CHAPTER 15

In-vitro maturation for the treatment of infertility with polycystic ovary syndrome

Hanadi Ba-Akdah, Hananel EG Holzer, Julie Lukic, and Seang Lin Tan

INTRODUCTION

The first live birth resulting from in-vitro fertilization (IVF) occurred in 1978[1] and, since then, over 2 million IVF babies have been born worldwide. IVF success rates have steadily improved over the years[2] and nowadays exceed spontaneous conception rates in fertile couples[3]. However, ovarian stimulation protocols are associated with high costs, daily injections of gonadotropins, and close monitoring, and carry a significant risk of causing ovarian hyperstimulation syndrome (OHSS)[4,5]. Papanikolaou et al.[6] found that the incidence of patients undergoing IVF requiring hospitalization for OHSS was 2%; in exceptional cases, where OHSS appropriate care is not given, it may even be fatal[7].

In-vitro maturation (IVM) is the maturation of immature oocytes in vitro from the germinal vesicle (GV) stage to the metaphase II (MII) stage of development, at which time they can be fertilized and subsequently undergo normal embryonic development. Clinical IVM is without ovarian stimulation. Therefore, IVM avoids OHSS, is clinically simple, and has a lower cost. As a result, it is a potentially useful treatment for infertility.

As GV oocytes will be successfully aspirated from about 60% of the tiny follicles during follicular collection, the number of antral follicles is the major predictor clinically for the success of IVM[8]. Since the high number of antral follicles found in patients with polycystic ovary syndrome (PCOS) makes them more likely to develop OHSS, they are also prime candidates for IVM treatment. This applies even if the appearance of PCOS on ultrasound scan is not associated with an ovulatory disorder; that is, if patients have ultrasound-only polycystic ovaries (PCO)[9,10].

IN-VIVO AND IN-VITRO MATURATION OF OOCYTES

Oocytes are formed in the ovaries of a human female during fetal development but are arrested at the prophase I stage of meiosis. At birth, there are approximately 1 million primordial follicles in the ovaries[11]. Although large numbers of follicles can leave the primordial pool and begin to grow, very few will be selected to mature and to ovulate for potential fertilization. In response to rising levels of gonadotropins, the follicles will grow and become fully mature, but only after the onset of puberty will they be released into the fallopian tube by ovulation. During a woman's reproductive life, only about 400 to 500 mature

oocytes will be released from the ovaries for potential fertilization. The process of follicular development within the ovary is directly influenced by gonadotropins, namely follicle stimulating hormone (FSH) and luteinizing hormone (LH). Gonadotropins (FSH and LH) are necessary for follicular development in vivo. The first meiotic division will occur in preovulatory follicles following the preovulatory LH surge, where the chromosomes progress from the metaphase I (MI) to the telophase I stage. After the first meiotic division and first polar body extrusion, the second meiotic division begins and a secondary metaphase plate (metaphase II) is formed at which time the oocyte is mature, enabling fertilization and early embryonic development to occur.

Hreinsson et al.[12] showed that the use of recombinant human chorionic gonadotropins (hCG) or recombinant LH is equally effective in promoting oocyte maturation in vitro. In addition, it is known that culture medium supplemented with a physiologic concentration of FSH or LH stimulates steroid secretions (estradiol and progesterone) from cultured granulosa and cumulus cells[13]. Therefore, it is likely that one of the actions of gonadotropins is mediated by either estradiol or progesterone, which may control oocyte maturation in vitro.

POLYCYSTIC OVARY SYNDROME (PCOS)

PCOS is a very heterogeneous syndrome and is the most common cause of anovulatory infertility. In the past, diagnosis of PCOS was made according to the National Institutes of Health (NIH) Consensus Criteria. However, more recently, the Rotterdam Consensus has revised the prerequisites whereby the diagnosis of PCOS now requires the presence of two out of the following three criteria: (1) oligo- or anovulation, (2) clinical and/or (3) biochemical signs of hyperandrogenism and polycystic ovaries at ultrasound scan[14].

Patients with PCOS might present with hirsutism, obesity, or frequently with cycle abnormalities (oligomenorrhea) and infertility (Figure 15.1). PCOS is the most widespread endocrinologic disorder among women of reproductive age as well as the most common cause of anovulatory infertility[15] and has been shown to exist in from 4% to 10% of the general population. Fertility treatments for women with PCOS include lifestyle management, administration of insulin-sensitizing agents, ovulation induction, ovarian stimulation, and IVF.

