IVM is an alternative for patients with PCO after failed conventional IVF attempt

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Abstract

Purpose To determine if IVM of oocytes from unstimulated cycle is a treatment option for patients who did not deliver after standard IVF-ET.

Method Twenty three women with PCO, thirteen of them with normal cycles and all <35 years old, who failed IVF served as their own control. During the control IVF cycle patients were stimulated with 1730.7 ± 639.5 IU recombinant FSH, a long Buserelin acetate protocol was used and embryo transfer was performed on day 2 or 3 after ICSI. After failed IVF immature oocytes were aspirated transvaginally from antral follicles during spontaneous menstrual cycle. Embryo transfer was performed 2 or 3 days later.

Result 11.4 ± 4.8 mature oocytes and 6.7 ± 3.2 embryos were produced with IVF, which served as the control, compared to 9.7 ± 4.5 mature oocytes and 6.2 ± 3.2 embryos with IVM. There was one clinical pregnancy in the IVF group which did not result in a live birth where as five singleton and one pair of twins with healthy live births and one miscarriage in the IVM group.

Conclusion IVM does not involve ovarian stimulation with possible financial and health consequences. It may be an useful treatment after unsuccessful IVF.

Keywords IVM · Live birth rate · Unstimulated cycle · Unsuccessful IVF attempt

Introduction

Women undergoing IVF treatment who had an unsuccessful result to gonadotropin stimulation present a challenge for reproductive physicians. Several studies have explored reasons for dropout, including poor prognosis, financial or physiologic burden. Emotional distress and poor prognosis of outcome have been identified in a new study as the main reasons for dropping out of IVF [1]. Different strategies for managing subsequent treatment cycles have been reported, and these include: changing the ovarian stimulation protocol, natural cycle IVF or oocyte donation. None of these management strategies, however, are entirely satisfactory. In vitro maturation (IVM) of immature oocytes aspirated from unstimulated ovaries is a relatively new assisted reproductive technology, which has shown promising results in women with polycystic ovaries [2–4]. Although the classical indication for IVM is PCOS/PCO, it is used also for other indications today: poor responders, fertility preservation of cancer patients, normal responders with history of poor oocyte/embryo quality, as well as for oocyte donation. Cryopreservation of immature oocytes or ovarian tissue and embryos, coupled with IVM, is a potential way to preserve fertility [5, 6].

We reported a series of 25 cycles in 20 women with PCOS undergoing IVM treatment [3]. An average of 10.3 immature oocytes was retrieved, with a clinical pregnancy rate of 40% per cycle commenced. In an another prospective observational study we investigated the differences in immature oocyte maturation, fertilization, and pregnancy rates among women with unstimulated normal

Capsule In vitro maturation of oocytes from unstimulated ovaries after unsuccessful in vitro fertilization attempt

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ovaries, polycystic ovaries and PCOS [7]. The number of immature oocytes retrieved is proportional to the number of antral follicles present [8]. Consequently, fewer oocytes are retrieved from normal as compared with polycystic ovaries.

Although the number of oocytes and embryos produced in an IVM cycle may be low, we hypothesized that offering IVM treatment to women, who did not deliver after standard IVF-ET, could be attractive for some couples because it may result in live birth. If so, IVM treatment could offer an alternative to a further stimulated cycle in some women who have an unsuccessful result after an IVF cycle. This study therefore compared the outcome for women who did not deliver after standard IVF-ET in one cycle, and who, in their next cycle, underwent IVM treatment.

Materials and methods

Patients

After obtaining IRB approval, we recruited for the study 23 women who did not deliver after standard IVF-ET with a long Buserelin acetate protocol. A failed IVF attempt was defined as a cycle which did not reach pregnancy with live birth. Live birth was defined as a living child. All 23 women are Caucasian with PCO/PCOS, a mean FSH of 7.3 and a mean BMI of 24. Thirteen reported normal cycles, 8 had oligomenorrhea and 2 were amenorrhoic. Their antral follicle count (AFC) was > 20 on their baseline ultrasound scan. An ovary was classified as polycystic when on transvaginal ultrasound performed during the early follicular phase ≥ 10 small (2–8 mm) cysts were found arranged around the periphery or scattered through an enlarged echogenic stroma [9]. Four of the couples were recruited to the IVF program because of male factor infertility and four women were smokers. Nine teen women did not deliver after three cycles of controlled ovarian hyperstimulation with rec-FSH together with intrauterine insemination and at least one IVF attempt. The mean age of twenty three women was 30.8 ± 3.2 . Each patient gave informed written consent before undergoing each treatment. All IVM cycles were performed between March 2007 and December 2009.

