47 Follicle aspiration

Carl Wood

The initial studies on the maturation of human oocytes *in vitro* were carried out on oocytes that were obtained when ovaries or pieces of ovaries were acquired by laparotomy.[1] By 1970, Steptoe and Edwards had developed a laparoscopic method for aspirating oocytes from their graafian follicles that yielded oocytes from about one-third of follicles.[2] Initially, they used a needle and syringe to provide the suction, but later they developed an aspiration device which provided continuous suction, with control being exerted by the assistant's finger on the bypass valve. A similar technique using a Venturi system activated by a foot-operated "on-off" valve was utilized by Lopata *et al.*[3]

Following the first few births from *in vitro* fertilization (IVF) and embryo transfer, attention was focused on the instruments used for oocyte recovery. Equipment, including a variety of needles and regulated aspiration pumps, became commercially available in the early 1980s: for example, a Teflon-coated needle was developed that resulted in oocyte collection rates of > 90%.[4] The next major development was the change from laparoscopic to transvaginal ultrasound-guided aspiration.[5]

Experimental and physical aspects of oocyte retrieval

Apart from the comparison of manual and mechanical suction on the effect of zonal damage,[6] surprisingly little has been published on the theory of oocyte collection until the studies performed by Cook Medical Technology, Brisbane.[7]

A number of factors may affect oocyte collection and/or damage the ova. These include variables such as pump vacuum flow, velocity, needle lumen size and length, follicular pressure and size, and collection techniques. In order to study factors influencing the success of oocyte collection and the cause of trauma to oocytes, Cook Medical Technology, Brisbane, developed appropriate equipment.[7]

Velocity and flow rate through the needle and attached lines were calculated from the pressure difference between the collection tube and the needle tip. The velocity and flow rate were slightly underestimated, especially at the moment the needle punctured the wall of the follicle. The studies were done on bovine follicles.

Application of vacuum to a follicle

Vacuum applied after needle entry into the follicle

Upon the application of vacuum, the vacuums throughout the system equilibrate to steady flow conditions, the period of time depending on the follicle volume, the vacuum used and the capacity of the needle. During this time the follicle wall collapses as the fluid volume decreases until the follicle totally collapses and blocks the needle tip.

Maximum flow is achieved during the steady state, then slows dramatically as the follicle collapses, blocking the needle tip. In some cases the fluid continues to flow very slowly up the needle, possibly owing to air being sucked into the follicle around the point of entry of the needle (the system was not fully closed).

Vacuum deactivated before exit from the follicle

Changes in vacuum and flow occur if the regulated vacuum is discontinued while the needle tip is still in the follicle, providing the system remains closed (there are no air leaks). After the pump is deactivated and the pressure in the collection tube returns to atmospheric, there is a backflow of fluid towards the follicle. The magnitude of the backflow depends on (i) how much air enters the system, and (ii) the height of the collection tube above the needle tip.

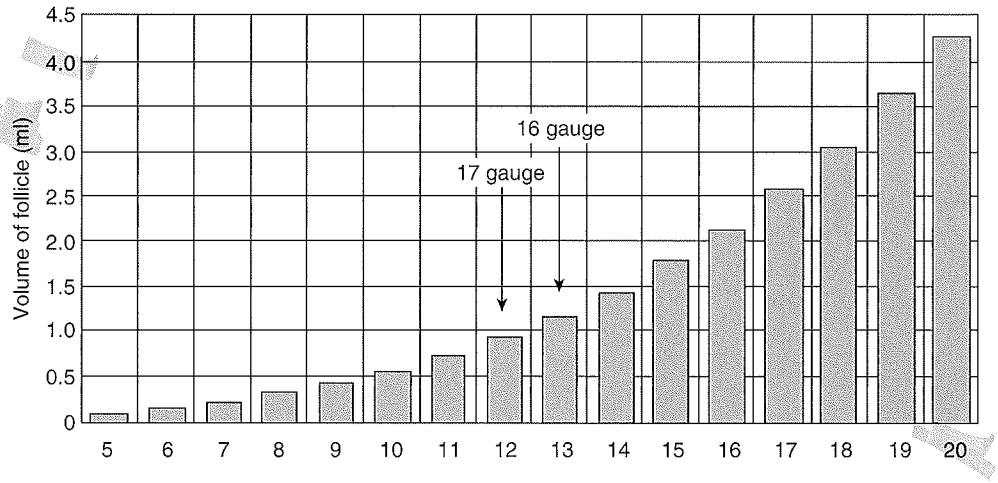

Fig 47.1 Relation between diameter of follicle and volume of follicle. Arrows indicate the internal volume of standard 16-gauge and 17-gauge needles and lines (100 cm length).