POLYCYSTIC OVARIES (PCO)

The definition of ultrasound-only polycystic ovaries (PCO) is the presence of 12 or more follicles in each ovary measuring 2–9 mm in diameter and increased ovarian volume (>10 ml) without any other manifestation of PCOS (Figure 15.2)[10]. Isolated PCO morphology without a full picture of PCOS has an incidence of 16–23%[16–18]. On ultrasound examination, women with ultrasound-only PCO were found to produce more follicles, oocytes, and embryos than women who had normal ovarian morphology[9,19].

The high number of antral follicles in patients with PCO makes them prime candidates for IVM

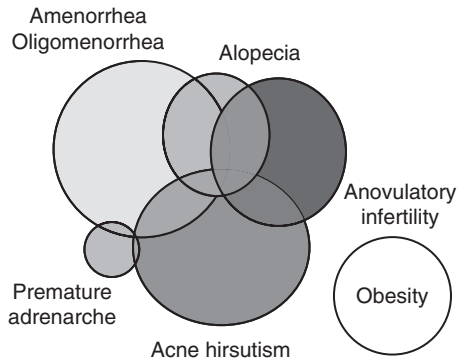

Figure 15.1 The spectrum of presentation in polycystic ovary syndrome (see color plate section)

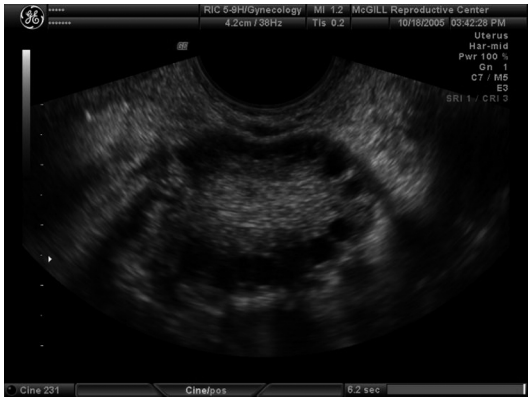

Figure 15.2 Ultrasound appearance of polycystic ovary

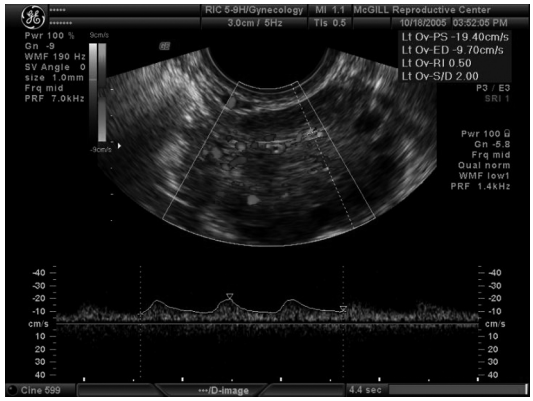

Figure 15.3 Stromal blood flow in polycystic ovary (see color plate section)

(treatment, even if the appearance of PCO in the scan is not associated with an ovulatory disorder. Indeed, the main clinical determinant of the success rates of IVM treatment is antral follicle count[8,20].

RISKS ASSOCIATED WITH IVF IN PCO/PCOS WOMEN

As previously mentioned, patients with PCO/PCOS have a greater risk of developing severe OHSS from gonadotropin stimulation than those who have normal ovaries[9]. Patients with PCO or PCOS are particularly more prone to develop OHSS, with an incidence of up to 6%[9]. The most severe manifestation of OHSS involves massive ovarian enlargement, multiple cysts, hemoconcentration, and third-space accumulation of fluid. The syndrome may be complicated by renal failure and oliguria, hypovolemic shock, thromboembolic episodes, and adult respiratory distress syndrome (ARDS), which, in extreme cases, may even be fatal, if appropriate care is not given. Ovarian volume, stromal volume, and stromal peak blood flow velocity are all significantly higher in the ovaries of women with PCO and PCOS (Figure 15.3). The reason for increased blood flow is probably because of the increased vascular endothelial growth factor (VEGF) levels in PCO/PCOS patients. Serum VEGF seems to be a major capillary permeability factor in the development of OHSS ascites[21–24].

