Birth of a healthy infant after *in vitro* oocyte maturation and ICSI in a woman with diminished ovarian response: Case report

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*In vitro* maturation of oocytes (IVM) has been developed as a treatment option for subjects with good prognosis in assisted reproduction. We present successful IVM treatment in connection with a woman from whom low numbers of embryos were obtained after repeated failed conventional IVF cycles. A 35 year old woman, after 5 years infertility and two intrauterine insemination and three conventional IVF cycles, underwent first an IVM cycle with low dose FSH stimulation, and after failure, another natural IVM cycle. Three oocytes were obtained. After 36 h of IVM the oocytes had reached metaphase II stage, and fertilization using ICSI resulted in one 4-cell stage embryo, which was transferred 2 days later. The result was an uneventful pregnancy and birth of a healthy female infant weighing 4150 g. IVM may be an option for women from whom only low numbers of oocytes are obtained after gonadotrophin stimulation.

**Key words:** diminished ovarian response/*in vitro* maturation/live birth/natural cycle

**Introduction**

*In vitro* maturation of oocytes (IVM) has been introduced as a technique particularly suitable for women with a known high risk of ovarian hyperstimulation syndrome (OHSS). Women with polycystic ovaries and women who have a previous history of a hyperstimulation reaction during conventional controlled ovarian stimulation have been regarded as particularly good candidates for IVM. The protocols used for IVM include utilizing a natural menstrual cycle (Tan and Child, 2002) or priming with low dose FSH stimulation (Mikkelsen et al., 1999). Women who do not respond well to gonadotrophin stimulation might comprise another group which could benefit from IVM. If gonadotrophin stimulation does not help in increasing the number of oocytes surviving to maturity, it might be better to aspirate the oocytes before they are atretic, and then carry out IVM. It would be easier for the woman if she could avoid the costly high dose hormone treatment.

At our clinic when beginning the IVM programme, we offered IVM as the first alternative before IVF if the woman was at high risk of OHSS, or if she was aged <36 years, and healthy, with good prognosis in her treatment. We compared recombinant human (r)LH and rhGC in the maturation of oocytes in vitro (Hreinsson et al., 2003), and then applied IVM more widely among couples with an expected good number of oocytes. The pregnancy rate has increased and was 30% per embryo transfer in 2003 (Hreinsson, 2003). We have now started to consider it as an alternative for the women who do not seem to benefit from gonadotrophin stimulation.

One couple where the female partner belonged to a poor prognosis group and was likely to have diminished ovarian reserve was treated using IVM. She had three failed IVF treatments and a high serum concentration of FSH. The couple underwent two IVM cycles, the latter resulting in pregnancy and birth of a healthy child.

This is the first case report regarding a pregnancy and delivery of a healthy child after retrieval of immature oocytes in a natural cycle, and *in vitro* maturation of the oocytes followed by ICSI in a woman with a poor prognosis in IVF due to diminishing ovarian response.

**Case report**

A 35 year old woman with a 5 year history of infertility was referred to our centre for IVF treatment. At that time she had regular ovulatory cycles with 26 day intervals. She had normal serum levels of FSH, thyroid-stimulating hormone and prolactin, a normal hysterosalpingogram and the husband’s semen analysis was normal before her treatment cycles were initiated. The couple had undergone two unsuccessful cycles of intrauterine insemination (IUI) using the husband’s sperm.
The couple then underwent a treatment cycle with conventional IVF. The starting dose of recombinant FSH was 225 IU (Gonal-F; Serono Nordic, Solna, Sweden) after down-regulation with a GnRH agonist (Suprecur; Aventis Pharma, Stockholm), starting at the mid-luteal phase of the previous cycle. Seven oocytes were retrieved and four became fertilized, but only one of these cleaved. This embryo was transferred on day 3 at the 8-cell stage with an average score. No conception occurred. Only two follicles were seen in each ovary in the beginning of a cycle in an ultrasound scan after that treatment. During the second cycle, only one oocyte was obtained after stimulation with FSH doses of 450 IU per day.

The woman was re-evaluated, and serum FSH on menstrual cycle day 3 was assayed. This was 16 IU/l, indicating impending ovarian failure. The number of small follicles in her ovaries was again low, a total of four. An alternate flare-up protocol using high dose GnRH antagonist, cetrorelix (Cetrotide), 3 mg, in the mid-luteal phase on day 23 of the cycle to synchronize the cohort of growing follicles (Fridén and Nilsson, 2005), was then tried. This resulted in the development of eight oocytes. ICSI was performed, and the woman had two good quality 4-cell embryos transferred on day 2 after ICSI. No conception occurred.

