Pregnancy following transfer of cryopreserved-thawed embryos that had been a result of fertilization of all in vitro matured metaphase or germinal stage oocytes. Case report

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Summary

Purpose: To see if pregnancies can be achieved after performing intracytoplasmic sperm injection (ICSI) of in vitro matured metaphase I or germinal vesicle stage oocytes and then cryopreserving them at the 2 pronuclear stage.

Methods: Transfer of frozen/thawed hatched day 3 embryos to two women. All embryos were a result of in vitro maturation of immature oocytes followed by ICSI.

Results: Both women conceived. One has delivered and one has successfully completed the first trimester.

Conclusion: These two cases represent only the second and third reported cases of pregnancies following frozen embryo transfer where the embryos resulted from fertilizing immature oocytes by ICSI.

Key words: Frozen embryo transfer; Immature oocytes; Pregnancy.

Introduction

Fertilization by intracytoplasmic sperm injection (ICSI) requires mature metaphase-II oocytes. One case was reported where a pregnancy and successful birth resulted from the in vitro maturation of germinal-vesicle stage oocytes followed by ICSI [1].

A case was also reported of successful pregnancy following frozen embryo transfer (ET) of in vitro matured metaphase I and germinal-vesicle stage oocytes [2].

Reported here are the second and third cases of pregnancies following frozen ET derived from retrieval of immature oocytes that were first matured in vivo for 24 hours before ICSI was performed.

Case Report

Case 1:

A 29-year-old woman with secondary infertility (her only pregnancy had been ectopic) sought IVF. The ectopic pregnancy was treated by salpingectomy and the remaining tube was also removed at the same time for hydrosalpinx and occlusion. She had had four previous IVF cycles in another institution that had not been successful.

Her first IVF cycle at our IVF center was performed in November, 1997. Her day 3 serum FSH was 3 mIU/ml with a serum E2 of 44 pg/ml. She was on a follicular phase leuprolide acetate controlled ovarian hyperstimulation regimen using 0.75 mg leuprolide acetate (Lupron, Tap Inc.) from day 2 of the cycle until injection of hCG. Gonadotropins, including 150 IU human menopausal gonadotropin (hMG) (Humegon, Organon Inc.) and 150 IU of FSH (Fertinex, Serono Inc.) were given until two dominant follicles with an average diameter of 20 mm each was reached. The hCG was given when the serum E2 was 1,531 pg/ml but only six mature oocytes and three immature oocytes were retrieved.

Though the semen analysis was normal, ICSI was used because she had had relatively poor fertilization in her previous IVF attempts. Intracytoplasmic sperm injection was performed as previously described [3]. She fertilized three of the six mature oocytes and she transferred three embryos (morula, 5-cell, and 4-cell) 72 hours later. The three immature oocytes were cultured for one more day and ICSI was performed. Two fertilized and both were cryopreserved at the 2 pronuclear stage (2 PN) using a simplified freezing protocol [4]. She failed to conceive.

For her second cycle she used the microdose flare protocol (follicular phase leuprolide acetate 0.1 mg daily in 2 divided doses) with 225 IU hMG (Humegon, Organon Inc.) and 225 IU recombinant FSH (Follistim, Organon Inc.). Her baseline FSH was 9 mIU/ml with a serum E2 of 1,100 pg/ml. There were seven eggs retrieved with three mature and four immature. All three mature oocytes fertilized and an 8-cell, 7-cell, and 6-cell embryo was transferred three days later but she failed to conceive. Three of the four immature oocytes were matured another 24 hours in culture and ICSI was performed one day after retrieval. Three fertilized and were frozen at the 2PN stage.

She then proceeded to frozen ET. All five frozen embryos that were the result of culturing immature oocytes one day more followed by ICSI were thawed. Though all five survived the thaw, only three cleaved to an embryo with adequate blastomeres (4-cell, 5-cell, and 10-cell) to transfer. The thawing procedure involved a one-step removal of the cryoprotectant 1,2 propanediol [4]. Assisted embryo hatching was performed immediately prior to transferring the 72-hour-old embryos [5]. All embryos had 25% or less fragmentation. She conceived and delivered a healthy full-term baby girl.

The immature oocytes that were used in this transfer were metaphase I (n=3) and germinal vesicle stage (n=2).