Several methods have been used to reduce the risk of OHSS, including starting stimulation with a lower dose of FSH, close monitoring by ultrasound scans and serum estradiol (E_2) level measurements, coasting, withholding or decreasing the hCG dose for final follicular maturation, administration of intravascular volume expanders, use of glucocorticoids[25], and decreasing the number of embryos transferred or freezing all the embryos and transferring in a later frozen embryo replacement cycle[26]. Despite many years of clinical experience, no method has been developed that will completely prevent severe OHSS after ovarian stimulation[27].

In assisted reproduction, the risk of multiple-follicle ovulation and subsequent multiple pregnancies in PCO/PCOS women is also of crucial importance[9,28]. When the outcome of in-vitro fertilization and embryo transfer (IVF-ET) in 76 patients with PCO diagnosed on pretreatment ultrasound scan was compared with that of 76 control patients who had normal ovaries, it was found that there was a 10.8% risk of OHSS in the PCO group compared with none (0%) in those with normal ovaries[9].

ADVANTAGES OF IVM

Because no expensive gonadotropin stimulation and no extensive monitoring scans are required, the cost of IVM treatment is lower than that of IVF. Furthermore, the side-effects of medications used in IVF, although mild, may be unpleasant.

The IVM treatment schedule is shorter, causing less stress, and it is not necessary to wait for 2 to 3 months between treatment cycles because no stimulation is involved. The risk of OHSS can be avoided by IVM treatment, especially in women with PCO/PCOS[5].

OUTLINE OF AN IVM TREATMENT CYCLE FOR WOMEN WITH PCO AND PCOS

The first pregnancy and delivery of a healthy baby after IVM of immature oocytes in a patient with PCOS was described by Trounson et al.[29]. In the following year, Barnes *et al.*[30] reported another pregnancy in a patient with PCOS treated with IVM combined with intracytoplasmic sperm injection (ICSI) and assisted hatching. In these early studies, the rates of maturation, fertilization, and cleavage were initially found to be higher in regularly ovulating women compared with irregularly ovulatory or anovulatory women with PCO; however, later studies have shown comparable maturation and fertilization rates[31,32]. When IVF was compared with IVM cycles in PCO women, the rates of fertilization and embryo cleavage were found to be similar[5].

Previous studies indicated that although immature oocytes recovered from unstimulated PCOS patients could be matured, fertilized, and developed in vitro, the implantation rate was still disappointingly low[5,33–35]. To compensate for this, endogenous priming with FSH or hCG has been suggested before oocyte retrieval and IVM.

Ultrasound

A baseline scan is performed between days 2 and 5 of the menstrual cycle to measure ovarian volume, ovarian stromal blood flow velocity, antral follicle count (AFC), size of the follicles, and endometrial thickness, and to detect whether any ovarian or uterine abnormalities are present. If the patient is amenorrheic, a withdrawal bleed with progestogens is induced. Although AFC, ovarian volume, and ovarian stromal maximal blood velocity are all predictors of the number of retrievable oocytes, we have found that when the other factors are controlled by multiple regression analysis, the antral follicle count is the only reliable predictor[8]. Between days 6 and 8 of the cycle, a second scan is performed to measure AFC and endometrial thickness. We and others have recently reported that atresia does not occur in the non-dominant follicles, even when a dominant follicle is present[36–38]. For this reason, we no longer cancel the procedure for patients who are found to have a dominant follicle. hCG is administered (see below) when the endometrial thickness is greater than 8 mm.

hCG priming and pretreatment with FSH

While the results of some studies indicate that pretreatment with FSH during the early follicular phase enhances both the number of oocytes retrieved and their rate of maturation[39], others have found that pretreatment with FSH produces no tangible benefits[40,41]. Based on the results of the latter studies, at our center we do not stimulate the ovaries with FSH prior to IVM oocyte collection.

Interest in the effects of hCG priming arose when morphologic and molecular differences were found between immature oocytes collected from stimulated cycles and those collected by cesarean section[42]. In 1999[43,44], we reported that the maturation rate of immature

oocytes collected from women with PCOS was improved by administering 10 000 IU hCG 36 h prior to retrieval. Results of a randomized prospective study indicated that besides improving the maturation rate, priming with hCG also shortened the maturation process[44]. We determined that oocytes that had been matured for 24 h following collection produced better quality embryos than those that had been matured for 48 h[44]. Furthermore, our findings seem to indicate that hCG priming could increase the number of MII stage oocytes retrieved, resulting in a clinical pregnancy rate of >35% per cycle in young women up to 35 years of age[12,45–49]. Moreover, induced luteinization with hCG might enhance endometrial preparation, resulting in improved synchronization of embryonic development within the endometrium[50].

