

An evaluation of couples with failure of fertilization *in vitro*

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Attempts at in-vitro fertilization (IVF) may be used as a method of evaluating whether in a given couple, the inability of the sperm to fertilize the oocyte may be the cause of infertility. We evaluated all IVF patients in our practice who had at least one cycle with no fertilization to determine how often this was an isolated event or was repeated in multiple cycles; would poor semen quality be found as a frequent cause; and how well can a donor sperm or oocyte 'probe' uncover which of the two is the problem? Of 35 couples who used their own gametes exclusively, 30 (85.7%) had at least one cycle with zero fertilization; 42.5% of those failing to fertilize in cycle 1 and 35% of those failing in cycle 2 had a subnormal concentration of motile spermatozoa, morphology or hypo-osmotic swelling test scores. The pregnancy rate per cycle with both husband's and wife's gametes was only 2.3% (3/130), but was 8.3% for those using donor spermatozoa (3/36) and 18.2% (2/11) for donor oocytes. Thus, failing to fertilize in a given cycle does not necessarily predict failure to fertilize in a subsequent cycle, but does predict a poor fertility outcome unless donor gametes are used.

Key words: donor oocytes/donor sperm/zero fertilization failure

Introduction

Previous studies on the cause of poor fertilization have focused on the use of sophisticated laboratory tests for assessing gamete function (Tesarik and Testart, 1989). Studying the spermatozoa, Check *et al.* (1989, 1990a,c, 1991a, 1992a) have reported difficulties in predicting fertility based on the motile concentration of spermatozoa sperm morphology even using strict criteria, and computer assisted analysis. Attempts to use the sperm penetration assay (SPA) with zona-free hamster oocytes as a predictor of fertility have had mixed results. Some investigators have found it to have no predictive value for fertility (Wickings *et al.*, 1983; Check *et al.*, 1986) and those who have found some predictive value in distinguishing fertile from sub-fertile men admit that there

is too much crossover to be clinically useful (Karp *et al.*, 1981; Rogers, 1985, 1986).

In a study designed to evaluate oocyte quality, Tesarik (1989) used the zona-free human oocyte penetration test. His data suggest that defective oocytes might be responsible for 40% of couples who have idiopathic fertility associated with failure of fertilization. This was based on the fact that 92% of donor sperm preparations penetrated zona-free oocytes taken from failed fertilization cycles in couples with a suspected male factor, but in only 60% of unexplained infertility cases did donor spermatozoa penetrate the zona-free eggs. This penetration test of zona-free human oocytes might not accurately distinguish a sperm versus oocyte defect in that any problem relating to a defective zona pellucida, which is removed during the test, would not be detected.

In-vitro fertilization (IVF) offers another method for evaluating the cause of infertility in any given couple. In this study, we have evaluated all patients in our IVF programme who underwent multiple IVF cycles and had at least one cycle with no fertilization. The questions of interest were whether failure of fertilization is an isolated event or repeated in multiple cycles; whether poor semen quality would be found as a frequent cause of zero fertilization; and how well a donor sperm or oocyte 'probe' can uncover which of the two gametes is the problem.

Materials and methods

The charts of all IVF patients attending our practice from January 1988 to October 1990 were reviewed to identify any couples who underwent at least two cycles of IVF ET in which at least one cycle had no fertilization. Cycles where oocytes were not successfully retrieved were excluded.

Patients registering for IVF in our practice have the option of choosing from one of three protocols. The first protocol (standard) requires the use of the couples' own gametes. Each patient was started on leuprolide acetate, 1 mg beginning on day 21 of the cycle for 10 days; the dosage was decreased to 0.5 mg on the 11th day. Human menopausal gonadotrophin (HMG) (300 IU/day) was started on the 11th day of leuprolide acetate for 4 days and then reduced to 225 IU. Further changes were made in accordance with the results of serum oestradiol measurements and the number and size of follicles obtained by pelvic sonography using an ATL Ultramark 4 unit (Advanced Technology Laboratories, Bothell, WA, USA) equipped with a 5 MHz endovaginal transducer. Human chorionic gonadotrophin was given when at least two leading follicles attained an average diameter of 18 mm and the serum oestradiol level was at least 300 pg/ml per follicle.

