

Poor fertilization may be related to oocyte or zona pellucida recognition defects specific to certain hyperstimulation regimens and limited to some males but not others: a case report

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Objective: To determine if the controlled ovarian hyperstimulation (COH) regimen may cause sperm to appear subfertile only to improve considerably by changing the COH protocol.

Design: Case report with retrospective review of previous fertilization rates according to COH protocol used.

Main Outcome Measures: Fertilization and pregnancy rates (PRs) after IVF-ET.

Results: Known fertile donor sperm failed to fertilize any of 11 oocytes compared with 14 of 18 for processed retrograde ejaculate using the same oocyte pool. Retrospective analysis of other cycles for different female patients but same donor found 16.6% fertilization rate whenever luteal phase leuprolide acetate (LA)-hMG regimen was used compared with 70.6% with short-flare regimen.

Conclusion: Some COH regimens may cause oocyte or zona pellucida changes that create recognition defects for some sperm but not others. Interestingly, the sperm with the binding defect with the luteal phase LA-hMG COH protocol exhibited good fertilization rates with oocytes prepared with the short-flare protocol and demonstrated high in vivo PRs after IUI. Fertil Steril 1995;63:1341-3

Key Words: Poor fertilization, donor sperm, hyperstimulation regimen, IVF

Poor or failed fertilization may be related to either a sperm or oocyte (or both) defect. Determination of which gamete is involved may be accomplished by inseminating a portion of the oocytes retrieved by known fertile donor sperm (1, 2). The assumption is made that if the donor sperm demonstrates good fertilization whereas the male partner's sperm results in poor or failed fertilization, then a male factor problem is established (3); failed or poor fertilization by both donor and male partner would establish an oocyte factor or combined oocyte and male factor problem (which could only be determined when inseminating donor oocytes) (1).

The case presented demonstrates for the first time that failed or poor fertilization may be related to a particular controlled ovarian hyperstimulation (COH) regimen apparently modifying oocyte or zona structures and making them not recognizable by one man's sperm with known fertility potential yet recognizable by another man's sperm. Further evaluation of more IVF cycles with the same luteal phase leuprolide acetate (LA)-hMG protocol versus the short-flare COH regimen and evaluating pregnancy rates after IUI allowed the conclusion that the sperm recognition problem of this donor was specific for oocytes prepared using a longer course of LA.

CASE REPORT

A 29-year-old female and a 28-year-old male presented to the Cooper Center for IVF with a 3-year

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history of infertility seeking another IVF cycle. They further requested that a portion of the oocytes be inseminated with donor sperm.

The couple's infertility seemed to be related predominantly to retrograde ejaculation. However, they failed to conceive despite seven cycles of IUI. They also failed to conceive after two IVF-ET cycles at another infertility center in which they had fertilized 18 of 20 oocytes in the first IVF cycle and 6 of 19 in the second (the center they used did not have cryopreservation facilities). In the previous IVF center the husband's sperm had been collected by urination preceded by ejaculation. The bladder had been prepared not only by oral ingestion of sodium bicarbonate but also by rinsing the catheterized bladder with an alkaline solution. The sperm then was separated quickly from the urine.

The sperm collection technique used by the Cooper Center for IVF had the husband first urinate and then take 10 sodium bicarbonate pills (each one containing 300 mg), wait 2 hours, and then urinate again. Another 10 sodium bicarbonate pills were taken and 1 hour later the male partner ejaculated followed by urination. The urine was mixed with a HEPES-buffered medium (human tubal fluid [HTF], Irvine Scientific, Irvine, CA) supplemented with 0.5% bovine serum albumin (BSA). After centrifugation at $300 \times g$ for 5 minutes, the sperm pellet was resuspended in 2 mL of HTF. The final result was a sperm concentration of 11×10^6 sperm/mL with 36% motility (20% with fast and linear motion, 60% with slower and/or nonlinear motion, and 20% moving in place). The male partner's semen analysis showed 10% with normal morphology using strict criteria compared with 16% for the donor. The sperm was next processed with a three-layer Percoll discontinuous gradient containing 1 mL each of 90%, 60%, and 45% isotonic Percoll in HTF and BSA. After the sperm suspension was layered gently onto the Percoll gradient it was centrifuged at $300 \times g$ for 20 minutes; subsequently it was washed two more times ($300 \times g$ for 5 minutes). After processing, the volume was 0.1 mL with a sperm concentration of 9×10^6 /mL and a motility of 78% with 50% with fast linear motion and only 10% moving in place. The frozen-thawed donor specimen also was processed with the three-layer Percoll technique. The concentration was 38×10^6 /mL with 60% motility (40% with rapid linear motion and none moving in place) and postprocessing was 14.5×10^6 /mL with 90% motility (60% fast linear).