In-vitro maturation cycle

The IVM protocol used in our center has been reported previously [2]. Patients underwent a baseline ultrasound scan on day 2 or 3 of menstrual bleeding to measure the number and the sizes of antral follicles, the endometrial thickness, and to exclude the presence of any ovarian cysts. Transvaginal ultrasound scans were repeated on day 8 to

exclude the development of a dominant follicle. The size of all follicles on ultrasound scan had to be < 10 mm on diameter and the endometrial thickness ≥ 6 mm on day of hCG. All patients received 10 000 IU hCG (Pregnyl; Organon, Turkey) s.c. 38 h before oocyte retrieval. We reported the IVM aspiration technique previously [10]. Transvaginal ultrasound-guided oocyte collection was performed using a specially designed 19 G single-lumen aspiration needle (K-OPS-7035-RWH-ET, Cook, Australia) with reduced aspiration pressure of 7.5 kPa. Aspiration of small follicles was performed under general anesthesia. Oocytes were collected in culture tubes containing warm Quinn's Advantage medium with HEPE S with 2 IU/ml heparin. Immature oocytes were collected in a culture dish containing 2.0–2.5 ml oocyte washing medium (SAGE IVM media-ART-1600-A, Medek, Turkey). These oocytes were incubated in a culture dish containing 1 ml oocyte maturation medium (SAGE IVM media -ART-1600-B, Medek, Turkey), 75 IU/ml FSH+LH (Menegon; Fehring, Turkey) at 37°C in an atmosphere of 5% CO_2 and 95% air with high humidity. Following culturing, the maturity of the oocytes was determined under the microscope at 12 h intervals for up to 48 h. Mature oocytes, determined by the presence of a first polar body extrusion, were denuded of cumulus cells and fertilized by intracytoplasmic sperm injection (ICSI) using the husband's sperm. Following ICSI, each oocyte was transferred into a 20 μl droplet of Embryo Maintenance Medium (SAGE IVM media -3 ART-1600-C, Medek, Turkey) in a tissue culture dish under mineral oil. Fertilization was assessed 18 h after ICSI by examining the oocytes for the appearance of two distinct pronuclei and two polar bodies. Oocytes with two pronuclei were further cultured in Embryo Maintenance Medium. Embryos were transferred on day 2 or 3 after ICSI. We reported the endometrial preparation protocol used previously [11]. Briefly, patients received oral 17 β estradiol (Estrofem; Novo Nordisk, Turkey), 6–10 mg daily, starting on the day of oocyte retrieval; the dose depending on the endometrial thickness on that day. Luteal support was provided by 200 mg of intravaginal progesterone (Progestan, Koçak, Turkey) t.i.d., from the day of ICSI. A pregnancy test was considered positive when a serum hCG taken 14 days after embryo transfer had a concentration of greater than 25 IU/L. (Our laboratory defines a serum hCG positive if greater than 25 IU/L). Clinical pregnancy was defined as an intrauterine gestation with a fetal heartbeat seen by transvaginal ultrasound scan 3 weeks after positive serum hCG.

Comparison of IVF and IVM cycles

The outcome varies in the number of mature oocytes, embryos produced and pregnancies achieved.

Statistical analysis

The statistical significance of the differences between the means of the groups was determined using non parametric McNemar Test and Wilcoxon Signed Ranks Test. A *p* value of < 0.05 was considered statistically significant.

Results

Twenty three women who did not deliver after standard IVF-ET underwent IVM treatment. All 23 women had AFC > 20 on baseline ultrasound scanning. 4 of them were recruited to the IVF program because of severe male factor infertility. The outcome of the treatment cycles are summarized in Table 1.

IVF cycles

Twenty three patients underwent 29 IVF cycles before entering the study. The control for each IVM cycle was defined as the last IVF cycle of the same patient. Twenty two women underwent fresh embryo transfer. One of the patients underwent frozen embryo transfer which resulted in a clinical pregnancy without a live birth. She had 15 cryopreserved embryos because of severe OHSS. The median (range) gonadotropin dose was 1730 (750–3187) IU. The mean number of mature oocytes retrieved and embryos produced per cycle commenced 11.4 ± 4.8 and 6.7 ± 3.2 respectively (Table 1).

