J Assist Reprod Genet (2014) 31:1409–1419
DOI 10.1007/s10815-014-0334-9

REVIEW

Approaches to oocyte retrieval for advanced reproductive technology cycles planning to utilize in vitro maturation: a review of the many choices to be made

B. I. Rose

Received: 13 July 2014 / Accepted: 1 September 2014 / Published online: 12 September 2014

Abstract
Purpose To evaluate the minutiae associated with oocyte retrieval for use in human in vitro maturation IVF cycles. Many of the relevant features of oocyte retrieval were identified by the Trounson group in the first publication on successful in vitro maturation using transvaginal oocyte harvesting and these were a major focus of this review.
Methods Published human and animal studies, together with topics from mathematics and mechanics, were used to try to understand the importance of different choices that could be made in structuring a transvaginal oocyte retrieval procedure in humans.
Results The published literature suggests that the highest oocyte recovery rate occurs using higher pressures and thicker needles, but this comes at the cost of damaging the cumulus oocyte complex. It is likely that this damage is caused by the sheer stress forces exerted on the cumulus oocyte complex due to parabolic forces associated with laminar flow within the needle and is likely worsened by irregular forces during intervals of turbulent flow occurring with entry into the needle. Larger needles also cause more pain and may be associated with more blood loss. Higher velocity entry into the follicle, needle rotation to prevent premature blockage of the lumen, and carefully timed applications of aspiration pressure theoretically optimize oocyte retrieval technique.
Conclusions Oocyte retrieval for in vitro maturation is effected by the interaction of the many choices that need to be made in planning for the procedure. The most difficult decision involves aspiration pressure or fluid flow rate and needle size.

Keywords Transvaginal oocyte retrieval · In vitro maturation · IVM · Reduced pressure oocyte collection · IVM aspiration needle set · Oocyte damage · Techniques for optimizing IVM retrievals

Capsule Subtle differences in aspiration technique may have a significant impact on IVM. Patient related issues, needle size, and aspiration pressure need to be considered for optimal oocyte recovery.

B. I. Rose (✉)
Infertility Solutions, P. C., 1275 South Cedar Crest Boulevard, Allentown, PA 18103, USA
e-mail: brucerose50@gmail.com

B. I. Rose
Department of Obstetrics and Gynecology, St. Luke's University Health Network, 801 Ostrum Street, Bethlehem, PA 18015, USA

Introduction

There is a growing awareness of advanced reproductive technology (ART) alternatives to conventional IVF. In conventional IVF, patients are treated with exogenous gonadotropins with the objective of obtaining a cohort of oocytes that are mature (have extruded polar bodies) at the time of oocyte retrieval. The decision to induce maturity (usually with an hCG injection) is made when an adequate number of follicles have diameters greater than 17 or 18 mm. Oocyte retrieval is undertaken about 36 h later and the largest diameter follicle is usually at least 20 mm in diameter. Oocytes are fertilized and the best embryos are transferred into the uterus.

In an advanced reproductive technology cycle in which in vitro maturation will be used (IVM), oocytes are removed from much smaller follicles and most oocytes will still be immature (germinal vesicle intact). The decision to proceed to oocyte retrieval will be made when the largest follicle is less than 10 mm [1], 12 mm [2], or 14 mm [3], depending on the philosophy of the program. Some programs wish to remove oocytes before any follicles exert dominance, which is thought to begin when the lead follicle grows to a diameter of approximately 7 mm [4]. Other programs want to maximize the impact of granulosa cell growth on oocyte competence, but wish to remove oocytes before the dominant follicle causes significant apoptosis of granulosa cells and before it decreases the size of non-dominant follicles as it modifies the blood flow

when the ovary moves into mid-follicular phase [4]. With IVM, oocytes in prophase I are cultured until they become metaphase II. They are then fertilized and the best embryos are transferred into the uterus as with conventional IVF.

The procedure of oocyte retrieval is more difficult in an IVM cycle than an IVF cycle since the follicles are smaller. A follicle's volume varies by the cube of its radius. Thus a 10 mm follicle contains 1/8th the volume of a 20 mm diameter follicle and a 5 mm follicle contains about 1/64th the volume of a 20 mm diameter follicle (Table 1). Antral follicles as small as 3 mm may contain a competent oocyte, but will have approximately 1/300th the amount of fluid of a normal dominant follicle. Thus although the same actions occur in both IVF and IVM retrievals, follicle volume differences have a number of practical consequences.