The second protocol offered allows the couple to use donor spermatozoa and/or oocytes for their first IVF cycle if either the semen parameters showed poor chance of fertilization, or the wife had premature ovarian failure. Those using donor spermatozoa were also given the option to have half of their eggs inseminated with the donor spermatozoa and the other half with the husband's spermatozoa in a single cycle so that if both fertilized, transfer would be of husband-fertilized embryos, and the donor-fertilized embryos would be cryopreserved.

The third protocol is designed for couples who wish to participate in our donor/recipient programme. In this programme, patients are given the option of sharing half of their retrieved oocytes with a recipient who had premature ovarian failure, in exchange for assistance in paying for the donor's IVF cycle. All oocytes retrieved are then distributed equally according to morphological criteria between the donor and the recipient. The long leuprolide acetate-HMG regimen is used for ovarian stimulation of the donor (Meldrum *et al.*, 1989). Recipients received a gradually increasing dose of oestradiol (Estrace-2 tablets) starting on the donor's sixth day of leuprolide acetate, and then 50 mg progesterone i.m. starting with the HCG injection of the donor.

Data recorded for each IVF cycle included the age of the women, the number of eggs retrieved, the fertilization rate, source of gametes, outcome of conception and motile concentration of the male partners' semen specimen obtained on the day of oocyte retrieval. Fertilization was considered to have occurred when pronuclei were observed by the embryologist. A baseline analysis for sperm morphology using strict criteria (Kruger *et al.*, 1988) and antisperm antibody using the immunobead assay, were obtained prior to the first IVF cycle for the majority of the male partners. A motile sperm concentration $<10 \times 10^6/\text{ml}$ or normal sperm morphology $<4\%$ were considered abnormal.

Statistical analysis of the data was descriptive in nature. Relative frequencies, means and standard deviations were computed as appropriate. To investigate the patterns by which no fertilization occurred in multiple cycles, patients were classified by the cycle in which zero fertilization first occurred, i.e. their first, second or third IVF cycles. The relative frequencies of each pattern were computed. To investigate the relationship of semen parameters to zero fertilization patterns, the relative frequencies of men with poor semen quality were compared according to the above pattern.

The fertilization outcome per cycle was also compared by sperm quality and fertilization pattern. Spermatozoa were considered normal if all available semen parameters fell within the normal ranges: motile concentrations $>10 \times 10^6/\text{ml}$, strictly normal morphology $>4\%$, and negative antisperm antibodies ($<50\%$). Any sperm sample with at least one semen parameter within the subnormal range (i.e. motile concentration $<10 \times 10^6/\text{ml}$, strict normal morphology $<4\%$ or positive antisperm antibodies) was considered subnormal.

The source of the gametes was also considered in relation to fertilization parameters. Fertilization outcomes were analysed for all cycles by source of gametes (i.e. donor or partner spermatozoa, donor or own oocytes) and quality of spermatozoa. Finally, the pregnancy rate for this group of patients was

calculated and the treatments used to achieve the pregnancy recorded.

Results

Sixty-three couples underwent at least two cycles of IVF in our practice between January 1988 and October 1990 in which fertilization did not occur in at least one cycle. These couples were studied for a total of 181 cycles (average 2.9 cycles per couple). Although eight of the couples had failure of fertilization in all their IVF cycles, 87.3% attained fertilization in at least one cycle. A profile of the couples, classified cumulatively according to the IVF cycle in which failed fertilization first occurred, is presented in Table I. The frequencies of the source of gametes relative to the pattern of failed fertilization are given in Table II.

The most common pattern observed was failure to fertilize in the first IVF cycle attempted. In this first cycle, the average age of the women was 35.0 ± 4.4 years. An average of 6.0 ± 4.5 oocytes were retrieved, with seven of the women having a low yield of only one or two oocytes. Subnormal semen parameters were found in 42.5% of the males. In the failed cycle, 31 of the 35 used their own gametes, four used some donor gametes. On subsequent cycles, 27 (77.1%) of these couples were able to achieve fertilization, 14 using their own gametes and 13 using donor gametes. Six pregnancies were achieved in this group.