Retrieval of immature oocytes

Oocyte retrieval is performed under spinal anesthesia or intravenous sedation using 1 to 2 mg of fentanyl and midazolam. Intravenous fentanyl is administered at intervals of 15 to 20 minutes to a maximum dosage of 150 to 200 mg. In order to reduce the discomfort of the multiple needle punctures that are required by this procedure, 0.5% bupivacaine is infiltrated locally into the vagina. Oocyte retrieval is then performed under ultrasound guidance using a 19G, single-lumen aspiration needle. Because the aspiration pressure is reduced to 7.5 kPa and such a small gauge needle is used, bloodstained aspirate can frequently block the needle. Therefore, the needle is withdrawn from the vagina after aspirating a few follicles and is flushed to clear any obstruction. The procedure is repeated until all follicles seen are aspirated. Follicular fluid is collected in culture tubes containing a solution of 0.9% saline supplemented with 2 U/ml of heparin. Because immature oocytes are enclosed in tightly packed cumulus cells, curettage of the follicular wall is performed in order to dislodge the cumulus–oocyte complex. Over the past 5 years, we have increased the average number of GV oocytes we collect per cycle from 10 to 15.

IVM and fertilization

Immature oocytes are incubated in a culture dish containing maturation medium supplemented with 75 mIU/ml of FSH and LH at 37°C in an atmosphere of 5% carbon dioxide and 95% air with high humidity and are checked for maturity 24 and 48 hours following culture. The oocytes are denuded of granulosa cells following retrieval, and mature oocytes (detected by the presence of an extruded polar body) are fertilized using intracytoplasmic sperm injection (ICSI). However, it has been demonstrated that when the sperm parameters are normal, ICSI may not always be required for the fertilization of oocytes collected through IVM[51]. Nevertheless, ICSI is generally performed on in-vitro matured oocytes because it reduces the risk of unexpected poor fertilization as compared with IVF. After ICSI, the oocytes are transferred into 1 ml of IVF medium in a tissue culture dish. Fertilization is determined 18 hours later by examining the oocytes for the appearance of two distinct pronuclei and two polar bodies.

Embryo transfer

The fertilized oocytes are further cultured up to day 2 or 3, following which an embryo transfer is performed. Prior to transfer, assisted hatching is performed to avoid reduced implantation due to a hardened zona pellucida. When a large number of embryos have formed, two alternatives are possible: a double transfer or a blastocyst transfer[52]. The former is performed on day 3 and repeated on day 5–6, while the latter involves extending the culture to the blastocyst stage at day 5 or 6 and transferring at that stage only. The embryo transfer technique is the same as that used for conventional IVF.

Endometrial preparation and luteal support

To achieve optimal endometrial growth, exogenous 17β-estradiol (micronized) is administered, starting on the day of oocyte retrieval. The dosage used depends on the endometrial thickness on the day of oocyte retrieval. When endometrial thickness is less than 6 mm, 12 mg a day is started; if the thickness is between 6 and 8 mm, then 10 mg a day is started; and if the thickness is more than 8 mm, then 6 mg is used – all in three divided doses. When an extremely thin endometrium (i.e. <4 mm) is recorded on ultrasound scan prior to collection, we have recently begun to administer estradiol treatment before oocyte collection. We are currently investigating an alternative approach whereby in-vitro matured oocytes are vitrified when the endometrial lining is very thin[53]. The endometrium is then prepared in an artificial cycle and, once it reaches a thickness of 8 mm, the oocytes are thawed, fertilized, and transferred. In an IVM treatment cycle, luteal support with daily intramuscular injections of progesterone in oil or Prometrium 200 mg (Schering Canada) three times per day, is started on the day that oocyte maturation is achieved and ICSI is performed. Estradiol and progesterone supplementation is continued until the twelfth week of pregnancy.

