CHAPTER 19

Immature oocyte collection

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INTRODUCTION

In-vitro maturation (IVM) of immature oocytes retrieved from women without any ovarian stimulation is a promising new treatment especially for women with polycystic ovary syndrome (PCOS) and/or polycystic ovaries (PCO), with many successful pregnancies reported worldwide. Although Cha et al.[1] reported the first birth using IVM of immature oocytes collected at cesarean section within an oocyte donation program in 1991, in 1994 it was Trounson and colleagues[2] who put IVM into clinical practice when they reported the first pregnancy using a woman's own immature oocytes collected by transvaginal ultrasound-guided follicle aspiration from a patient with polycystic ovaries.

Although the classic indication for IVM is PCOS/PCO, it is also for other indications today: poor responders, fertility preservation of cancer patients, normal responders with a history of poor oocyte/embryo quality, as well as for oocyte donation.

DEVELOPMENT OF OOCYTE RETRIEVAL FOR IVF

Before the first successful birth following in-vitro fertilization (IVF) and embryo transfer, the first culture and maturation of human oocytes in vitro were carried out on oocytes that were obtained by laparotomy. Similar experiments were done by Steptoe and Edwards and they introduced a laparoscopic method for aspirating oocytes from the ovarian follicles[3]. Laparoscopic oocyte retrieval may be performed either by the 2- or 3-puncture technique depending on the type of laparoscope and the experience of the surgeon. Each follicle is punctured and aspirated at an avascular site (Figure 19.1). The needle may be left in the follicle to permit flushing and re-aspiration in case an oocyte is not identified in the initial aspirate.

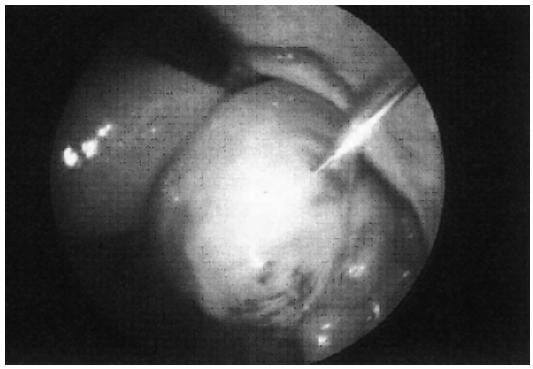

Figure 19.1 Laparoscopic oocyte retrieval (see color plate section)

Following those first few births via laparoscopically aspirated oocytes, ultrasound-guided oocyte collection techniques were described. While laparoscopic oocyte retrieval is generally performed under general anesthesia with endotracheal intubation, the major advantages for the ultrasound-guided oocyte retrieval include decreased exposure to general anesthesia, lower risk for operative complications, and feasibility of performing on an outpatient basis. In addition, laparoscopy identifies visible follicles on the ovarian surface whereas ultrasonography identifies intraovarian follicles, as well.

There are many different ultrasound-guided oocyte collection techniques. The first follicular puncture under transabdominal ultrasound guidance was described in 1981 and the same group introduced transabdominal transvesical ultrasound-directed oocyte recovery for the oocytes from the ovarian follicles a year later (Figure 19.2)[4,5]. Transabdominal oocyte retrieval under ultrasound guidance is still indicated in women with vaginal/müllerian agenesis[6]. Oocyte retrieval for IVF by ultrasonically guided needle aspiration via the urethra was proposed by Parsons and coworkers in 1985 (Figure 19.3)[7]. The above techniques were in routine use during the days of conventional external ultrasound transducers. Gleicher et al.[8] described the first oocyte retrieval via the vaginal route; today's method using the transvaginal approach for both the scanning and the oocyte retrieval was introduced by Wickland et al.[9] and has become the worldwide method for oocyte recovery[10].