Vacuum activated and deactivated outside the follicle

A comparison of flow rates during aspiration of a 15 mm follicle, where both activation and deactivation of the vacuum occur outside the follicle-shows that flow decreases as the follicle collapses, but there is a sudden rapid flow towards the collection tube as the needle is withdrawn from the follicle. This may assist in emptying the follicle. This may be the best technique.

Damage to oocytes

The effects of flow rates and maximum velocities achieved in the aspiration system using a 17 gauge needle at various vacuums on the morphology of the oocyte cumulus mass show that all oocytes have lost their cumulus mass after aspiration at 20 kPa (150 mmHg).

Vacuum profiles in aspiration system

Usually, for oocyte collection, the vacuum in the collection system is < 20 kPa (150 mmHg). It is estimated that, using 20 kPa (150 mmHg), it will take up to five seconds for the system described above to stabilize to the selected vacuum.

Large follicles have a small positive pressure of 3.75–75 mmHg. Follicular pressure is dependent on the size (and hence the maturity), shape and position of the follicle, and pressure increases with increases follicular size.

The pressure of the fluid in the follicle at the moment of penetration of the needle may be much higher than the normal follicular pressure. As the needle tip is being forced into the wall, the deformation of the surface of the follicle will cause the pressure to rise. The more blunt the needle, the higher the pressure will become (up to 60 mmHg) and consequently, the larger the amount of fluid that spurts out of the follicle when punctured. Some of this fluid will flow up the needle, while the remainder escapes between the outer needle wall and the follicular wall. If a vacuum has already been applied before the needle punctures the follicle, little follicular fluid is lost.

Follicular and needle volumes

With an increased interest in oocyte collection from immature follicles, it is important to note that the volume of such follicles is small (Fig 47.1). For example, an immature follicle with a diameter of 5 mm has a volume of ~0.065 ml. It would take the contents of over 17 such follicles to fill the lumen of a standard 16 gauge needle and line (total length 100 cm).

Application of vacuum

Once a needle punctures the follicle, the pressures within the follicle and needle equilibrate. The follicular wall will generally make a tentative seal around the needle and, in the absence of an applied vacuum, resistance within the needle will bring the fluid to rest. As the regulated vacuum is applied, the vacuums throughout the system tend to equilibrate to steady flow conditions.

In the above situation, where a good seal exists around the needle tip, if the regulated vacuum is discontinued while the needle tip is still in the follicle,

there is a backflow of fluid toward the follicle. In this closed system the magnitude of the reverse flow is similar to the maximum flow toward the collection tube but only lasts for a fraction of a second and slows rapidly.

If the needle is withdrawn from the follicle while the vacuum is still applied, there is a dramatic surge of fluid toward the collection tube. The needle tip goes from the high vacuum of the follicle to atmospheric pressure. If the oocyte is contained in the last fraction of the collected follicular fluid, or comes from an immature follicle where the volume is small, it can be subjected to speeds well above those expected. It can also be subjected to increased turbulence in both the needle and the collection tube.

Damage within the needle/vacuum lines

There is a vacuum gradient down the collection system, with the vacuum at the needle tip being only 5% of that selected at the vacuum pump. The ovum is therefore exposed to an ever-increasing vacuum during its travel along the collection system. This may cause the ovum to swell and the zona to crack.

High velocities may strip the cumulus of the oocyte. Even in laminar flow, there will be a significant difference between the velocity of the fluid in the centre of the needle and that towards the periphery. Thus the outer layers of the cumulus may be subjected to "drag," which may strip them.

The longer the needle, or the smaller its internal diameter, the greater the vacuum required to maintain the same velocity and the greater the risk of damaging the oocyte. If turbulent flow is present the ovum may be tossed about, which could result in either stripping off of the cumulus or cracking of the zona.