Alan Trounson et al., created the first IVM program in which oocytes removed from a patient transvaginally led to a successful birth [5]. His program was adapted and refined by a number of practitioners over the years. Some of his choices and the reasoning supporting them have continued to be applied unchanged even though they have not been shown to be the best approaches experimentally. Others have been adapted and improved upon. In terms of oocyte retrieval, Trounson et al. recommended the following differences for IVM compared to conventional IVF: reduction in needle length, shortening the bevel of the needle, changes to the needle to make it more rigid, and decreased aspiration pressure.

The objective of this review is to look at some of the particulars involved in the IVM retrieval. These details include needle choice, aspiration pressure, follicle flushing, and needle handling technique. There are choices to be made by the IVM program in how to approach these minutiae. We will review the available evidence as well as the advantages and disadvantages of these choices.

Issues raised by Trounson

Trounson et al., found that needles routinely used for IVF were unsuccessful for IVM retrievals and they designed a needle that overcame what they found to be the problems with traditional needles. These special needles were made by Cook Australia and were shorter than the needle used for conventional IVF, had a shorter bevel, and were made to be stiffer [5]. The currently available descendant of this needle is Cook's 19-gauge immature ovum aspiration needle (K-OPS-7035-RWH-ET, Cook Medical, Spencer IN). However, as can be seen in Table 2, experienced programs are successful with a range of different sized needles.

Early studies in the history of IVF suggest that oocyte recovery was higher when larger gauge needles are used. The follicles aspirated were 14 mm or larger in diameter. In these studies 14 and 16-gauge needles were the best choices for oocyte recovery [6, 7].

It makes intuitive sense to use a smaller gauge needle to enter a smaller structure. This approach is routine throughout medicine. A smaller gauge needle is used for venipuncture in an infant or a small child compared to that used for venipuncture in an adult. This analogy is impaired since the ratio of the cross sectional area of a needle used for venipuncture to the cross sectional area of a vein is much larger relative to the ratio of the cross sectional area of an IVF needle to a follicle. To enter a follicle for aspiration, the needle should be directed at a right angle to the surface of the cyst, but entry may be successful if the needle passes anywhere through the upper hemisphere of the follicle. The 2 % difference in the ratio of the cross sectional area of a 17-gauge needle (1.7 mm^2) to the surface area of a hemisphere with a radius of 5 mm (52.4 mm^2) compared to the ratio of the cross sectional area of a 19-gauge needle (0.9 mm^2) to the ratio of a hemisphere with a radius of 5 mm (52.4 mm^2) is not clinically significant (using data from [8]). Thus the fact that the target follicles in IVM are smaller than the target follicles in IVF, by itself, is not a good reason to use a smaller gauge needle for IVM than for IVF.

Table 1 Follicle diameters and fluid volumes

Follicle diameter (mm)	Fluid volume (ml)	Hemisphere surface area (mm^2)
4	0.034	25
6	0.113	56
8	0.268	101
10	0.524	157
12	0.905	226
14	1.437	308
20	4.189	625

IVM needles

Given the same needle bevel length (measured along the outer horizontal barrel edge), a smaller gauge needle will form a smaller angle (off the horizontal) and thus be sharper. A sharper needle requires less force to pass through tissue. It will deform the follicle less during entry making placement of the end of the needle in the center of the follicle easier. The bevel length for many conventional IVF needles is about 3 mm (Table 3). If a needle badly deforms a follicle, it may cut a path through the tissue significantly to the side of the follicle so that the needle tip is at least partially outside of a small follicle. This is an important issue for small follicles. If a needle enters a follicle axially and it passes through the follicle's center, then it has the opportunity to occupy a portion of the entire diameter of the follicle. If it enters at an angle to