CURRENT TECHNIQUE FOR IVF OOCYTE RETRIEVAL

Anesthesia/analgesia

Today almost all oocyte retrievals are performed transvaginally. The transvaginal route is used both for the scanning and the retrieval. Generally, spinal or epidural anesthesia may be used for the procedure; however, in most cases intravenous sedation, opioid analgesia, and cervical blockage with local anaesthesia is sufficient for effective

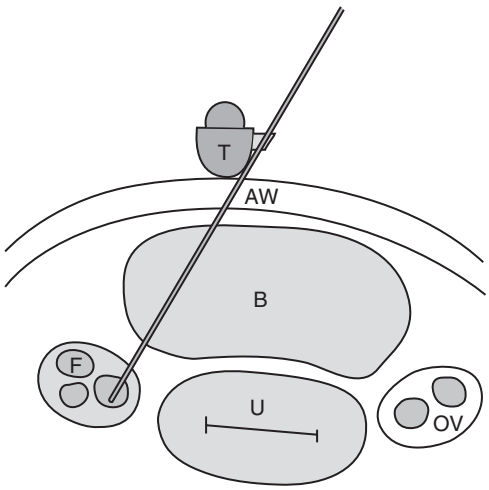

Figure 19.2 Transabdominal ultrasound-guided oocyte retrieval
T, transducer; AW, abdominal wall; B, bladder; F, follicle; U, uterus; OV, ovary

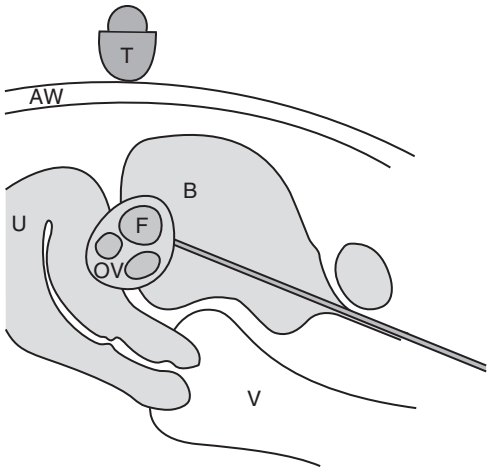

Figure 19.3 Transurethral oocyte retrieval under transabdominal ultrasound guidance
T, transducer; AW, abdominal wall; B, bladder; F, follicle; OV, ovary; U, uterus; V, vagina

pain relief. In the McGill Reproductive Center, 1–2 mg midazolam for intravenous sedation, 25 mg fentanyl every 10 minutes or according to the needs of the patient for analgesia, and 0.5% bupivacaine 10–20 ml for the cervical block are used and effective pain relief is maintained in most of the patients. Rarely, non-steroid anti-inflammatory drugs alone may provide sufficient analgesia in cases with a few follicles or in natural IVF cycles where there is only one follicle to be aspirated.

Cleaning and antisepsis

Normal saline is used for vaginal cleaning before the oocyte retrieval. When compared to vaginal cleaning with povidon iodine, no increase in infection risk is observed. However, povidon iodine may decrease the pregnancy rate[11], probably by an adverse effect on oocytes. Routine antibiotic prophylaxis is not recommended, however it may be administered in selected cases with endometriomas or hydrosalpinges.

Materials needed for the IVF oocyte retrieval

An ultrasound scanner with a 6 MHz or 7.5 MHz transvaginal probe, a sterile condom or plastic sheath to cover the probe, sterile ultrasound gel, a needle guide, a 15 or 16G single/double lumen aspiration needle, suction pump, test tubes, 1% heparinized saline, a heater block for the tubes, an inverted microscope plus heating plate, and petri dishes for oocyte identification in the laboratory are the materials necessary for an IVF oocyte retrieval. The test tubes, heparinized saline, and petri dishes are preheated to 37°C before the collection.

Needles

Single or double lumen needles of 15/16G are used for aspiration. Double lumen needles allow the follicle to be flushed through a separate route. Although routine flushing does not offer any advantage in terms of the number of retrieved oocytes, it may be needed in cases with one or a few follicles.

Ultrasound technique

Transvaginal scanning and oocyte retrieval is the routine oocyte retrieval method today. The procedure is easy to learn for physicians who have already been trained in transvaginal ultrasound examination. A 9.3 MHz transvaginal transducer is used for oocyte retrieval in our center. The high resolution is particularly helpful in IVM retrieval in which small follicles of less than 10 mm in diameter are aspirated. In IVF oocyte retrievals 6 MHz or 7.5 MHz ultrasound transducers and two-dimensional visualization are successfully used. Doppler or three-dimensional ultrasound may be helpful, but they are not essential.

Aspiration technique

The transvaginal ultrasound guided follicular aspiration technique has become the method of choice because of refinements in the instrumentation and the clinicians' growing experience. Over the past few years automatic aspiration and washing systems have been introduced and the follicles can be aspirated and, if necessary, washed with various systems.