Damage within the follicle

The ovum has to be accelerated from a resting state within the follicle to the velocity of the fluid within the needle. Moreover, it has to accelerate to this velocity as it enters the needle tip. This rapid acceleration may strip off the cumulus. In theory, this damaging effect should be greatest in smaller follicles, especially immature ones, where there may be some adherence of oocytes, necessitating the use of higher suction vacuums. Additionally, the oocyte will be drawn closer to the needle tip as the follicle collapses. This means that it could be subjected to an increasing accelerative force once it detaches from the wall. This may cause the cumulus to tear off from the oocyte. In addition, there is a rapid increase in vacuum at the needle tip, which may also affect the oocyte.

Damage to the cumulus

The above results indicate that an intact cumulus may be an important factor in the resistance of oocytes to damage. The morphology of cumulus is not changed after *in vitro* aspiration at vacuums and velocities above those normally used *in vivo*, providing the cumulus is regular, compact and refractive. The cumulus is less resistant if it is damaged or degenerate.

Conclusion

The above findings highlight two important issues relating to oocyte collection. Firstly, maintenance of suction: follicular fluid (and oocytes) may be lost if entry into and exit from the follicle are made in the absence of suction. This gain, however, may be offset by possible damage due to the dramatic forward flow of fluid toward the collection tube.

Secondly, movement of the needle tip within the follicle: damage to the oocyte, particularly the cumulus, may occur because of collection technique. It is a common practice during oocyte collection to "spin" the needle within the follicle. It is possible that significant damage may occur as the oocyte is "scraped" from the follicular wall by the edge of the needle, particularly in small follicles or in the collapsed follicle, where the needle size becomes large compared to the follicular volume.

There is a need to undertake further studies on the effect of needle movement in follicles on oocyte quality and subsequent blastocyst development. One possible solution, however, may be to combine flushing of follicles with lower suction vacuums.

Clinical aspects of oocyte retrieval

Timing of oocyte retrieval

The development of the ovarian follicles is monitored by vaginal ultrasound scanning of the ovaries and measurement of serum estradiol. The clinical criteria for administration of human chorionic gonadotropin (hCG) will vary with the stimulation protocol. For instance, in gonadotropin releasing hormone/follicle stimulating hormone (GnRH/FSH) stimulated cycles, a cohort of at least three follicles with a diameter of more than 17 mm is required. In addition, serum estradiol concentrations should approximate 800–1000 pmol/l per follicle. For natural cycles, on the other hand, one mature follicle is all that can be expected. When the criteria are met, the final maturation of the oocytes is initiated by an intramuscular injection of hCG (5000–10 000 IU) to

Table 47.1 Anaesthetic protocol of IVF procedures.

Fentanyl
1–2 g/kg i.v. (average dose 100 g)

Midazolam
0.05–0.1 mg/kg i.v. (average dose 2–5 mg)

If required, add Propofol
1–2 mg/kg

Monitor oxygen saturation and administer oxygen as indicated

PREPARATION

The success of oocyte retrieval is dependent on good visualization, accessibility of both ovaries, and on the materials and methods used for collection.

Pretreatment diagnostic laparoscopy will reveal difficult vaginal access to one or both ovaries and should be corrected prior to controlled ovarian hyperstimulation. When confronted with inoperable pelvic adhesions the feasibility of transvaginal retrieval is assesssed by transvaginal ultrasound. Occasionally it may be necessary to perform laparoscopic oocyte pick-up because the ovaries are adherent high on the lateral pelvic sidewall.

mimic the endogenous luteinizing hormone (LH) surge. After the administration of hCG, the oocytes are expected to ovulate about 37 hours later. The oocyte retrieval is scheduled to precede ovulation, about 34–36 hours after the hCG injection. During this period cytoplasmic changes take place in the oocyte and meiosis is resumed. The intercellular cytoplasmic connections between the granulosa cells and the oocyte are interrupted. The cortical granules, vesicles synthesized by the Golgi-apparatus, migrate towards the oolemma. The prophase of the first meiotic division is resumed and the oocyte progresses to the metaphase of the second meiotic division, at which stage it becomes arrested for the second time. During this process the nuclear envelope has broken down and the first polar body has been expelled.