IVM cycle

All 23 women who began cycle monitoring for IVM treatment proceeded to oocyte retrieval. A mean of 12.8 ± 6.9 germinal vesicle oocytes was retrieved per patient (Table 1). This resulted in a mean of 9.7 ± 4.5 mature oocytes and 6.2 ± 3.2 embryos per patient. Twenty three

women had a mean of 3.3 ± 1.0 embryos transferred. There were fewer collected oocytes, metaphase II oocytes, fertilized oocytes and cleaving embryos in the IVM group which did not reach statistically significance. The mean number of transferred embryos, clinical pregnancies and live birth rate in the IVM group were significantly different than in the IVF group (Table 1). There were five singleton and one pair of twins with healthy live birth and one miscarriage.

Discussion

In vitro fertilization and embryo transfer is an established and successful form of treatment for couples suffering from infertility. However a significant number of couples discontinue treatment without achieving a live birth. Although some of them may respond to a subsequent trial of gonadotropin stimulation for IVF, this success is achieved at expense of two major complications, namely ovarian hyperstimulation syndrome (OHSS) and multiple births. Severe OHSS affect 1–2% of all women undergoing ART and up to 6% of women with PCO or PCOS [12]. The only way to avoid OHSS totally is to avoid ovarian stimulation [13]. Therefore there is an increasing desire for milder ovarian stimulation using either lower amounts of gonadotropins, or, ideally, dispensing with ovarian stimulation altogether [14]. An alternative approach is to use natural cycle IVF [15]. However, since there is generally only one preovulatory follicle in a natural cycle, many women will not have an oocyte collection or embryo transfer [15]. It has been shown that IVM is a successful method of treatment in women who have polycystic ovarian syndrome [3, 7, 16]. Since the high number of antral follicles found in patients with PCO makes them more likely to develop OHSS, they are also prime candidates for IVM treatment. This applies even if patients have ultrasound-only polycystic ovaries without an ovulatory

Table 1 Comparison of outcomes between IVM and IVF groups

	IVM	IVF	<i>p</i>
Number of cycles commenced	23	23	
Age (years) (mean $\pm$ SD)	30.3 ± 3.5	29.9 ± 3.3	NS ^a
Number of oocytes retrieved (mean $\pm$ SD)	12.8 ± 6.9	13.7 ± 6.6	NS ^a
Number of M II oocytes (mean $\pm$ SD)	9.7 ± 4.5	11.4 ± 4.8	NS ^a
Number of oocytes fertilized (mean $\pm$ SD)	6.9 ± 3.6	7.5 ± 3.1	NS ^a
Number of embryos (mean $\pm$ SD)	6.2 ± 3.2	6.7 ± 3.2	NS ^a
Number of ET (mean $\pm$ SD)	3.4 ± 1.4	2.7 ± 0.8	$<0.05^b$
Clinical pregnancies (%)	7 (30.4%)	1 (4.3%)	$<0.05^b$
Live birth (%)	6 (26.1)	0	$<0.05^b$
Miscarriage (%)	1 (4.3%)	1 (4.3%)	NS ^b
OHSS (%)	0	4 (17.3%)	NS ^b

^a Wilcoxon Signed Ranks Test

^b McNemar Test

disorder; [12, 17]. That is the case in fifty six percent of our patients who reported normal cycles despite polycystic ovaries.

The other known disadvantages with gonadotropin stimulation are high drug costs, daily injections, frequent monitoring and potential side effects such as abdominal bloating, breast tenderness, mood swings and nausea.

We hypothesized that IVM may be potentially easy and useful in treating women with at least one previous unsuccessful IVF attempt, provided an antral follicle count (AFC) > 20 are seen in the ovaries on ultrasound scan. In vitro maturation is a useful treatment option, particularly for women with polycystic ovaries. However, to our knowledge this is the first study that compares IVM with IVF in the same patient. The number of immature oocytes collected from an unstimulated cycle is related to the number of antral follicles present [8]. We expected, and found fewer oocytes in this group of women in the present study.

Secondary to their structural similarity hCG has been used in ART to imitate the endogenous LH surge. It has been demonstrated in IVM cycles, that hCG priming increases both the percentage [3] and the rate of immature oocyte maturation [2]. However a prospective, randomized, controlled trial did not demonstrate an improvement in oocyte maturation rates with 20 000 IU of hCG compared with 10 000 IU of hCG [18]. Therefore all the patients in this study were primed with 10 000 IU of hCG.

