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CLINICAL ASSISTED REPRODUCTION

Extending the Coincubation Time of Gametes Improves In Vitro Fertilization

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KEY WORDS: Assisted reproductive technology; coincubation; embryo development; in vitro fertilization; sperm–oocyte interaction.

INTRODUCTION

Altering the coincubation time for spermatozoa and oocytes in vitro leads to varying results. Some authors suggest that longer exposure of spermatozoa to oocytes through coincubation is detrimental to fertilization and/or embryo development, possibly because of reactive oxygen species produced by the spermatozoa (1–5). Other studies indicate that a decreased exposure time does not alter fertilization rates (3,6–9) and may even improve such rates (10–13). In this report, we describe the results of our randomized study on the effects of two coincubation times on in vitro fertilization (IVF) rates and embryo quality.

MATERIALS AND METHODS

Patient Population

We obtained gametes from 20 couples in this study; the average female age was 32.8 years (range, 23–40 years). The primary diagnoses among the women included tubal factor (40%), ovulatory dysfunction (20%), endometriosis (15%), pelvic factor (15%), uterine factor (5%), and advanced maternal age (5%). Our institutional review committee approved the study, and patients signed consent forms indicating awareness that their data might be used to monitor and possibly improve methodologies used for in vitro production (IVP).

The prewashed semen values for the 20 men in this study were as follows: volume, 2.96 ±1.34 ml (mean ± SD); spermatozoal concentration, $113.4 \times 10^6 \pm 84.1 \times 10^6$/ml; spermatozoal motility, 60.3 ± 18.0%; strict spermatozoal morphology, 34.2 ± 12.7%; spermatozoal motility index, 225.2 ± 67.8 (determined by multiplying the percentage rapid-moving spermatozoa by 4, the percentage medium-moving spermatozoa by 3, and the percentage slow-moving spermatozoa by 2, and adding these three values together); and spermatozoal straight-line velocity, 46.9 ± 8.8 μm/sec (14). The postwash values for these semen specimens were as follows: spermatozoal concentration, $91.9 \times 10^6 \pm 75.4 \times 10^6$/ml; spermatozoal motility, 84.8 ± 11.0%; and spermatozoal straight-line velocity, 56.7 ± 9.0 μm/sec.

In Vitro Production Conditions

We have published the details of our IVP procedures (15). Briefly, all female partners underwent ovulation induction with the use of gonadotropins, usually in conjunction with a midluteal leuprolide acetate suppression protocol. Through transvaginal

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ultrasound and the measurement of serum estradiol levels, we monitored the response to the gonadotropins. When two or more follicles reached ≥16 mm in size, the patient self-administered 10,000 IU of hCG intramuscularly. Follicle aspiration occurred 34 to 35 hr later, and we evaluated the recovered oocytes for maturity.

Each patient provided at least 9 oocytes (maximum of 28 oocytes) that we randomized into two treatment groups: 3 or 19 hr of spermatozoa and oocyte coincubation. We selected these two times to coincide with work schedules of the laboratorians. In addition, we chose 19 hr based on a previous report indicating that human oocytes were observed 15 to 20 hr postinsemination (16).

Once we randomized metaphase II oocytes to 50-μl drops of Human Tubal Fluid (HTF; Irvine Scientific, Santa Ana, CA), we placed 20,000 motile spermatozoa in each of these drops. For Group I, we rinsed the spermatozoa from the short-exposure oocytes after 3 hr and placed the oocytes in 50 μl of fresh HTF. After 16 hr in the fresh medium, we removed the cumulus and corona cells and moved the denuded oocytes into fresh medium. For Group II, we exposed the oocytes to spermatozoa for 19 hr and then removed their cumulus and corona cells. Following the stripping process, we moved the oocytes into fresh medium. Two or more pronuclei in the cytoplasm after 19 hr confirmed fertilization.