Egg pick-up technique

Anesthesia

The use of analgesia and anesthesia varies in different countries and different patients. Light anesthesia is most acceptable as the patient is asleep, has no memory of the procedure, wakes within 5 minutes of completion of the oocyte collection, and is able to return home within 1–2 hours.

Preoperative counseling and physical examination are necessary. Because it is a low-risk surgical procedure routine checks have occasionally been omitted. The procedure should be cancelled or performed under local anesthesia if an upper respiratory infection is present or a fever of unknown cause is evident.

Depending on the procedure (transvaginal or laparoscopic) and specific requests from patients the degree of anesthesia may vary.

For transvaginal procedures pain relief may be obtained with a paracervical block (for example xylocaine or mepivacaine) or mild sedation (diazepam given intramuscularly or intravenously) in combination with opioid analgesics (pethidine hydrochloride). Alternatively, spinal or general anesthesia may be used.

One anesthetic protocol is outlined in Table 47.1. An excellent degree of relaxation is obtained, allowing for a quick and safe procedure and immediate recovery at conclusion of the operation. On average, the oocyte retrieval takes no longer than 10 minutes, minimizing the exposure of the oocytes to the anesthetic agents which accumulate rapidly in follicular fluid during the procedure.[8] There is little evidence that sedative and anesthetic agents have an adverse effect on the postconceptional development of the exposed human oocyte.[9] Even though the concentrations of these drugs in the follicular fluid are much lower than their serum concentrations, it is advisable to reduce the procedure time to a minimum.[8] For a detailed description and discussion of anesthesia and analgesia for oocyte retrieval please refer to Chapter 56.

Materials checklist for transvaginal oocyte retrieval (Figs 47.2–47.6)

Dry block heater with thermostat (Thermoline Scientific Equipment, Wetherill Park, Australia)

Warm blocks (U-Lab, Melbourne, Australia)

Falcon test tubes 2047 (Becton Dickinson, Sydney, Australia)

Glass syringe with blunt needle (Lab Supply, Melbourne, Australia)

Glass beaker (Lab Supply, Melbourne, Australia)

Thermometers (Lab Supply, Melbourne, Australia)

Automated pumps for flushing (William A Cook Australia, Brisbane, Australia)

Aspirating needle, 17 g, single/double lumen (William A Cook Australia, Brisbane, Australia)

Suction pump with vacuum regulator (William A Cook Australia, Brisbane, Australia)

Ultrasound scanner with a 7.5 MHz transvaginal probe and needle guide (Acuson 128 and EV519 transducer; Acuson, Mountain View, CA, USA)

K-Y Jelly (Johnson & Johnson, Arlington, TX, USA)

Latex probe cover (G.E. Medical Systems, Melbourne, Australia)

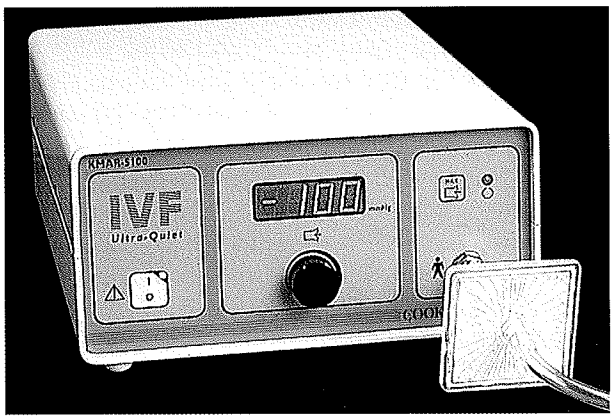

Fig 47.2 K-MAR-5100 aspiration unit (Cook IVF).

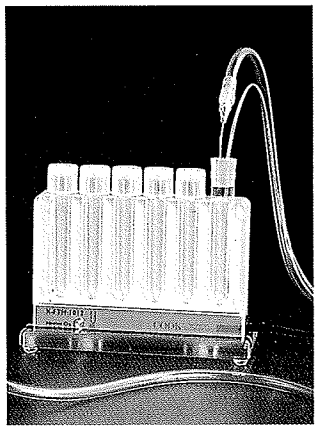

Fig 47.3 K-FTH 1012 Falcon tube heater and connecting tubing (Becton Dickinson).