The COH regimen used 1 mg SC LA beginning 1 week after ovulation and this was reduced to 0.5 mg after 10 days when both serum E_2 and P levels were suppressed; then hMG was initiated at 300 IU/d. There were 33 oocytes retrieved and 4 were consid-

ered immature; 18 oocytes were incubated with male partner's sperm and 11 were incubated with donor sperm. The sperm concentrations were adjusted so that each oocyte (whether male partner's or donor sperm was used) was inseminated with 10,000 progressively motile morphologically normal sperm. Fertilization was determined by performing corona removal with a finely drawn needle 16 hours after insemination.

The fertilization rate using male partner's sperm was 77.7% (14/18) but was 0% using donor sperm. The husband's sperm was used on 13 oocytes retrieved from the right ovary and 10 (77%) were fertilized and on 5 oocytes from the left ovary and 4 (80%) were fertilized. After the transfer of 4 fresh embryos (10 were cryopreserved), the female partner conceived.

DISCUSSION

The wife of the donor who was selected delivered two natural children (the youngest aged 17 months). Other frozen-thawed specimens that he produced for therapeutic donor insemination had resulted in pregnancies, both after IVF and IUI.

The donor performance in nine previous IVF cycles was evaluated. There had been 33 oocytes inseminated using the luteal phase LA-hMG technique and 23 using the short-flare protocol; the percent fertilization had been 16.6% versus 70.6%, respectively. He had been used in four cycles where the same luteal phase LA-hMG protocol was used and in five in which the protocol was the short-flare technique (4). Failed fertilization occurred in two of four cycles where the LA-hMG regimen had been used and, if the cycle for the present case report is included, the failed fertilization rate for this COH regimen was 60%. In contrast, there were no failed fertilization cycles using the short-flare protocol. Because the donor had been selected previously by females who were considered poor responders (only 6.6 oocytes retrieved with luteal phase LA-hMG protocol versus 4.6 with short flare), the cycles with poor fertilization were considered related to defective oocytes. Pregnancies were achieved in two of five short-flare cycles but none where the COH was luteal phase LA-hMG. This donor sperm had achieved pregnancies in 3 of 10 previous IUI cycles. The mean time of cryopreservation before thaw of the donor sperm in short-flare cycles was 9.8 versus 8.1 months for the luteal phase LA-hMG protocol.

It would have been interesting to see if the husband's frozen-thawed sperm the next day would fertilize oocytes that failed to fertilize on the 1st day but, because of so many ($n = 15$) embryos from the first day, reinsemination was not performed. Simi-

larly, it would be of interest to compare fresh versus frozen donor sperm with oocytes developed by the LA-hMG regimen but, because of risk of sexually transmitted diseases, all donor specimens used were frozen and quarantined.

These data strongly suggest that the luteal phase-hMG COH protocol can produce oocytes that are recalcitrant to fertilization by certain sperm, but not others. Interestingly, these same sperm with apparent oocyte or zona recognition defects can achieve good fertilization when using a different protocol or in non-IVF circumstances.

Whether this defect is related to the longer use of LA or whether the FSH:LH ratio used for stimulation is important (short-flare uses both pure FSH and hMG) is not known. However, the two cycles with LA-hMG where fertilization did occur, though initially using the same 10 days of exclusive LA, required less hMG to achieve follicular maturation and thus overall used less LA than in the three failed fertilization cycles. In none of the cycles with failed fertilization was there demonstration of sperm binding to the oocyte.

Thus, if one gets poor fertilization after IVF in one

cycle, especially if the luteal phase LA-hMG COH protocol is used, it would be best to consider repeating one more cycle using a different COH regimen before going to donor sperm or donor oocytes or attempting micromanipulation procedures.

The mechanism to explain poor fertilization with one COH protocol and not another is unknown at this time. Possibly there may be some changes in zona protein receptors, leading to poor sperm binding by some sperm but not others.

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