Table 2 Aspiration techniques used in selected programs

Reference (country and year)	Needle gauge	Needle type	Aspiration pressure
Trounson et al. (Australia 1994)	Not specified	Single lumen	7.5 kPa (56 mm Hg)
Junk et al. (Australia 2012) [3]	16	Double lumen	Not specified
Lin YH et al. (Taiwan 2003) [33]	17	Double lumen	7.5 kPa (56 mm Hg)
Child et al. (Canada 2001) [2]	17	Single lumen	7.5 kPa (56 mm Hg)
Guzman et al. (Belgium 2012) [34]	17	Single lumen	70 mm Hg
Zhao et al. (China 2008) [35]	17	Single lumen	75-80 mm Hg
Gremeau et al. (England 2012) [36]	17	Single lumen	85 mm Hg
Mikkelson and Lindenberg (Denmark 2001) [1]	17	Single lumen	Syringe aspiration
Vitek et al. (United States 2013) [37]	19	Single lumen	80 mm Hg

the surface, it must occupy a smaller cord. For example, using geometry, if a needle enters a 6 mm diameter follicle at 45 degrees off perpendicular, it will have only √18 mm or 4.24 mm of space inside the follicle. Thus, if the tip of the needle is in the follicle, some or all of the ultrasound scoring on the needle used to help the surgeon know where the needle is, will be outside of the follicle (Table 3). The scoring may begin at the tip of the needle or at the top of the bevel, but can only be seen easily at the top of the bevel. In the 17-gauge Wallace needle (ONS1833LL-500, Smiths Medical, Kent, UK) (Table 3), scoring begins at the tip of the needle covering the 3 mm to the top of the bevel and then continues 20 mm more on the barrel of the needle. The surface area of the scoring on the bevel is 50 % of a segment of the needle barrel of that length, but can only be seen in the orientation where the back of the needle faces the probe. The surgeon has to adjust his or her idea of where the end of the needle is and if the needle is not axial, it could easily lie in the wall of the follicle. For Cook's 19-gauge immature ovum aspiration needle set (K-OPS-7035-RWH-ET, Cook Medical, Spencer IN), the ultrasound scoring begins 2 mm above the top of the bevel.

A smaller gauge needle is sharper for a given length bevel, since the angle of the bevel to the barrel of the needle is smaller. Using the length of the bevel and the outer diameter needle of different gauges, trigonometry can be used to calculate the angles of different gauge needles with different bevels. For example, with a bevel of 3 mm the angle of a 17-gauge needle would be 26.2 degrees and the angle of a 19-gauge needle would be 19.6 degrees. If length of the bevel is shortened, then the angle of the bevel increases. If the length of the bevel were decreased by half to 1.5 mm, then the angle of the bevel of a 19-gauge needle would increase to 35.4 degrees. Shorter beveled needles were one of the recommendations made by Trounson et al. [5]. There is a trade-off between the length of the bevel of a needle that requires space in order to be contained within a follicle and the sharpness of a needle. A sharper needle makes needle placement easier and more precise. Experimental data on tissue-needle interaction for passing a needle into a biological structure shows that when a beveled needle has a smaller angle, there is less tissue deformation before the needle pierces that structure [9].

Some beveled needles utilize a diamond cut to make them sharper without increasing the bevel length (Table 3). This is done by making two cuts at right angle to the barrel at the end of the needle. This makes the needle sharper by reducing its thickness at the end. A minor disadvantage of this is that it makes a scored tip of the needle slightly harder to see by ultrasound.

There appear to be few published studies that look at needle tip bevel. An exception is a study by Bols et al., which looks at short and long beveled 20-gauge needles for retrieval from bovine ovaries [10]. Two thousand follicles from bovine ovaries obtained from slaughtered cattle were used in one of their experiments. A higher recovery rate from follicles was found with the longer gauge bevel needle than with the shorter. However, at high pressure they also found more damaged oocytes with the longer compared to the shorter bevel needle. This may be due to different characteristics of fluid flow into the needle tip with aspiration pressure changes as will be discussed in a later section. Earlier in the history of human IVF, the needles used had a shorter bevel. For example, the needles used by Aziz et al. had a 45 degree angle bevel [11].