Heating plate covering the microscope stage (Thermoline Scientific Equipment, Wetherill Park, Australia)

Microscope WILD MS (Leica, Heerburgg, Switzerland)

92*21 mm Petri dishes (Medos Company, Melbourne, Australia)

Glass Pasteur pipettes (Crown Scientific, Melbourne, Australia)

Small injection needles 25 g (Terumo, Melbourne, Australia)

Most materials in this list except the dry heater block are preheated at 37°C in a warming box. Immediately prior to the procedure, the heating stage with thermostat control is positioned on a trolley covered with a sterile drape. The heating stage, set at 37°C, is covered with a transparent plastic drape to minimize contact with textile fibers. The "warm blocks" with the test tubes, glass syringe, and glass beaker are placed in the heating stage. The thermometer is placed in one of the test tubes filled with handling medium and the temperature is checked and

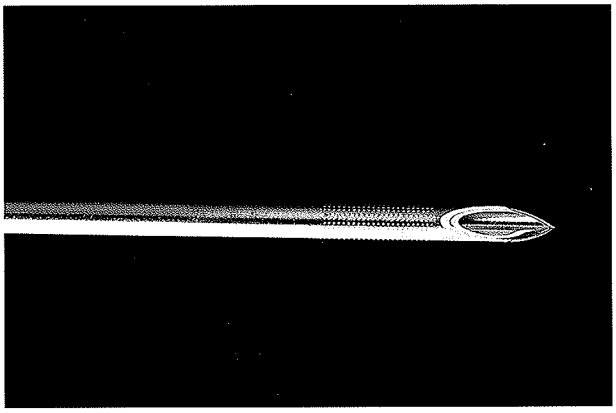

Fig 47.4 Needle showing echo tipping to obtain a bright image on ultrasound (Cook IVF).

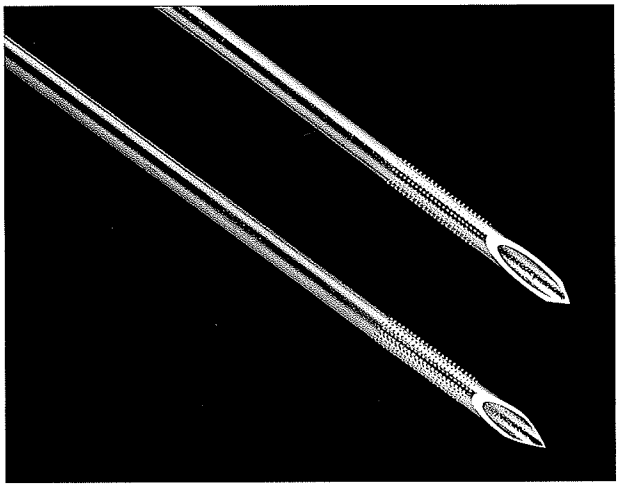

Fig 47.5 Needle with different bevels (Cook IVF).

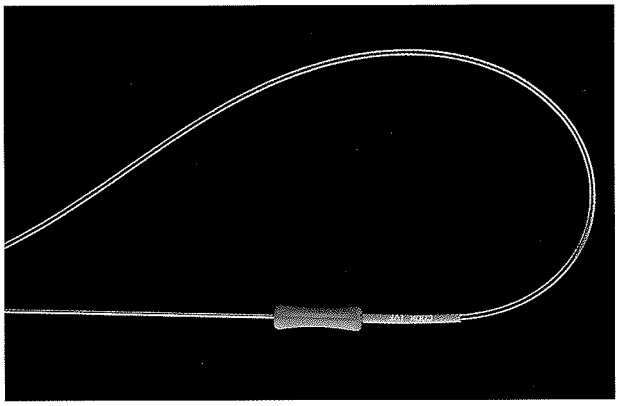

Fig 47.6 Handle on ovum pick-up needle—handpiece allows rotation of needle tip to maximize fluid and oocyte collection (Cook IVF).

adjusted. The glass syringe and beaker are filled with handling medium and kept ready for flushing, remaining in the warm blocks at all times. Glass syringes are still used to avoid possible toxicity associated with rubber plungers and silicone lubricants.

Automated pumps facilitate the flushing procedure. These pumps also provide preset flushing volumes and injection rates.