Although the number of embryos available for transfer following IVM was less compared to the number available in the previous IVF cycle, there was still enough embryos available for transfer. The mean number of embryos transferred in the IVM group was significantly greater than that in IVF group. It seems to be difficult to draw conclusions when more embryos are transferred in IVM than IVF cycles, but our previous study revealed that the implantation rate of IVF-derived embryos was higher (17.1% versus 9.5%) than that for IVM ($P<.01$) [16]. Therefore it is our routine to transfer one embryo more in average in the IVM cycles compared to IVF cycles.

These embryos were produced without the need for prolonged, expensive and potentially dangerous gonadotropin therapy. That is attractive for those couples who self finance their treatment expenses and cannot afford conventional IVF secondary to high drug costs and who are under psychological stress. The level of stress in infertility patients tends to increase as treatment intensifies and as duration of treatment continues. Therefore, a population of IVF patients would be expected to experience more stress than women early in their infertility evaluation [19]. Most IVF patients state that treatment is more of a psychological than a physical stressor [20]. Infertility specialists traditionally have assumed that patients drop out of treatment for two

reasons: active censoring and finances. The psychological burden of the procedures appears to be the major reason for dropout [21–23].

Any new development in ART must also provide data concerning congenital abnormality and perinatal outcome. Based on current limited data IVM does not increase the risk of congenital abnormality or perinatal outcome [24–28] over that already accepted for IVF or ICSI [29, 30]. In our small group neither of the babies born after IVM treatment had any congenital abnormalities.

Even if IVM only produces a comparable outcome to conventional IVF in ‘previous unsuccessful IVF attempts’, it would be an attractive treatment secondary to avoidance of large expenses of gonadotropin therapy and its concomitant side effects.

Conclusion

This small, preliminary study demonstrates that women with AFC > 20 on the baseline ultrasound scan who exhibit an unsuccessful attempt to previous stimulated IVF can produce a similar number of oocytes in a subsequent unstimulated IVM cycle. IVM has the potential to be of clinical significance for women who cannot afford conventional IVF, who are prone to ovarian hyperstimulation, for whom the emotional toll of conventional IVF is crushing.

References

1. Brandes M, van der Steen JO, Bokdam SB, et al. When and why do subfertile couples discontinue their fertility care? A longitudinal cohort study in a secondary care subfertility population. *Hum Reprod.* 2009;24(12):3127–35.
2. Chian RC, Buckett WM, Tulandi T, Tan SL. Prospective randomized study of human chorionic gonadotrophin priming before immature oocyte retrieval from unstimulated women with polycystic ovarian syndrome. *Hum Reprod.* 2000;15:165–70.
3. Chian RC, Gulekli B, Buckett WM, Tan SL. Priming with human chorionic gonadotropin before retrieval of immature oocytes in women with infertility due to the polycystic ovary syndrome. *N Engl J Med.* 1999;341:1624–6.
4. Cha KY, Han SY, Chung HM, Choi DM, Lim JM, Lee WS, et al. Pregnancies and deliveries after in vitro maturation culture followed by in vitro fertilization and embryo transfer without stimulation in women with polycystic ovary syndrome. *Fertil Steril.* 2000;73:978–83.
5. Tucker MJ, Wright G, Morton PC, et al. Birth after cryopreservation of immature oocytes with subsequent in vitro maturation. *Fertil Steril.* 1998;70:578–9.
6. Chian RC, Gulekli B, Buckett W, et al. Pregnancy and delivery after cryopreservation of zygotes produced by in-vitro matured oocytes retrieved from a woman with polycystic ovarian syndrome. *Hum Reprod.* 2001;16(8):1700–2.
7. Child TJ, Abdul-Jalil AK, Gulekli B, Tan SL. In vitro maturation and fertilization of oocytes from unstimulated normal ovaries,