At this stage, her ovarian response and reserve were regarded as diminished on the basis of the high serum FSH concentration, low numbers of embryos (1, 0 and 2) obtained during the three IVF cycles and the low numbers of follicles seen in her ovaries (two in each). A decision to perform IVM was made.

**In vitro maturation treatment**

The couple underwent one IVM cycle using our earlier protocol with low dose FSH (37.5 IU daily of Gonal-F) treatment on cycle days 2–6 (Hreinsson et al., 2003). Only one immature oocyte was retrieved on cycle day 10. The oocyte was matured in vitro in Tissue Culture Medium 199 (TCM-199; Invitrogen-Gibco, Paisley, UK) supplemented (10%) with pyruvate (P-4562; Sigma–Aldrich, Stockholm), starting at the mid-luteal phase of the previous cycle. Seven oocytes were retrieved and four became fertilized, but only one of these cleaved. This embryo was transferred on day 3 at the 8-cell stage with an average score. No conception occurred. Only two follicles were seen in each ovary in the beginning of a cycle in an ultrasound scan after that treatment. During the second cycle, only one oocyte was obtained after stimulation with FSH doses of 450 IU per day.

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**Discussion**

The recovery of immature oocytes from unstimulated or low dose FSH-stimulated ovaries followed by IVM represents an alternative to conventional controlled stimulation in IVF protocols, especially for subjects at risk of ovarian hyperstimulation, in spite of somewhat lower pregnancy rates per cycle (Mikkelsen et al., 2000; Yoon et al., 2001; Child et al., 2002). Priming in vivo with low dose FSH during the early follicular phase seems to have no beneficial effects on results among women with normal ovarian function (Mikkelsen et al., 2000). Adequate cumulative pregnancy rates with IVM, compared with conventional techniques, can, however, be achieved, since couples can undergo many consecutive cycles without any adverse medical effects.

Diminished ovarian reserve with a poor response to controlled ovarian stimulation is a frustrating condition in IVF practice, yielding low success rates. Poor ovarian response in IVF has been characterized as a low number of follicles seen in ultrasound scans and high basal serum FSH concentrations and fewer than five oocytes obtained in a stimulated cycle (Bancsi et al., 2003; Goverde et al., 2005; Hendriks et al., 2005; Penarrubia et al., 2005). In recent literature, a follicle count less than five at the beginning of the cycle has been used as a predictor of poor response in IVF (Durmusoglu et al., 2004; Kailasam et al., 2004; Klinkert et al., 2005).

Many different approaches have been tried to treat poor ovarian response. Flare-up protocols using oral contraceptive pills and shorter down-regulation with GnRH agonists are well known, although the results are conflicting and somewhat disappointing. Protocols involving co-treatment with steroids, growth hormones and anti-diabetics have also been tried (Surrey and Schoolcraft, 2000; Tarlatzis et al., 2003; Loutradis et al., 2003). Natural cycle with aspiration of mature oocytes does not increase the pregnancy rate in poor responders (Kolibianakis et al., 2004). One approach has been the use of IVM for immature oocytes obtained during stimulated cycles before cancelling the treatment (Liu et al., 2003).

Our patient initially initially had seven oocytes after FSH stimulation, but only one cleaved embryo. In the next attempt only one oocyte was obtained in spite of the high dose of FSH (450 IU). Using a particular poor responder programme, eight oocytes were subsequently obtained, but no more than two embryos. She had fewer than five follicles in her ovaries at the beginning of her cycles, and high basal concentration of FSH towards the end of the treatment period. She can therefore be regarded as a patient with poor prognosis and diminishing ovarian response.

What was even more striking with our patient was the low fertilization rate and low number of embryos obtained (1/7, 4150 g (Apgar 9, 10, 10), was delivered by spontaneous vaginal birth.
0/1 and 2/8), probably also reflecting poor oocyte quality. It has been suggested that in all women, even towards the end of the reproductive period, there are some oocytes of good quality among poorer ones and that gonadotrophin stimulation may not result in increased numbers of good quality oocytes in these women and is therefore of no advantage (Khalaf et al., 2002). This appears to have been the case with our patient.

Use of IVM in natural cycles in low-responding women may bring benefits. The numbers of oocytes retrieved from women with poor response were comparable using IVM or stimulated cycles, and directly comparable to the numbers of follicles > 5 mm in the ovaries (Requena et al., 2000). Apart from being more economical, it is possible to retrieve non-atretic oocytes in repeated, consecutive IVM cycles without the discomfort and cost of hormone treatment. The treatment therefore may increase the chances of finally achieving a good quality oocyte for fertilization.

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References


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