The needle is first introduced in the follicle closest to the transducer through the vaginal vault with controlled stabbing, resembling popping a balloon. Vacuum pressure should be less then 150 mmHg to aspirate the follicular fluid and a suction pump is used to maintain the constant vacuum pressure. The tip of the needle is echogenic, so it can be watched on the ultrasound screen while the follicular fluid is aspirated. When follicular walls collapse onto the needle, the needle can be retracted and rotated to aspirate all the follicular fluid (Figure 19.4). Then the next follicle is stabbed and the needle is gently moved from one follicle to another

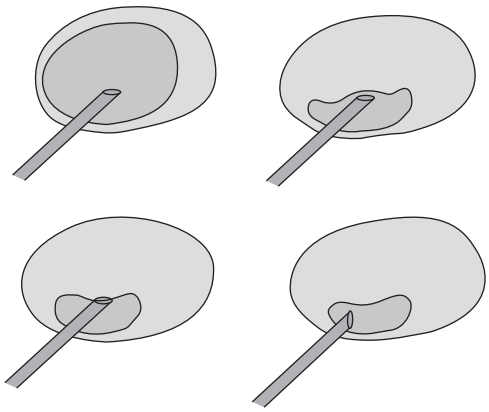

Figure 19.4 IVF oocyte retrieval: aspiration technique

after fully aspirating each follicle. Usually, only a single ovarian puncture is needed to aspirate all the follicles in one ovary. When all the follicles of one ovary have been aspirated the needle is withdrawn and flushed with the handling medium. If the needle is withdrawn before all the follicles in the ovary have been aspirated, it is flushed before puncturing the ovary a second time. The same procedure is repeated for the other ovary.

Flushing is not routinely recommended because no apparent improvement has been observed in the pregnancy rate[12]. However, as noted above, it may be needed in patients with few follicles.

Once a follicle has been aspirated the collection tube is handed over to the laboratory staff. If no oocyte is identified in the aspirate then the follicle may be flushed with the handling medium. Although the flushing can also be done with a single lumen needle, the oocyte may move back and forth in the dead space of the needle by repeated flushes and aspirations. Therefore it is recommended to use a double lumen needle in cases with a few follicles where flushing may be needed. The flushing volume should not exceed the aspiration volume in order not to rupture the follicle. Flushing can be done manually by using a syringe or by an automatic pump.

Heparinized saline or heparinized culture medium is used as the handling medium since there is no significant difference in fertilization rates between oocytes obtained after saline and culture medium flushing[13].

Risks

The complications of ultrasonically guided transvaginal oocyte collection are rare. Infection risk is around 0.25%. Serious pelvic infection – such as pelvic or ovarian abscess – is seen almost exclusively in women with either severe tubal disease or severe endometriosis and endometriomas which have been inadvertently punctured during oocyte retrieval. Serious intraperitoneal or vaginal bleeding is even rarer – with an estimated risk of 0.01%. Laparoscopy and very rarely laparotomy have been reported in cases of severe intra-abdominal bleeding[14].

DEVELOPMENT OF IVM

Today IVM has started to be routinely offered either to patients with PCOS or to normo-ovulatory patients with a high risk of developing OHSS (i.e. patients with PCO). As noted above, it has also been used for other indications: poor responders, fertility preservation in cancer patients, normal responders with a history of poor oocyte/embryo quality, and oocyte donation cycles. In these conditions immature oocyte retrieval may be performed in the presence of a few follicles. The only significant factor predicting the number of oocytes collected in unstimulated cycles is the antral follicle count[15]. Although the oocyte recovery rate in stimulated cycles is 70–80%, it is usually about 50% in IVM cycles and this oocyte recovery rate depends on the experience of the surgeon[16]. However, the reason could be that not all the follicles may be easily aspirated and/or oocytes

may not be recovered from each follicle aspirated. Therefore good ultrasonographic visualization, accessibility of the ovaries, and the retrieval technique are even more important, particularly for those with a low antral follicle count.

IVM is improved by hCG priming[17]; hCG priming does not seem to increase the number of oocytes retrieved, but it reduces the time needed for IVM[18]. The dose of hCG was studied in a prospective, randomized, double blind study by our group and increasing the dose did not give any benefit in any of the outcomes, therefore current evidence suggests a benefit from 10 000 IU hCG administered 36 h before the collection[19].