- polycystic ovaries and women with polycystic ovary syndrome. *Fertil Steril*. 2001;76:936–42.
8. Tan SL, Child TJ, Gulekli B. In vitro maturation and fertilization of oocytes from unstimulated ovaries: predicting the number of immature oocytes retrieved by early follicular phase ultrasound. *Am J Obstet Gynecol*. 2002;186:684–9.
 9. Adams J, Polson DW, Franks S. Prevalence of polycystic ovaries in women with anovulation and idiopathic hirsutism. *Br Med J*. 1986;293:355–9.
 10. Gulekli B, Demirtas E, Buckett WM. Immature oocyte collection. In: TanSL, Chian RC, Buckett WM, editors. *In-vitro maturation of human oocytes basic science to clinical application*. Publisher: Informa Healthcare; 2007. p 253–263 UK.
 11. Child TJ, Gulekli B, Sylvestre C, Tan SL. Ultrasonographic assessment of endometrial receptivity at embryo transfer in an in vitro maturation of oocyte program. *Fertil Steril*. 2003;79:656–8.
 12. MacDougall MJ, Tan SL, Balen A, et al. A controlled study comparing patients with and without polycystic ovaries undergoing in vitro fertilization. *Human Reprod*. 1993;8:233–7.
 13. Buckett W, Chian RC, Tan SL. Can we eliminate severe ovarian hyperstimulation syndrome? Not completely. *Human Reprod*. 2005;20:2367.
 14. Edwards RG, Lobo RA, Bouchard P. Why delay the obvious need for milder forms of ovarian stimulation? *Hum Reprod*. 1997;12:399–401.
 15. Bassil S, Godin PA, Donnez J. Outcome of in-vitro fertilization through natural cycles in poor responders. *Hum Reprod*. 1999;14:1262–5.
 16. Child TJ, Philips SJ, Abdul-Jalil AK, Gulekli B, Tan SL. A comparison of in vitro maturation and in vitro fertilization for women with polycystic ovaries. *Obstet Gynecol*. 2002;100:665–7.
 17. Balen AH, Laven JSE, Tan SL, et al. Ultrasound assessment of polycystic ovary: international consensus definitions. *Human Reprod Update*. 2003;9:505–14.
 18. Gulekli B, Buckett WM, Chian RC, Child TJ, Abdul-Jalil AK, Tan SL. Randomized, controlled trial of priming with 10 000 IU versus 20 000 IU of human chorionic gonadotropin in women with polycystic ovary syndrome who are undergoing in vitro maturation. *Fertil Steril*. 2004;82:1458–9.
 19. Domar AD, Broome A, Zuttermeister PC, et al. The prevalence and predictability of depression in infertile women. *Fertil Steril*. 1992;58(6):1158–63.
 20. Eugster A, Vingerhoets AJ. Psychological aspects of in vitro fertilization: a review. *Soc Sci Med*. 1999;48(5):575–89.
 21. Land JA, Courtar DA, Evers JL. Patient dropout in an assisted reproductive technology program: implications for pregnancy rates. *Fertil Steril*. 1997;68(2):278–81.
 22. Hammarberg K, Astbury J, Baker H. Women's experience of IVF: a follow-up study. *Hum Reprod*. 2001;6(2):374–83.
 23. Rajkhowa M, McConnell A, Thomas GE. Reasons for discontinuation of IVF treatment: a questionnaire study. *Hum Reprod*. 2006;21(2):358–63.
 24. Buckett W. Pregnancy and neonatal outcome following IVM. In: TanSL, Chian RC, Buckett WM, editors. *In-vitro maturation of human oocytes basic science to clinical application*. Publisher: Informa Healthcare; 2007. p. 313–318 UK.
 25. Cha KY, Chung HM, Lee DR, et al. Obstetric outcome of patients with polycystic ovary syndrome treated by in vitro maturation and in vitro-embryo transfer. *Fert Steril*. 2004;83:1462–5.
 26. Buckett W, Chian RC, Barrington K, et al. Obstetric, neonatal and infant outcome in babies conceived by in-vitro maturation (IVM): initial five-year results 1998–2003. *Fert Steril*. 2004;82 Suppl 2:S133.
 27. Buckett W, Chian RC, Holzer H, et al. Congenital abnormalities and perinatal outcome in pregnancies following IVM, IVF, and ICSI delivered in a single center. *Fert Steril*. 2005;84 Suppl 2:S180.
 28. Mikkelsen AL, Ravn SH, Lindenberg S. Evaluation of newborns delivered after in vitro maturation. *Human Reprod*. 2003;8 Suppl 2:S133.
 29. Hansen M, Kurinczuk JJ, Bower C, et al. The risk of major birth defects after intracytoplasmic sperm injection and in vitro fertilization. *N Engl J Med*. 2002;346:725–30.
 30. Koivurova S, Kartikainen AL, Gissler M, et al. Neonatal outcome and congenital malformations in children born after in-vitro fertilization. *Human Reprod*. 2002;17:1391–8.