The aspirating needle can be either single or double lumen. The double-lumen needle is useful when multiple follicle flushes are needed (for example in natural cycle IVF). It must be remembered that the dead space volume of the single-lumen needle and its tubing is approximately 1 ml, and the oocyte may thus move backward and forward within this dead space during aspiration and flushing. The design of the double-lumen needle eliminates this problem, since the aspirating channel and the flushing channel are separated, ensuring a unidirectional flow in the aspirating channel. Prior to use, the aspiration needle and its Teflon tubing are flushed thoroughly with heparinized handling medium. The length of the Teflon tubing between the needle and the collecting test tube should be minimized to avoid unnecessary cooling of the oocytes.

A pedal-operated suction pump with vacuum regulator is used. A wide range of different models are now available. The maximum aspiration pressure is set at approximately –15 kPa or –125 mmHg.

Retrieval techniques

Laparoscopic oocyte retrieval

This technique may still be indicated when the ovaries are out of reach with the transvaginal approach either because of their elevated position in the pelvis or behind an enlarged uterus, or when the ovaries are too mobile, rendering the transvaginal technique impossible or unsafe. This procedure requires general anesthesia and endotracheal intubation, which is a major disadvantage.

The ovary is immobilized by holding it at the utero-ovarian ligament with a grasping forceps. Laparoscopic oocyte retrieval is only possible when the ovarian cortex is partly exposed and follicles are visible on the surface. After the follicles are identified, they are punctured with the aspiration needle, which has been flushed previously with heparinized flushing medium. To prevent the follicle wall from tearing and breaking the seal around the needle, the needle should be inserted where the follicular wall is slightly thicker. While the needle remains in the follicle, the aspirate in the collecting tube is handed to the embryologist and checked for the presence of an oocyte. When no oocyte is identified the follicle may be gently flushed until the oocyte is retrieved. The procedure is repeated until all accessible oocytes are aspirated.

Ultrasound-guided retrieval

In our center an Accuson 128 with a 7.5 MHz transvaginal probe is used for all transvaginal oocyte retrievals. After applying the conducting jelly to the tip of the transducer, the transvaginal probe is covered with a latex cover. Care is taken to position the needle guide correctly onto the probe. Regular checks to make sure that the indicator line on the imaging screen actually coincides with the path of the aspirating needle are essential to the safety of the procedure. The needle tip is specially treated to enhance echogenicity and shows up as a bright spot on the monitor. During the procedure it should follow the indicator line. When a lot of lateral tension is exerted on the needle, it may bend and the needle tip may leave the path of the indicator line. In such circumstances, the needle may be withdrawn and the curvature corrected manually, or better still, the needle may be replaced.

The ovaries are localized and lined up with the indicator line on the imaging screen. The follicle closest to the probe is entered with a short, controlled stabbing motion. A more progressive drilling motion may be indicated when follicles are localized at the posterior side of the ovary in the vicinity of major pelvic blood vessels or bowel loops.

The needle tip is kept at the centre of the follicle while the follicle wall collapses around it. The operator should make sure that the follicle is completely emptied. Rotating the needle around the longitudinal axis may help drain small pockets of follicular fluid. When the follicle is aspirated, the fluid is immediately sent to the embryologist. To reduce adverse effects of temperature fluctuations on the oocytes, the distance between the patient and the embryology laboratory should be minimal. Alternatively, the oocytes should be transported with the collecting tubes in warm blocks.

When follicles are flushed, the assistant injects the prewarmed heparinized handling medium through the Teflon tubing. The injected volume depends on the size of the follicle; to prevent rupture of the follicle it should not exceed the volume of the aspirated follicular fluid. While the medium is being injected, the operator can observe the follicle filling.

In many cases only one puncture through the ovarian capsule is needed to aspirate all or most follicles: this greatly reduces the risk of postoperative hemoperitoneum. Many follicles may be aligned in the path of the needle to minimize the number of puncture wounds in the ovary. When all follicles in one ovary are aspirated, the needle is withdrawn and flushed by holding the needle tip in a test tube filled with heparinized handling medium. Subsequently, the vaginal vault is punctured a second time to reach the contralateral ovary and the procedure is repeated. When all follicles have been aspirated in

both ovaries the pouch of Douglas is inspected for any fluid collection. Fluid is aspirated transvaginally and checked by the embryologist for the presence of spontaneously ovulated oocytes or oocytes lost at the time of puncture. When the pelvic aspirate contains mainly blood, this routine also helps to reduce postoperative pain caused by peritoneal irritation. After the needle is withdrawn it is flushed once again. All needle flushes need to be checked for oocytes. The vaginal vault is swabbed and checked for any bleeding. After each retrieval the transvaginal probe is cleaned with warm soapy water, rinsed, and dried. It is then soaked in 0.5% aqueous chlorhexidine for 10 minutes.