Thirty-two per cent of the couples achieved fertilization in their first IVF cycle but failed to fertilize in their second cycle. In this group of couples, the average age of the woman was 35.6 ± 5.8 years. In the second cycle an average of 5.4 ± 4.0

Table I. Comparison of patient characteristics by patterns of failed fertilization in multiple IVF cycles

Fertilization pattern	No. of patients	Average ^a age (years)	Average ^a no. cycles	Average ^a no. eggs retrieved
(Z,Z)	8	37.9 (4.7)	2.3 (.5)	6.1 (4.1)
(Z,F)	27	34.1 (4.0)	2.9 (1.4)	7.8 (4.7)
(F,Z)	13	33.3 (4.8)	2.0 (0)	6.8 (3.7)
(F,Z,Z)	3	43.3 (4.0)	3.3 (.6)	2.7 (1.2)
(F,Z,F)	4	37.0 (3.5)	4.0 (1.2)	5.2 (1.9)
(F,F,Z)	4	33.0 (5.8)	3.0 (0)	8.2 (1.4)
(F,F,Z,F)	4	35.0 (6.2)	4.7 (.9)	11.9 (6.7)

Z denotes zero fertilization, F denotes fertilization occurred.

^aData presented as mean (standard deviation).

Table II. Relative frequencies of the source of gametes for couples undergoing multiple IVF cycles according to their pattern of failed fertilization

Cycle when failure of fertilization first occurred	No. of couples using own gametes in all cycles	No. of couples undergoing cycles with own gametes and cycles with donor gametes	No. of couples using donor gametes in all cycles
1	15 (42.8)	17 (48.6)	3 (8.6)
2	14 (70.0)	2 (10.0)	4 (20.0)
3	6 (75.0)	1 (12.5)	1 (12.5)
Total	35	20	8

oocytes were retrieved, with five women having a low yield of oocytes. Thirty-five per cent of the males had at least one subnormal semen parameter. In the cycle with failed fertilization, 15 couples used their own gametes and five used donor gametes. Seven couples tried a third cycle, three failed again (two using their own gametes, one with donor) and four achieved fertilization (three with donor gametes, one with their own). Two pregnancies were achieved in this group.

Zero fertilization first occurred in the third cycle for eight couples. For these couples, the woman's average age was 34.0 ± 5.6 and an average of 6.9 ± 3.6 oocytes were retrieved with only one woman having a low yield of oocytes. At least one abnormal semen parameter was found in 12.56% of the males. In the failed cycle, seven couples used their own gametes, one used donor gametes. Four couples underwent subsequent cycles and all achieved fertilization, two with their own gametes and two with donor gametes. However, no pregnancies were achieved in this group.

Investigating the relationship of semen parameters and oocyte yield to failure to fertilize, we found that overall, 23 of the 63 (36.5%) couples had subnormal semen, while 40 (63.5%) couples still had failed fertilization with normal semen parameters. A low oocyte yield was observed in 13 cycles with failed fertilization; however six of the 13 had another cycle in which they achieved fertilization *in vitro* with a low oocyte yield. Thus, these parameters seem to be inconsistent with their ability to predict a poor outcome.

The source of gametes was also investigated as an indicator of the chances of fertilization. Overall, 35 couples continued to use their own gametes in all cycles and 28 couples tried donor gametes. Of the 35 couples who used their own gametes exclusively, 30 (85.7%) had at least one cycle with fertilization. Eight of the couples used donor gametes exclusively, with only one failing in all cycles. Of the 20 couples who tried cycles with their own gametes as well as donor gametes, 14 attained fertilization using donor gametes after failing with their own, two couples failed with both their own and donor gametes, four couples had fertilization using their own gametes but failed with donor gametes, and one attained fertilization with both their own and donor gametes.

The cycles involving donor gametes were further analysed to determine if the donor gamete could be used as a 'probe' to indicate whether the fertilization failure was due to poor sperm or oocyte quality.

Eighteen couples tried IVF cycles using donor spermatozoa. Twelve achieved fertilization using donor spermatozoa after failing with their own. Seven of the 18 men had at least one subnormal semen parameter; they were all able to achieve fertilization using donor spermatozoa after failing with their own. Thus, the semen parameters correctly identified the male factor in these cases. Of the 11 men with normal spermatozoa, five had failure of fertilization with their own spermatozoa whereas donor spermatozoa did lead to fertilization. It appears that semen parameters falsely identified five samples as normal.

Five patients used donor oocytes only, for a total of nine cycles. In all cases, the male semen parameters were normal. Three oocyte recipients had fertilization and two achieved pregnancies

(22.2% per cycle). All of the donors had fertilization in at least one of their cycles. Thus, for two of the patients, the problem seems more related to an undetected male factor than oocyte quality.