IVM OUTCOME

IVM pregnancy rates are correlated with the number of immature oocytes retrieved. The clinical pregnancy rate in women younger than 35 years, from whom we retrieved more than 10 immature GV oocytes, is 38% per cycle. As with IVF, clinical pregnancy and implantation rates decrease with increasing age[54]. In our practice, the clinical pregnancy rate for women younger than 35 years is 38% per oocyte retrieval and the implantation rate is 13%, while for women between 36 and 40 years old, the clinical pregnancy rate is 21% per retrieval and the implantation rate is 5%. Because of lower implantation rates, we transfer 1–2 more embryos compared with conventional IVF for women in comparable age groups without any increase in the multiple pregnancy rate.

In four centers performing IVM cycles, more than 1000 IVM cycles with hCG priming were done before oocyte collection, the pregnancy rates reached 30–35% and the implantation rates 10–15%[55].

In various published series, no increased rates of congenital malformations have been reported with IVM[56,57]. A recent analysis of the obstetric, neonatal, and infant outcome in our IVM conceptions showed a pregnancy outcome of 73% singleton, 24% twin, and 2.7% triplet. The median gestation age was 39 weeks for singletons and 37 weeks for multiple pregnancies. Similar reassuring results have been published by others[58].

IVM FOR OOCYTE DONATION

Oocyte donation is now a standard treatment for women who have diminished ovarian reserve and/or who are of advanced reproductive age; women affected by, or who are carriers of a significant genetic defect; and women with poor oocyte and/or embryo quality[59]. This method of treatment results in a high pregnancy rate for patients who would otherwise have a poor reproductive prognosis; the accumulated pregnancy rate may even increase up to 94.8% after four transfers[60]. Many potential oocyte donors may be deterred by the risk of OHSS, complications associated with oocyte collection, and concern about the inconvenience of a large number of hormone injections, as well as their possible long-term side-effects[61,62]. This perception has been supported by the results of a recent survey which indicate that three-quarters of potential donors declined after receiving information about the procedures involved[63]. Avoidance of

ovarian stimulation would obviously eliminate the associated risks to oocyte donors and would drastically reduce the costs of donation cycles[64]. The first reported IVM pregnancy was, in fact, conceived from immature oocytes retrieved and donated to a woman with premature ovarian failure[65]. At our center, 12 oocyte donors (age 29 ± 4) with high antral follicle counts (29.6 ± 8.7) underwent immature oocyte collection without ovarian stimulation. Out of a mean of 12.8 ± 5.1 GV oocytes collected, 68% matured and were fertilized using ICSI. Of a total of 47 embryos transferred to 12 recipients, six (50%) were successfully conceived, four of which have resulted in live births[66]. Based on the foregoing evidence, it would therefore appear that collecting immature oocytes from the unstimulated ovaries of oocyte donors is both prudent and worthwhile.

IVM AND PGD

Preimplantation genetic diagnosis (PGD) is a procedure that allows the in-vitro testing of embryos produced by couples who are potential carriers of an inherited disease or genetic defect, or by patients who have had three or more unexplained miscarriages. Patients can now select only those embryos diagnosed as being unaffected for implantation in the woman's uterus, thus improving the chance of a successful pregnancy. PGD patients are generally required to undergo IVF treatment in order to generate multiple embryos for genetic analysis. However, we have recently used IVM as an alternative for selected PGD patients with PCO/PCOS in order to avoid the side-effects of fertility drugs and to eliminate the risk of OHSS. We recently treated a 35-year-old patient with recurrent miscarriage who had unsuccessfully undergone two intrauterine insemination cycles and two IVF cycles in Germany. We collected 14 GV and 1 MII oocytes and biopsied the eight embryos generated. After the transfer of two normal embryos following aneuploidy screening, she became pregnant and we had the world's first live birth after combined IVM and PGD[67].

CONCLUSIONS

PCOS is the most common endocrinologic disorder among women of reproductive age as well as the most common cause of anovulatory infertility[15]. Patients with PCO or PCOS are particularly more prone to develop OHSS, with an incidence of up to 6%[9].

IVM is the maturation of oocytes in vitro from the GV stage to the MII stage of development. Because of the absence of OHSS and reduction in cost and complexity, IVM appears to be a promising treatment for infertility, especially for women with PCO or PCOS. The pregnancy rate in four centers performing IVM cycles in PCOS/PCO patients currently reaches 30–35%[54].