Another issue to consider when choosing which gauge needle to use is needle stiffness. A surgeon can feel the difference in the stiffness of a long 19-gauge compared to a 17-gauge needle although the wall thickness of the two needles differs by only about 5 %. The wall diameter of a 17-gauge needle is 0.203 mm whereas a 19-gauge needle has a wall thickness 0.191 mm. An obvious problem with decreased needle thickness occurs for those surgeons who find it helpful in some patients to apply external pressure on the ovaries. It is easier to bend a 19-gauge needle that has been inserted into an ovary than bend a 17-gauge needle in the ovary from external pressure. There are commercial alternatives that provide sharp thin needles within the ovary while providing a stiffer thicker needle for outside the ovary. Two of these are the Steiner-Tan pseudo-double lumen needles which provides a 21 or 19-gauge needle mounted on a larger needle

Table 3 Physical characteristics of selected needle sets

Manufacturer (identification number)	Gauge	Lumen	Needle length (cm)	Bevel length (mm)	Bevel angle (degrees)[1]	Needle dead space (ml)[1]	Total dead space (ml)	Length ultrasound scoring (mm)	Ultrasound scoring begins	Diamond Tip?	Flow rate (ml/sec)[2]
Cooper-Smith, Trumbull, CT (AR-N1695)	16	Single	35	5	18.3	0.392	1.57	22	tip	Angled bevel	0.78
Cook Medical, Spencer, IN (K-OPSD-1635-A-S-US)	16	Double	41	5	18.3	0.291[3]	1.31	5	1 mm above bevel top	No	0.45
Smiths Medical, Kent, UK (Wallace, ONS1733LL-500)	17	Single	36	3	26.2	0.322	0.93	23	tip	Yes	0.42
Smiths Medical, Kent, UK (Wallace, ONS1833LL-500)	18	Single	36	3	22.9	0.199	0.74	23	tip	Yes	0.31
Cook Medical, Spencer, IN (K-OPS-7035-RWH-ET)	19	Single	41	3	19.6	0.152	0.58	5	2 mm above bevel top	Yes	0.21
IVFETFLEX.com, Ganz, Austria (Steiner-Tan 21 gauge)	21	Pseudo-double	8	3	15.3	0.017	0.02[4]	9	0.5 mm above bevel top	Yes	0.30

[1] Using data from [8] and length measurement

[2] Using a Craft suction unit aspiration pump with pressure set at 100 mm Hg

[3] Measured

[4] Tubing and larger sheathed needle were not counted as dead space since they can be flushed

that does not enter the ovary (Steiner-Tan Needle 21 (19) gauge, IVFETFLEX.com, Graz, Austria) and Cook's Immature Ovum Aspiration Set (K-IOPS-2035-1730, Cook Medical, Spencer, IN) which uses a double needle. This aspiration set uses a 17-gauge needle to pass through the vagina and ovary and uses a 20-gauge inner needle to aspirate the follicles.

Likely a more important consideration in choosing the gauge of a needle to use is the amount of dead space in the needle. Dead space is the volume of the cylinder created by the cross sectional interior area of the needle and the length. This is more significant for IVM than for IVF in terms of comparing the volume of the follicle aspirated with the amount of dead space in the needle. Table 1 lists follicle diameters and their calculated volumes. Table 3 lists characteristics of selected needle sets with measured dead space of the needle set and calculated dead space of the needle. For larger gauge needles, several follicles may need to be aspirated before enough fluid is aspirated for fluid to enter the collecting tube (attached to the bung). For example, it would take the fluid from fourteen 5 mm follicles to just fill a Wallace 17-gauge needle and the tubing proximal to the collecting tube. It would take five such follicles just to see the fluid in the tubing where it is attached to the needle. Depending on the speed of the surgeon, prolonged residence of the aspirate in the dead space of the needle and tubing may lead to clotting in the needle and loss of oocytes or to exposure of oocytes to non-optimal environmental conditions. A smaller dead space is obviously an advantage over a larger dead space. The IVM needle and collection device with the smallest dead space is the Steiner-Tan needle, which also allows for simultaneous emptying of the needle outside the vagina and the tubing attached to the needle without removing the needle from the ovary [12]. The genuine dead space is limited to a 8 cm segment of 21-gauge needle and has a volume of 0.017 ml (Table 3). It also partly overcomes the stiffness issue and its bevel length is approximately 3 mm and bevel angle is 15.3 degrees (based on [8]).