We assigned zygotes one of two paths: cryopreservation or continued development for potential embryo transfer. Generally, if the cohort was 11 or fewer zygotes, we did not cryopreserve any zygotes. If the cohort was 11 or more zygotes, we froze a minimum of four zygotes.

Noncryopreserved zygotes remained in culture medium for an additional 48 hr prior to transfer, at which time they underwent evaluation for cell stage and quality as described previously (15). Briefly, the blastomere number constituted the cell stage, while cell quality ranged from a value of 1 to 5, 1 being the best and 5 being dead. The grading scale for cell quality reflected the size, shape, granularity, and fragmentation of the blastomeres. Those embryos selected for transfer represented the highest quality and the most advanced cell stage.

Statistical Analysis

We used Fisher's two-tailed exact test and McNemar's test to determine significance, with P values ≤ 0.05 considered significant.

RESULTS

We excluded any reinseminated oocytes or immature oocytes. Our data included polyploids in the fertilization information but excluded them in the cell stage and grade information.

One hundred seventeen of 165 oocytes (70.9%) were fertilized when exposed to spermatozoa for 3 hr, whereas 135 of 168 oocytes (80.4%) were fertilized when exposed to spermatozoa for 19 hr ($P = 0.001$). At transfer time, oocytes exposed to spermatozoa for only 3 hr averaged 6.5 cells per embryo, with a 2.4 average quality score. Similarly, oocytes exposed to spermatozoa for 19 hr averaged 6.6 cells, with a 2.4 average quality score. The differences between embryo cell stages and quality scores of the two groups were not significant ($P = 0.20$ and $P = 0.94$, respectively). The polyploid rate for the 3-hr exposure was 2.4% (4/165), while the polyploid rate for the 19-hr exposure was 6.5% (11/168) ($P = 0.110$).

DISCUSSION

The objective of this study was to determine if a difference in fertilization rates occurred when we coincubated spermatozoa and oocytes in vitro for 3 hr (Group I) or 19 hr (Group II). While numerous studies address the issue of exposure time (3,4,6–13), this is the first research to report a significant increase in the fertilization rate when the coincubation time is longer.

Even though a majority of the current papers indicate a decrease in fertilization rates with a decrease in exposure time (3,6–9), none of these studies indicate that the difference is significant. In studies where there is a short (1-hr) versus a long (18-hr) coincubation time, the short coincubation time has a fertilization rate that is only 91 to 92% of the long coincubation time (3,8). The fertilization rate increases to 94 to 96% with an additional hour (2 hr) of coincubation time (6,9). In the four studies (10–13) indicating a decrease in fertilization rate when the spermatozoa were left with the oocytes for an extended time period, none randomized the treatments within patients; the studies randomized only between the patients.

In other mammalian species, when greater than 500 oocytes are prospectively randomized and exposed to four or more different coincubation times, there is a positive linear relationship with at least the three

shortest time points (4,17–18). Thus, as the time is increased, the fertilization rate is increased. These same experiments indicate a maximum time after which there may actually be a decline in fertilization rate. However, reduced fertilization does not occur until after 12 hr of coincubation. Results of these studies with cattle and sheep oocytes beg that similar studies be performed using human gametes to determine if there is an actual decline in fertilization rates of primates after 12 hr of coincubation.

Unlike other reported studies, our study is randomized within patients to optimize fertilization potential. Unfortunately, we cannot compare our data with those of others whose entire cohort of embryos came from one treatment source. Data on our pregnancy rates from specific treatments cannot be obtained because (a) we randomized within patients, and (b) we did not allow some zygotes to develop but cryopreserved them, and therefore, they are not included in the embryo developmental data.

Probably the most detrimental effect of reduced fertilization rates is the loss of embryos available for cryopreservation or transfer. This loss of embryos alone makes a reduced fertilization rate worrisome.

In conclusion, these data indicate that a longer coincubation time for spermatozoa and oocytes does increase the fertilization potential and thus the total number of embryos available for transfer.

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