Ovarian endometriomas are usually visible on ultrasound when follicles are more than 15 mm in diameter, but may only be detected in smaller follicles when the follicular aspirate reveals chocolate-colored fluid. Similar fluid may sometimes be found in a hemorrhagic corpus luteum. The fluid aspirate may be embryotoxic, and thorough washing of the needle and aspirating system should be carried out before another follicle is aspirated. If cleansing is difficult a new needle and aspirating set are used.

If large endometriomas are aspirated at the time of egg pick-up an intravenous antibiotic is given to prevent the risk of pelvic infection, which may occur secondary to chemical peritonitis resulting from peritoneal spill of endometriotic fluid.

When the needle is in or close to the ovary, blood may be aspirated with follicular fluid. This may result from prior bleeding inside the follicle, which can be recognized by a speckled appearance on ultrasound, or by bleeding commencing after the needle punctures the wall of the follicle. Bleeding resulting from needle puncture is less likely when using a sharp (new) needle and entering the follicle at right-angles to its circumference. Tearing of the wall of the follicle is less likely, as is loss of fluid from the follicle before effective aspiration. The needle should be kept in the center of the follicle as contact between it and the inside of the follicle wall is avoided until the follicle is empty, reducing trauma, possible bleeding and blood in the aspirate.

Sometimes pure venous or arterial blood is seen in the aspiration needle, indicating an ovarian vessel has been entered. The needle should be withdrawn and both it and aspirating system flushed clean before reuse. Re-entry to the same follicle may be worthwhile when ultrasound indicates bleeding has stopped, and few follicles are available for oocyte retrieval.

Follicular flushing

The value of follicular flushing is debatable.[10,11] Its value is evident where low numbers of follicles are present, such as in patients on a natural or minimal stimulation (Clomid only) IVF cycle, or in poor responders to controlled ovarian hyperstimulation. However, when more than 10 follicles have been recruited during controlled ovarian hyperstimulation, the benefits of flushing are less clear. Flushing all follicles prolongs the procedure considerably, increasing the patient's discomfort and raising the overall cost of the procedure.[12] Flushing all follicles up to six times may increase the yield by 20%. The time factor can be minimized by flushing all follicles only once.

Interestingly, equal proportions of oocytes were found in the first aspirate and in the dead space of the needle and its tubing, indicating that the cumulus–oocyte complex is frequently aspirated when the follicle is almost completely collapsed.[12] This underlines the importance of aspirating the complete content of the follicle.

Both heparinized culture medium and heparinized normal saline can be used for follicular flushing. A randomized study[13] has shown that heparinized normal saline is an equally good but cheaper and more convenient medium than standard heparinized culture medium, and could replace it for flushing follicles during oocyte recovery for IVF-ET procedures.

Egg pick-up technique—important points

- Cleanse vagina of particulate matter before needle entry, as this reduces needle contamination and vaginal bacterial count.
- Vaginal ultrasound focused to maximize size of each follicle so needle can enter center of the follicle.
- Enter the follicle at its maximum diameter.
- Aspiration commenced before entering follicle to prevent leakage.
- Avoid excess aspiration pressure as cumulus may be torn from oocyte.
- Flush follicles at low pressure.
- Flush aspirating system after the first follicle is emptied to remove vaginal mucus or tissue.
- An empty follicle is determined by (i) several ultrasound views, and (ii) observation of aspiration of the tube.
- Aspiration is easier if the ovary is fixed by firm manual pressure with one hand—this reduces rotation of the ovary.
- Operator observes both ultrasound picture and tubal aspirate to coordinate movement of ultrasound probe inside follicle.

Egg pick-up—difficulties

- Transuterine needle puncture—minimize distance by manipulation of uterus or pressure on ovary—needle may bend or break.