REFERENCES

1. Steptoe PC, Edwards RG. Birth after the preimplantation of a human embryo. Lancet 1978; 312: 366.
2. Tan SL, Royston P, Campbell S et al. Cumulative conception and livebirth rates after in-vitro fertilisation. Lancet 1992; 339: 1390–4.
3. Engmann L, Maconochie N, Bekir JS et al. Cumulative probability of clinical pregnancy and live birth after a multiple cycle IVF package: a more realistic assessment of overall and age-specific success rates? Br J Obstet Gynaecol 1999; 106: 165–70.
4. Brinsden PR, Wada I, Tan SL et al. Diagnosis, prevention and management of ovarian hyperstimulation syndrome. Br J Obstet Gynaecol 1995; 102: 767–72.
5. Child TJ, Phillips SJ, Abdul-Jalil AK et al. A comparison of *in vitro* maturation and *in vitro* fertilization for women with polycystic ovaries. Obstet Gynecol 2002; 100: 665–70.
6. Papanikolaou EG, Tournaye H, Verpoest W et al. Early and late ovarian hyperstimulation syn-

drome: early pregnancy outcome and profile. Hum Reprod 2005; 20: 636–41.

7. Semba S, Moriya T, Youssef EM et al. An autopsy case of ovarian hyperstimulation syndrome with massive pulmonary edema and pleural effusion. Pathol Int 2000; 50: 549–52.

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 ultrasonography. Am J Obstet Gynecol 2002; 186: 684–9.

9. MacDougall MJ, Tan SL, Balen A et al. A controlled study comparing patients with and without polycystic ovaries undergoing in-vitro fertilization. Hum Reprod 1993; 8: 233–7.

10. Balen AH, Laven JSE, Tan SL et al. Ultrasound Assessment of the Polycystic Ovary: International Consensus Definitions. Hum Reprod Update 2003; 9: 505–14.

11. Baker TG. Oogenesis and ovulation. In Austin CR, Short RV, eds. Reproduction in Mammals, 2nd edn. Cambridge University Press, Cambridge, 1982: 17–45.

12. Hreinsson J, Rosenlund B, Friden B et al. Recombinant LH is equally effective as recombinant hCG in promoting oocyte maturation in a clinical in vitro maturation programme: a randomized study. Hum Reprod 2003; 18: 2131–6.

13. Chian RC, Ao A, Clarke HJ et al. Production of steroids from human cumulus cells treated with different concentrations of gonadotropins during culture in vitro. Fertil Steril 1999; 71: 61–6.

14. The Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 2004; 19: 41–7.

15. Carmina E, Lobo RA. Polycystic ovary syndrome (PCOS): arguably the most common endocrinopathy is associated with significant morbidity in women. J Clin Endocrinol Metab 1999; 84: 1897–9.

16. Polson DW, Adams J, Wadsworth J et al. Polycystic ovaries – a common finding in normal women. Lancet 1988; 331: 870–2.

17. Abdel Gadir A, Khatim MS, Mowafi RS, Alnaser HM et al. Implications of ultrasonically diagnosed polycystic ovaries. I. Correlations with basal hormonal profiles. Hum Reprod 1992; 7: 453–7.

18. Abdel Gadir A, Khatim MS, Mowafi RS, Alnaser HM et al. Implications of ultrasonically diagnosed polycystic ovaries. II. Studies of dynamic and pulsatile hormonal patterns. Hum Reprod 1992; 7: 458–61.

19. Engmann L, Maconochie N, Sladkevicius P et al. The outcome of in-vitro fertilization treatment in women with sonographic evidence of polycystic ovarian morphology. Hum Reprod 1999; 14: 167–71.

20. Tan SL, Child TJ. In-vitro maturation of oocytes from unstimulated polycystic ovaries. Reprod Biomed Online 2002; 4(Suppl 1): 18–23.

21. McClure N, Healy DL, Rogers PA et al. Vascular endothelial growth factor as capillary permeability agent in ovarian hyperstimulation syndrome. Lancet 1994; 344: 235–6.

22. Buckett WM, Bouzayen R, Watkin KL et al. Ovarian stromal echogenicity in women with normal and polycystic ovaries. Hum Reprod 1999; 14: 618–21.

23. Agrawal R, Conway G, Sladkevicius P et al. Serum vascular endothelial growth factor and Doppler blood flow velocities in in vitro fertilization: relevance to ovarian hyperstimulation syndrome and polycystic ovaries. Fertil Steril 1998; 70: 651–8.