In a typical IVM cycle all the follicles had to be <10 mm in diameter because data suggest that the presence of a dominant follicle at the time of immature oocyte retrieval is deleterious to the outcome in IVM[20,21]. The maximum diameter of the follicle on the day of oocyte recovery provides one of the main differences between IVF and IVM cycles. However, in natural cycle IVF/IVM treatments, in spite of the presence of a dominant follicle, where a mature oocyte is expected to be retrieved, immature oocytes have also been collected and matured in vitro and pregnancies and live births have been obtained[22]. The administration of short-term gonadotropins and hCG priming when the follicles reach 12–14 mm in diameter has also been suggested as another approach to IVM[23]. The combination of natural cycle IVF with IVM, or the short-term use of gonadotropin administration before immature oocyte retrieval, are modifications currently under investigation, and the criteria for their use are not yet well established[22,24].

Nevertheless, immature oocytes from follicles less than 10 mm in diameter should still be collected, even with these modifications. Thus in IVM collections, sometimes one or more follicles may be larger than 10 mm, and mature oocytes may be recovered from these follicles.

CURRENT TECHNIQUES FOR IVM OOCYTE RETRIEVAL

TVS-guided follicular aspiration has now become the preferred procedure of choice for oocyte retrieval in IVM cycles; it requires certain modifications compared to conventional IVF oocyte retrieval.

Anesthesia/analgesia

The mode of anesthesia is decided according to the accessibility of the ovaries. Although the initial cases were performed under general or spinal anesthesia, this is not needed for most cases.

Intravenous sedation with 2 mg midazolam and 50–200 mg fentanyl, and paracervical block with 20 ml of 1% bupivacaine, are used for immature egg retrieval in the McGill Reproductive Center. Intravenous propofol infusion may also be added in certain patients. However, because of the multiple ovarian punctures often needed in IVM, the immature oocyte retrieval is likely to be more painful than a conventional IVF oocyte collection. It has been shown that reducing the size of the needle used for oocyte collection from 15G (the standard IVF needle) to 19/20G reduces the pain without affecting the number of the oocytes collected.

Consequently, in patients who have had previous poor pain relief during oocyte retrieval or those where ovarian access is difficult, a general or spinal anesthesia may be more appropriate and, in our experience, is needed more frequently than for conventional IVF collections.

Cleaning and antisepsis

The same principles applied to IVF oocyte retrieval are also valid for IVM patients. Therefore the vagina is cleaned with sterile saline. In women at increased risk of pelvic sepsis – such as those with severe tubal disease or endometriosis – antibiotic prophylaxis is appropriate. It is

unclear whether there is a role for routine antibiotic prophylaxis.

Collection needle

As discussed above, a smaller gauge needle (19G or 20G) is preferable. This causes less pain and less damage to the smaller follicles, thereby allowing greater numbers of immature oocytes to be collected. However, the finer gauge needles are more susceptible to blockage with stromal tissue or blood clots, and therefore require frequent removal and flushing through with fluid. Similarly, if ovarian access is difficult and the needle needs to pass through uterine tissue, care needs to be taken in order to avoid bending.

Aspiration pressure

Because the intrafollicular pressure is already higher in small follicles, the aspiration vacuum pressure is reduced to 75–80 mmHg, which is approximately half the conventional IVF aspiration pressure. A higher aspiration pressure provokes an increase in the number of denuded oocytes.

Tubes and blocks

The aspiration tubes are prepared with 2 ml heparinized saline before collection in the warm blocks. Heparinized saline is also used as the aspiration medium for flushing the needle. Some centers use IVM culture media rather than heparinized saline in the tubes and for flushing.

Aspiration technique

In unstimulated ovaries the follicles are small and often widespread throughout the ovarian stroma (Figure 19.5). Furthermore, polycystic ovaries are frequently mobile and external abdominal pressure and intravaginal pressure with the probe may be needed to fix the ovary in IVM oocyte retrieval.