- Endometriosis fluid may be embryotoxic—leave endometriomas alone or aspirate endometriomas and flush cyst and needle repeatedly to clean.
- Bleeding—ovarian vessels: remove needle, bleeding stops.
  - — iliac vein: remove needle gently; if rapid bleeding perform laparotomy.
  - — vaginal bleeding: apply pressure for 2 minutes; if bleeding continues, suture.
- Infection—intravenous antibiotics if vaginal or cervical infection, pelvic infection in past history, bowel or pelvic adhesions.
- Hydrosalpinx—often contains embryo toxins, preferably removed before oocyte pick-up.
- If hydrosalpinx found at time of oocyte pick-up suggest aspirate all oocytes and subsequently aspirate hydrosalpinx and repeatedly flush with hypertoxic saline to reduce embryo toxins and further production of toxins.

Complications

The ultrasound-guided transvaginal technique is a very efficient and simple procedure. However, this should not distract from the fact that a number of potentially dangerous complications exist, consisting mainly of hemorrhage, trauma to pelvic anatomic structures, and infection.[14] For a detailed review on the complications inherent with egg retrieval the reader is referred to Chapter 62.

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48 The luteal phase: luteal support protocols

James P Toner

This review discusses the special need for luteal support in assisted reproduction and the options currently available to provide this support. After a review of the differences between natural cycles and those seen in assisted reproductive technology (ART) (stimulated and programmed), the elements estradiol (E) and progesterone (P), timing, and route of replacement are reviewed. Lastly, standard protocols for replacement are provided.

Progesterone (P) and estradiol (E) are required for successful pregnancy, both to prepare the uterus for embryo implantation and to stabilize the endometrium during pregnancy. The success of donor egg programs which replace only these two hormones has amply demonstrated the sufficiency of this approach.[1,2]

In the normal luteal phase of a nonpregnant woman, E and P production peaks about 4 days after ovulation and continues at this level for about a week, until falling several days before the next menses (Fig 48.1).[3] During this time, P is secreted in a pulsatile fashion every 1–4 hours, with measured levels ranging between 4 and 20 ng/ml during peak production. This P production is enormous: it is 40-fold maximal E production, some 25 mg daily vs. 0.6 mg for E.

In normal cycles, P and E production wanes about 10 days after ovulation. Menses follows that event about 4 days later unless a pregnancy occurs. A dip in ovarian P production can occur even during cycles of pregnancy, but in that case it is quickly reversed, with P production restored by human chorionic gonadotropin (hCG) stimulation of the corpus luteum.

A shift from ovarian to placental production of gonadal steroids occurs over a period of weeks. In one study, placental P production was detected as early as 50 days of gestational age (36 days after embryo transfer) in hormone-replaced cycles of donor egg recipients.[4] This timing accords well with the observed effects of surgically removing the corpus luteum in early pregnancy. Classic work by Csapo showed that lutectomy led to miscarriage in almost every case if performed before 7 weeks of gestational age, and almost never if performed after that time.[5]

The special problem of the luteal phase after ovarian stimulation

In stimulated cycles typical of IVF therapy, the luteal phase is different from the natural one in two important ways. First, since ovarian stimulation produces multiple corpora lutea, the levels of both E and P in the early part of the luteal phase are supraphysiological. Second, and perhaps more importantly, the duration of ovarian steroid production in stimulated cycles is usually shorter than normal by 1–3 days. This truncated luteal phase has been noted since the earliest days of IVF (see Fig 48.2),[6] and created concern that an early menses might prevent a successful implantation, since menses were on occasion observed to occur as early as 10 days after egg retrieval.

Moreover, the decline of serum E and P levels is also more abrupt than the rate of fall in natural cycles (compare Figs 48.1 and 48.2). This early and rapid fall was the reason luteal support was adopted in the early days of IVF therapy. With the advent of GnRH agonist use in the late 1980s, the problem of the short luteal phase became even more common. Recent study in GnRH antagonist cycles has documented inadequate luteal phases no matter what the nature of the ovulatory trigger; the impairment was most profound when GnRH agonist was used to induce final follicular maturation, intermediate when rLH was used, and least when hCG was used.[7] Multiple studies show the importance of some form of luteal support in such cycles.[8–11]