24. Zaidi J, Jacobs H, Campbell S et al. Blood flow changes in the ovarian and uterine arteries in women with polycystic ovary syndrome who respond to clomiphene citrate: correlation with serum hormone concentrations. Ultrasound Obstet Gynecol 1998; 12: 188–96.

25. Tan SL, Balen A, el Hussein E et al. The administration of glucocorticoids for the prevention of ovarian hyperstimulation syndrome in in vitro fertilization: a prospective randomized study. Fertil Steril 1992; 58: 378–83.

26. Awonuga AO, Pitroff R, Zaidi J et al. Elective cryopreservation of all embryos in women at risk of developing ovarian hyperstimulation syndrome may not prevent the condition but reduces the livebirth rate. J Assist Reprod Genet 1996; 13: 401–6.

27. Buckett WM, Chian RC, Tan SL. Can we eliminate severe OHSS? Not completely. Hum Reprod 2005; 20: 2367.

28. Homburg R. Management of infertility and prevention of ovarian hyperstimulation in women with polycystic ovary syndrome. Best Pract Res Clin Obstet Gynaecol 2004; 18: 773–88.

29. Trounson A, Wood C, Kausche A. In vitro maturation and the fertilization and developmental competence of oocytes recovered from untreated polycystic ovarian patients. Fertil Steril 1994; 62: 353–62.

30. Barnes FL, Crombie A, Gardner DK et al. Blastocyst development and birth after in-vitro maturation of human primary oocytes, intracytoplasmic sperm injection and assisted hatching. Hum Reprod 1995; 10: 3243–7.

31. Suikkari AM, Tulppala M, Tuuri T et al. Luteal phase start of low-dose FSH priming of follicles results in an efficient recovery, maturation and fertilization of immature human oocytes. Hum Reprod 2000; 15: 747–51.

32. Cavilla JL, Kennedy CR, Baltsen M et al. The effects of meiosis activating sterol on in-vitro maturation and fertilization of human oocytes from stimulated and unstimulated ovaries. Hum Reprod 2001; 16: 547–55.

33. Barnes FL, Kausche A, Tiglias J et al. Production of embryos from in vitro-matured primary human oocytes. Fertil Steril 1996; 65: 1151–6.

34. Trounson A, Anderiesz C, Jones GM et al. Oocyte maturation. Hum Reprod 1998; 13(Suppl 3): 52–62.

35. Cha KY, Han SY, Chung HM 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.

36. Paulson RJ, Sauer MV, Francis MM et al. Factors affecting pregnancy success of human in vitro fertilization in unstimulated cycles. Hum Reprod 1994; 9: 1571–5.

37. Thornton MH, Francis MM, Paulson RJ. Immature oocyte retrieval: lessons from unstimulated IVF cycles. Fertil Steril 1998; 70: 647–50.

38. Chian RC, Buckett WM, Abdul Jalil AK et al. Natural-cycle in vitro fertilization combined with in vitro maturation of immature oocytes is a potential approach in infertility treatment. Fertil Steril 2004; 82: 1675–78.

39. Wynn P, Picton HM, Krapez JA et al. Pretreatment with follicle stimulating hormone promotes the numbers of human oocytes reaching metaphase II by in-vitro maturation. Hum Reprod 1998; 13: 3132–8.

40. Mikkelsen AL, Smith SD, Lindenberg S. In vitro maturation of human oocytes from regularly menstruating women may be successful without follicle stimulating hormone priming. Hum Reprod 1999; 14: 1847–51.

41. Trounson A, Anderiesz C, Jones G. Maturation of human oocytes in vitro and their developmental competence. Reproduction 2001; 121: 51–75.

42. Chian RC, Park SE, Park EL et al. Molecular and structural characteristics between immature human oocytes retrieved from unstimulated ovaries. In Gomel V, Leung PCK, eds. In vitro Fertilization and Assisted Reproduction. Bologna: Monduzzi, 1997: 315–19.

43. Chian RC, Buckett WM, Too LL et al. Pregnancies resulting from in vitro matured oocytes retrieved from patients with polycystic ovary syndrome after priming with human chorionic gonadotropin. Fertil Steril 1999; 72: 639–42.