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Case 2:

A 35-year-old woman reconsulted concerning her problem of secondary infertility. She presented with secondary infertility related to severe male factor problems. Her problem of primary infertility had been corrected by conception on her first cycle of in vitro fertilization (IVF) with ICSI at age 30. She had first consulted our center at age 29. The semen analysis on that cycle initially showed a volume of 4.0 ml, a concentration of 3.50 million/ml, with 23% motility (0% grade A, 35% grade B, 65% grade C) and after preparation using a 3-layer Percoll technique, the concentration was 5.6 million/ml with 15% motility (29% grade A, 6% grade B, 65% grade C). With ICSI, 11 of 12 mature oocytes fertilized (1 other oocyte was immature and the other atretic). She had been on a luteal phase leuprolide acetate stimulation regimen (0.75 mg x 10 days from the mid-luteal phase by itself and then it was reduced to 0.5 mg when 150 units of human menopausal gonadotropin (hMG) was given I.M. twice daily) until two oocytes reached an average diameter of 20 mm and then 10,000 units of human chorionic gonadotropin was administered. Four embryos were transferred 72 hours later (a 10, 6, 5, and 4-cell with the first three with 25% or less fragmentation and 50% fragmentation for the 4-cell embryo). She conceived on that cycle and delivered a full-term baby girl. Seven embryos remained frozen.

She failed to conceive following frozen ET. She had another oocyte retrieval cycle in which she fertilized eight of ten mature oocytes by ICSI, but failed to conceive after transferring four embryos (8, 7, 7, and 5-cells). Subsequent frozen ET of three embryos was also not successful.

The woman attempted IVF with ICSI one more time. She used for controlled ovarian hyperstimulation leuprolide acetate (Lupron, Tap Inc.) beginning the mid-luteal phase at 0.5 mg for ten days only and this was stopped when 225 units of recombinant FSH (Follistim, Organon Inc.) was given in the a.m. and 225 units hMG (Repronex, Ferring Inc.) was given in the p.m. Prior to this retrieval cycle, one attempt at IVF was cancelled because of poor response. In this cycle, despite the fact that her early follicular phase serum FSH was only 6 mIU/ml, she only reached a serum estradiol of 954 pg/ml (her serum E2 was beginning to plateau) and 10,000 units of human chorionic gonadotropin (hCG) was given. There were seven oocytes retrieved but six of seven were immature. The one mature oocyte failed to fertilize with ICSI. The six immature oocytes were cultured one more day and then ICSI was performed. There were three oocytes fertilized and cryopreserved. Though three embryos were transferred two months later when they were three days old (8, 8, and 5-cells) they all had more than 50% fragmentation..

Nevertheless, the woman attained a pregnancy and has successfully completed the first trimester. The oocytes originally retrieved in this successful transfer that formed the embryos used for transfer were at the metaphase I stage (n=2) and germinal vesicle stage (n=1).

Discussion

There has been only one previously published case report of fertilization of immature oocytes with in vitro maturation and subsequent successful pregnancy following transfer of frozen-thawed embryos [2]. This is the second and third case report of successfully frozen ET following ICSI of in vitro matured ooctyes. Case 2 differed from the previous published case in that the earlier case had had good fertilization with ICSI of the mature oocytes but failed to conceive following fresh ET and the only frozen embryos remaining were those resulting from in vitro matured oocytes [2]. In contrast, in case 2, the only mature oocyte failed to fertilize by ICSI and only the embryos formed from in vitro matured oocytes were available for transfer. Fresh transfer was deferred purposely because we believed that the development of the embryos would be asynchronous with uterine development. The circumstances of case 1 were similar to the one described by Edirisinge *et al.* [2].

For both cases 1 and 2 the embryos had been cryopreserved using a simplified freezing protocol with a one-step removal of cryoprotectant [4]. Assisted hatching was performed prior to transfer as previously described [5]. Thus, these two cases represent the first reported pregnancies following transfer of embryos formed by ICSI after in vitro maturation of oocytes that had been frozen using this particular cryopreservation protocol [4].

These two cases and the previously published two cases, demonstrate the importance of trying to get a better embryo yield from a particular oocyte harvest by attempting to fertilize immature oocytes the next day with ICSI. These reported cases only show that pregnancies are possible using embryos resulting from continued culture of immature oocytes followed by ICSI and that normal babies have been born. A couple given this information could decide that since there has been a precedent set, that they will transfer these type of embryos rather than keep them cryopreserved, or may opt for repeat IVF-ET with fresh ET.

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