Another consideration in selecting needle gauge size is that a smaller needle is likely to result in less tissue trauma and thus less bleeding. This may be particularly important in ART settings in which limited anesthesia is available for the patient. Sayhan et al. compared IVM patients in cases using a 19-gauge needle to IVF patients in cases using a 16 or 17-gauge needle [13]. Records on 375 patients were reviewed retrospectively. There were several approaches to anesthesia, but 233 patients received conscious sedation with midazolam and fentanyl together with a paracervical block. Patients ranked the amount of pain they experienced during the procedure on a scale of one to ten. There was no difference in the pain experience of the groups. Note that in addition to different gauge needles, this study compared different aspiration procedures since IVF required passage of the needle through the

vagina and into each ovary only once; whereas, IVM required a number of punctures in each ovary. The authors viewed the results as showing that a smaller gauge needle was less traumatic since multiple insertions of the 19-gauge needle caused no more pain than two insertions of the larger needle. Several studies from the IVF literature also suggest that smaller needles cause less pain for women who are lightly sedated during retrieval [11, 14].

Prudent surgical practice suggests that using the thinnest needle available to optimally accomplish the surgical objectives is best for patients since it is less traumatic to the ovaries than thicker needles. Conventional IVF oocyte retrieval is a bloody operation in which a patient's blood loss is not easily visualized. Dessole et al. used pre and 24 h post retrieval hemoglobin measurements on a sample of 220 normal patients to estimate an average blood loss of about 230 ml [15] for an uncomplicated oocyte retrieval procedure. Limiting blood loss may be especially important in IVM cases since the subgroup at greatest risk for ovarian hemorrhage requiring surgery after conventional IVF retrievals is lean PCO patients [16]. In this report, Liberty et al. used a 17-gauge Wallace oocyte retrieval set. A multiple regression analysis for risk factors for this bleeding complication found an odds ratio of 50 after retrieval in lean patients with PCO compared to all other patients.

Needle gauge or thickness also needs to be considered in the context of other parameters that effect the aspiration procedure. The most obvious of these is the aspiration pressure since needle thickness is the primary variable determining the pressure that an oocyte experiences at the needle tip (for a given pump aspiration pressure) [17, 18]. That needle tip pressure should vary so significantly with needle diameter is predicted by the Hagen-Poiseuille law for steady flow through pipes, even though this physical law does not fully explain the more complicated systems used for oocyte aspiration [17].

Aspiration pressure

Most IVM programs use a reduced aspiration pressure for IVM oocyte retrieval compared to the pressure they use for IVF retrieval (Table 2). Most commonly this is 7.5 kPa (approximately 56 mm Hg). This pressure and this approach follows Trounson et al's, original paper. They stated that "this [lower pressure] improved the recovery of immature oocytes in preliminary studies" [5].

Looking at aspiration pressure as a single isolated variable, this contradicts several targeted experiments on IVM retrieval using bovine slaughterhouse ovaries, which consistently show that increasing aspiration pressure improves the recovery rate (in the range of pressures tested). The vast majority of oocytes aspirated in these in vitro experiments were in follicles 2 to 4 mm in diameter and 90 % of all oocytes recovered had compact cumulus rather than expanded cumulus. The remaining follicles were 5 to 15 mm in size. The pressures tested were 25 to 130 mm Hg and the needles used were 20 to 17-gauge in caliber. Although these studies used a bovine in vitro model, they share a number of features of clinical IVM retrieval in humans [10, 18, 19].

For example, Fry et al. aspirated 5827 follicles from 720 ovaries with 17 and 20-gauge needles. More than 5000 of these follicles were 2–4 mm in diameter. The rest were 5–15 mm. With 17-gauge needles, 56 % of the follicles yielded oocytes, but with 20-gauge needles, recovery dropped to 45 %. The highest recovery also occurred with the highest aspiration pressures. The pressures evaluated ranged from 25 to 100 mm Hg. Recovery was 46 % at 25 mm Hg and 59 % at 100 mm Hg [18]. Bols et al. aspirated 3000 follicles 3 to 8 mm in diameter with 18, 19 and 21-gauge needles using aspiration pressures of 50 to 130 mm Hg. Oocyte recovery increased from 52.7 % for 21-gauge needles to 74.4 % for 18-gauge needles. Recovery was only 55.5 % with a pressure of 50 mm Hg, but increased to a maximum at the three highest pressure levels (67 % at 90 Hg, 69.5 % at 110 mm Hg, and 67 % at 130 mm Hg) [19].