44. Chian RC, Gulekli B, Buckett WM et al. 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.

45. Chian RC, Buckett WM, Tulandi T et al. 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.

46. Chian RC, Gulekli B, Buckett WM 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: 1700–2.

47. Hwang JL, Lin YH, Tsai YL et al. Oocyte donation using immature oocytes from a normal ovulatory

woman. Acta Obstet Gynecol Scand 2002; 81: 274–5.

48. Nagele F, Sator MO, Juza J et al. Successful pregnancy resulting from in-vitro matured oocytes retrieved at laparoscopic surgery in a patient with polycystic ovary syndrome: case report. Hum Reprod 2002; 17: 373–4.

49. Son WY, Yoon SH, Lee SW et al. Blastocyst development and pregnancies after IVF of mature oocytes retrieved from unstimulated patients with PCOS after in-vivo HCG priming. Hum Reprod 2002; 17: 134–6.

50. Buckett WM, Chian RC, Tan SL. Human chorionic gonadotropin for in vitro oocyte maturation: does it improve the endometrium or implantation? J Reprod Med 2004: 49: 93–8.

51. Soderstron AV, Makinen S, Tuuri T et al. Favourable pregnancy results with insemination of in vitro matured oocytes from unstimulated patients. Hum Reprod 2005; 20: 1534–40.

52. Philips SJ, Dean NL, Buckett WM et al. Consecutive transfer of day 3 embryos and of day 5–6 blastocyst increases overall pregnancy rates associated with blastocyst culture. J Assist Reprod Genet 2003; 20: 461–4.

53. Chian RC, Kuwayama M, Tan L et al. High survival rate of bovine oocytes matured in vitro following vitrification. J Reprod Dev 2004; 50: 685–96.

54. Chian RC. In-vitro maturation of immature oocytes for infertile women with PCOS. Reprod Biomed Online 2004; 8: 547–52.

55. Mikkelsen AL, Andersson AM, Skakkebaek NE et al. Basal concentrations of oestradiol may predict the outcome of in-vitro maturation in regularly menstruating women. Hum Reprod 2001; 16: 862–7.

56. Buckett WM, Chian RC, Holzer H et al. Congenital abnormalities and perinatal outcome in pregnancies following IVM, IVF, and ICSI delivered in a single center. 61st Annual Meeting of ASRM, Montreal, Canada. Fertil Steril 2005; 84(Suppl 1): S80–1.

57. Buckett WM, 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. 60th Annual Meeting of ASRM, Philadelphia, USA, Fertil Steril 2004; 82(Suppl 2): S133.

58. Cha KY, Chung HM, Lee DR et al. Obstetric outcome of patients with polycystic ovary syndrome treated by in vitro maturation and in vitro fertilization–embryo transfer. Fertil Steril 2005; 83: 1461–5.

59. The American Society for Reproductive Medicine. Guidelines for oocyte donation. Fertil Steril 2004; 82: s13–15.

60. Remohi J, Gartner B, Gallardo E et al. Pregnancy and birth rates after oocyte donation. Fertil Steril 1997; 67: 717–23.

61. The American Society for Reproductive Medicine. Repetitive oocyte donation. Fertil Steril 2004; 82: s158–9.

62. Bennett SJ, Waterstone JJ, Cheng WC et al. Complications of transvaginal ultrasound-directed follicle aspiration: a review of 2670 consecutive procedures, J Assist Reprod Genet 1993; 10: 72–7.

63. Murray C, Golombok S. Oocyte and semen donation: a survey of UK licensed centres. Hum Reprod 2000; 15: 2133–9.

64. Scharf E, Chian RC, Abdul Jalil K et al. In vitro maturation of oocytes: a new option for donor oocyte treatment. Fertil Steril 2004; 82(Supp 2): 514.

65. Cha KY, Koo JJ, Ko JJ et al. Pregnancy after in vitro fertilization of human follicular oocytes collected from nonstimulated cycles, their culture in vitro and their transfer in a donor oocyte program. Fertil Steril 1991; 55: 109–13.

66. Holzer H, Chian RC, Scharf E et al. IVM oocyte donors: oocyte donation without ovarian stimulation. Fertil Steril (submitted).

67. Ao A, Jin S, Rao D et al. First successful pregnancy outcome after preimplantation genetic diagnosis for aneuploidy screening in embryos generated from natural-cycle in vitro fertilization combined with an in vitro maturation procedure. Fertil Steril 2006; 85: 1510 e9–e11.