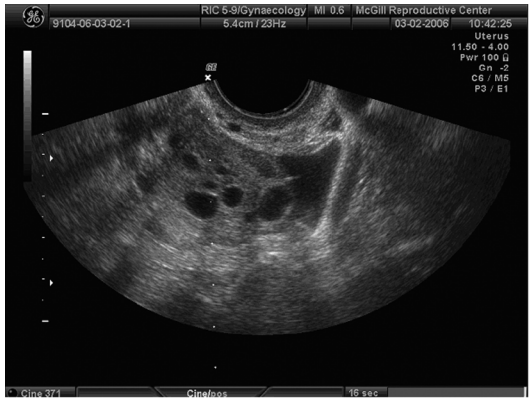

Figure 19.5 Ovary prior to commencing IVM collection. Note the widespread follicles throughout the stroma ranging from 4 mm to 11 mm mean diameter. A good quality ultrasound machine is needed at high magnification

The needle is introduced into the follicle with the bevel facing the larger part of the follicle, however it is probably less important than first thought. Because the needle slips easily into the surrounding stroma it should be stabbed into the follicle at 90° to the follicle wall. Also the needle is frequently removed to re-align it with the small follicles. The follicle should be completely emptied; rotating the needle could be of help. Although only a single puncture of the ovary is generally enough for IVF oocyte retrieval, multiple ovarian punctures are generally needed for IVM retrieval. The reason for this is that it is almost always impossible to reach all the follicles from the same puncture site (Figures 19.6, 19.7, and 19.8). In addition, the volume of the fluid aspirated from the follicles is very small and the single lumen aspiration needle tends to block frequently when passing through the dense ovarian stroma and follicular flushing is not performed. So, the needle is generally withdrawn after several follicles have been aspirated, and it is flushed with the heparinized saline. The collection takes, on average, longer than IVF oocyte retrieval because of the repeated flushing

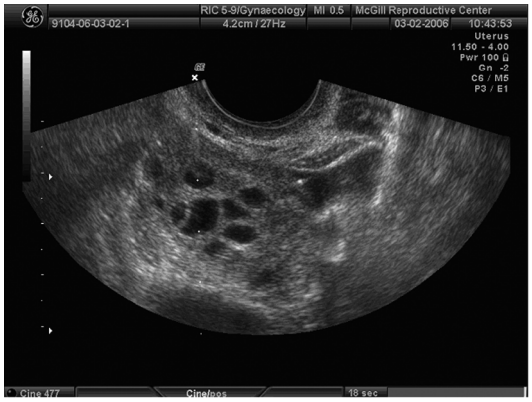

Figure 19.6 The commencement of an IVM oocyte retrieval. The needle tip is within the first follicle (6 mm) and a further three follicles can be aspirated on this puncture

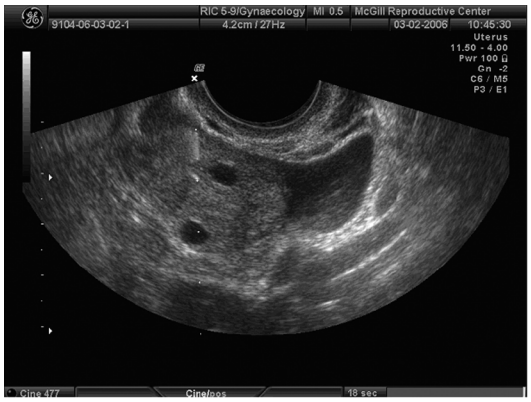

Figure 19.7 Towards the completion of one side. Two further antral follicles remain which will need to be aspirated with separate punc-

of the needle and the tubing in order to prevent the blockage[25,26].

Good ultrasonographic visualization is the key point for successful immature oocyte retrieval. Color-flow Doppler can help to differentiate intra-ovarian vessels from small antral follicles. The follicular sizes vary and certain follicles may be difficult to aspirate or, even if they are aspirated, no oocytes may be recovered, especially from the very small size follicles (<4 mm).

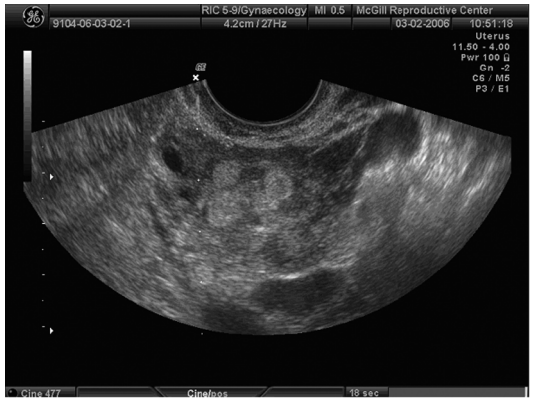

Figure 19.8 Final follicle (6 mm) to be aspirated. Note the hematomas in the previously emptied follicles

Follicles are isolated from follicular aspirates collected in tubes containing heparinized saline in the laboratory by using a stereomicroscope. The follicular aspirate is then filtered and rechecked for oocytes.

