome, from which develops a microtubular aster (Schatten, 1994), so the spermatozoon may also contribute to this type of fertilization defect.

While the majority of activated oocytes had a

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female PN and 2 PB, some had 2 PN but only 1 PB, which indicates that the second PB was retained and formed the second female PN. It also emphasizes the need for scoring 2 PN and 2 PB before an oocyte is recorded as fertilized. A small number of activated oocytes were at arrested anaphase II or telophase II, and one oocyte had 2 PB, an unformed female PN and a decondensed sperm head.

A variety of fertilization anomalies were found after ICSI, although most were due to digyny or abnormal PN. Palermo *et al.* (1993) originally speculated that 3 PN form after ICSI because the oocyte fails to extrude the second PB and consequently both sets of maternal chromosomes develop into pronuclei. This was verified by Flaherty *et al.* (1995a,b) and was confirmed again in this study. Failure to extrude the second PB has also been reported in routine IVF oocytes (Selva *et al.*, 1991; Plachot and Crozet, 1992). While most digynic fertilized oocytes had 3 PN and 1 PB, some also exhibited PN anomalies, including micro PN and possibly PN fusion, resulting in one very large PN and one normal sized PN. Various PN anomalies were also recorded in oocytes which were fertilized, but were not digynic. The most common anomaly was classified as an unformed PN, and this occurred despite the presence of a normal PN in the same oocyte; it was impossible to determine whether the unformed PN were of male or female origin. Micro PN and different sized PN were also encountered, and it would be interesting to study the chromosome complement in these oocytes to determine whether they had marked non-disjunction (aneuploidy) and unequal segregation of chromosomes in the PN and micro PN.

In conclusion, most couples consistently achieve high rates of fertilization after ICSI, and failed fertilization occurs rarely and is usually associated with the injection of only one or two oocytes. However, there are some couples who consistently achieve poor or failed fertilization and, in our experience, this is due to gamete defects which result in failure of oocyte activation after ICSI. Furthermore, most randomly selected unfertilized oocytes had not activated after injection, and there are a range of fertilization anomalies that occur after ICSI, most of which are due to retention of both sets of maternal chromosomes (digyny).

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