Of the five couples who tried various combinations of donor gametes for a total of 14 cycles, some possible sperm and oocyte defects were uncovered. One failed to fertilize using donor spermatozoa but achieved fertilization using donor spermatozoa and donor oocytes; two failed using donor spermatozoa but succeeded using donor oocytes; one succeeded using donor spermatozoa but failed using donor oocytes and the last one had mixed results both failing and succeeding with their own and donor gametes.

Five of the women in the study elected to participate in our donor oocyte programme. Comparison of their fertilization results with those of the recipients' is another method of assessing oocyte quality. Their oocytes were used in a total of 10 cycles. In six of the cycles, both the donor and recipient achieved fertilization indicating good oocyte quality. In three cycles, both the donor and the recipient had no fertilization; these were all preceded by the donor failing to fertilize in cycle 1 when they did not share the oocytes (the recipient gambled that the problem was defective spermatozoa). In one cycle, the donor had no fertilization while the recipient had good fertilization, suggesting a possible undetected male problem.

Eight of the 63 women (12.7%) became pregnant even though they experienced a cycle with no fertilization. The pregnancy rates per cycle according to the source of gametes used in the cycle were 2.3% (3 of 130) using the couple's own gametes; 8.3% (3 of 36) for cycles using donor spermatozoa with the female partner's oocytes; 18.2% (2 of 11) for cycles using the male partner's spermatozoa with donor oocytes; and 0% for the four cycles using both donor spermatozoa and donor oocytes. Seven of the eight pregnancies had successful deliveries, one conceiving with the couple's own gametes had an empty sac.

Discussion

Attempts at IVF may be considered as a method to evaluate unexplained infertility. If fertilization does not occur, it would suggest that there is a problem with one or both of the gametes as the cause of failed fertilization. If in a subsequent cycle, the husband's spermatozoa fail to fertilize again but some of the oocytes are fertilized by donor spermatozoa, it may be concluded that there is a problem with defective sperm function whether the semen parameters are normal or not. In contrast, when failure to fertilize with either the husband's or donor spermatozoa occurred, the conclusion may be drawn that a defective oocyte is probably responsible. However, in 35 couples who used their own gametes exclusively, 85.7% subsequently had a cycle with fertilization (data not given). Thus doubt is cast on the simple explanation considered above, that defective spermatozoa or oocytes are the cause of fertilization failure or that these defects will persist in all subsequent cycles.

There were 18 couples who were willing to use donor sperm and these were from a group where semen parameters were clearly poor and/or repeated failure to fertilize with their own

spermatozoa occurred. In this group, 10 of the 18 (58.5%) achieved fertilization using donor spermatozoa whereas they failed using their own spermatozoa, thus indicating possible defective spermatozoa as the cause of fertilization failure. However, since some couples continued to fail to fertilize using donor spermatozoa and some had mixed fertilization results using donor semen the oocyte would seem to be defective in these cases despite the assumption of normal oocytes and spermatozoa.

Although fertilization was achieved with donor semen for all seven men who failed with their own abnormal spermatozoa, the low pregnancy rate of 7.7% still raises concern about some concomitant oocyte factor.

Even patients with a history of fertilization failure are given the opportunity to be oocyte donors. Recipients informed of the donor's poor history are frequently willing to gamble that the problem was defective spermatozoa rather than oocytes. (This is especially true for recipients at the end of a long waiting list.) The fact that the recipient had positive fertilization in seven of 10 cycles indicates that this is still a reasonable gamble for the recipient. In six of the 10 cycles, the donor also fertilized, indicating that one of the difficulties in interpreting the data is that apparent male or female factors may be transient.

The results of this study support previous data on the inconsistency of semen parameters in identifying the subnormal male. Even when selecting couples where no fertilization occurred in at least one cycle, only 36.5% of the men had subnormal spermatozoa. Thus, 63.5% of the men with normal spermatozoa still failed to fertilize oocytes in their IVF cycle (*a priori*, one would expect a much higher proportion of men with subnormal spermatozoa in this group).

Though a cycle with no fertilization does not predict the likelihood of invariable subsequent failure, there were only three pregnancies in 130 cycles (2.3% per cycle) with the couple's own gametes compared to three of 36 cycles with donor spermatozoa (8.3% per cycle). The best pregnancy results were achieved with donor oocytes, but this does not necessarily indicate that an oocyte factor is more common than a male factor since higher pregnancy rates are achieved in oocyte donation cycles than regular IVF cycles (Check *et al.*, 1990b, 1992b).

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