CONCLUSIONS

Although IVM oocyte collections may appear more challenging than conventional IVF oocyte collections, following the above guidelines and with appropriate experience they are relatively straightforward. The learning curve is relatively short and highly dependent on the clinician's already established skills in conventional IVF. The current techniques have been described here. However, one would expect further refinements over the next few years.

The basic principles for IVM oocyte retrieval can be summarized as the following:

- Adequate analgesia
- Multiple ovarian puncture
- Fine-bore aspiration needle
- Good quality ultrasound
- Frequent flushing of the needle and tubing.

REFERENCES

1. 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.
2. Trounson AC, Wood C, Kausche A. In vitro maturation and the fertilization and developmental competence of oocytes recovered from untreated polycystic ovarian patients. Fertil Steril 1994; 64: 353–62.
3. Steptoe PC, Edwards RG. Birth after the reimplantation of a human embryo. Lancet 1978; 312: 366.
4. Lenz S, Lauritsen JG, Kjellow M. Collection of human oocytes for in vitro fertilisation by ultrasonically guided follicular puncture. Lancet 1981; 317: 1163–4.
5. Lenz S, Lauritsen JG. Ultrasonically guided percutaneous aspiration of human follicles under local anesthesia: a new method of collecting oocytes for in vitro fertilization. Fertil Steril 1982; 38: 673–7.
6. Damario MA. Transabdominal–transperitoneal ultrasound-guided oocyte retrieval in a patient with mullerian agenesis. Fertil Steril 2002; 78: 189–91.
7. Parsons J, Riddle A, Booker M et al. Oocyte retrieval for in-vitro fertilisation by ultrasonically guided needle aspiration via the urethra. Lancet 1985; 325: 1076–7.
8. Gleicher N, Friberg J, Fullan N et al. EGG retrieval for in vitro fertilisation by sonographically controlled vaginal culdocentesis. Lancet 1983; 322: 508–9.
9. Wikland M, Enk L, Hamberger L. Transvesical and transvaginal approaches for the aspiration of follicles by use of ultrasound. Ann NY Acad Sci 1985; 442: 182–94.
10. Sunde A, von During V, Kahn JA et al. IVF in the Nordic countries 1981–1987: a collaborative survey. Hum Reprod 1990; 5: 959–64.
11. van Os HC, Roozenburg BJ, Janssen-Caspers HA et al. Vaginal disinfection with povidon iodine and the outcome of in-vitro fertilization. Hum Reprod 1992; 7: 349–50.
12. Kingsland CR, Taylor CT, Aziz N et al. Is follicular flushing necessary for oocyte retrieval? A randomized trial. Hum Reprod 1991; 6: 382–3.
13. Biljan MM, Dean N, Hemmings R et al. Prospective randomized trial of the effect of two flushing media on oocyte collection and fertilization rates after in vitro fertilization. Fertil Steril 1997; 68: 1132–4.
14. Dicker D, Ashkenazi J, Feldberg D et al. Severe abdominal complications after transvaginal ultrasonographically guided retrieval of oocytes for in vitro fertilization and embryo transfer. Fertil Steril 1993; 59: 1313–15.
15. Tan SL, Child TJ. In-vitro maturation of oocytes from unstimulated polycystic ovaries. Reprod Biomed Online 2002; 4(Suppl 1): 18–23.
16. 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.
17. 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.
18. 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.
19. Gulekli B, Buckett WM, Chian RC et al. 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.
20. Russell JB. Immature oocyte retrieval combined with in-vitro oocyte maturation. Hum Reprod 1998; 13(Suppl 3): 63–70, 71–5.
21. Cobo AC, Requena A, Neuspiller F et al. Maturation in vitro of human oocytes from

unstimulated cycles: selection of the optimal day for ovum retrieval based on follicular size. Hum Reprod 1999; 14: 1864–8.

22. 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: 1675–8.

23. Lim KS. IVM/F-ET in stimulated cycles for the prevention of OHSS. Fertil Steril 2002; 78(Suppl 1): S11.

24. Chian RC, Buckett WM, Tan SL. In-vitro maturation of human oocytes. Reprod Biomed Online 2004; 8: 148–66.

25. Child TJ, Abdul-Jalil AK, Sulekli B et al. 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.

26. 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.