These bovine studies raise a different issue not commonly addressed in clinical human studies of IVM. As aspiration pressures increased, the recovered oocytes were increasingly denuded of cumulus cells [10, 18, 19]. This loss of cumulus cells also occurred at lower aspiration machine pressures using larger diameter needles compared to smaller diameter needles, suggesting that needle gauge and pressure interdependently contributed to outcome. Prior to the common use of electric aspiration pumps with gauges, the high pressures caused by aspirating with hand held syringes were shown to cause detrimental fractures of the zona pellucida [20]. The type of damage caused by the relatively lower pressures used in these studies appears to be more unique to IVM in that oocytes tightly enclosed by granulosa cells have these cells stripped off them. Naked oocytes are harder to visualize than cumulus enclosed oocytes (and may be overlooked by less experienced embryologists). Such naked oocytes are unusable for bovine IVM. In human IVM, naked oocytes are less likely to mature, fertilize or to cleave as embryos [21, 22]. Fig. 1, constructed using data from [10], illustrates this relationship and demonstrates that this damage was caused by the aspiration process. In this study, bovine ovaries were sliced to recover 800 compact cumulus oocyte complexes (CCOC) without exposing them to aspiration. These CCOCs were placed in dishes containing follicular fluid and the fluid was aspirated using the different needles and pressures indicated in Fig. 1. Note that the negative impact of increasing aspiration pressure is greater in the larger gauge needles than in the smaller gauge needles [10, 18, 19]. In an experiment in which recovered oocytes were cultured to blastocysts, Bols at al showed that the percentage of recovered oocytes that became

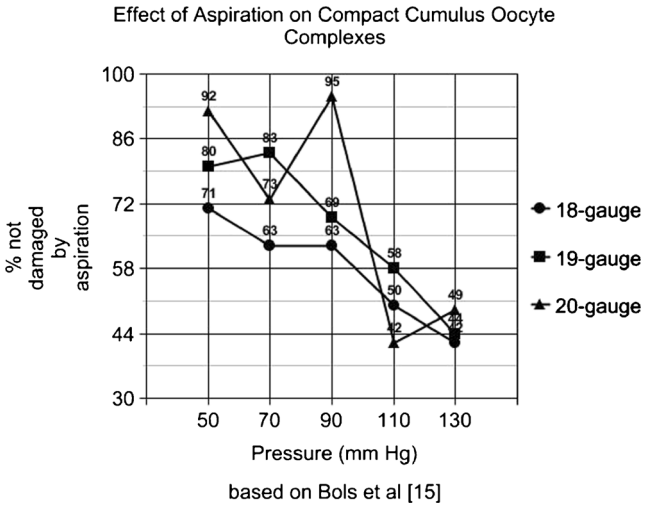

Fig. 1 Effect of aspiration on compact cumulus oocyte complexes

blastocysts decreased as pressure increased [14]. Fry et al. concluded that the optimal pressure to maximize recovery of bovine CCOCs was 55 mm Hg with a 17-gauge needle and 77 mm Hg for a 20-gauge needle [18].

A small study with human subjects (43 cycles) compared oocyte recovery for IVM using a 20-gauge needle with aspiration pressures of 180 or 300 mm Hg [23]. These aspiration pressures are both higher than the pressures used in the bovine studies sited above and most published clinical human IVM studies (Table 2). More oocytes were retrieved with more cumulus cells with the lower pressure retrievals. Patient selection for the pressures used was based on the date of the IVM procedure. Half of the patients in the lower pressure aspiration group received FSH for priming. FSH priming increases growth of the granulosa cells in follicles, making the antral follicle easier to visualize and aspirate since it increases the number of granulosa cells in the follicle [24].

A study from the pre-history of IVF looked at oocyte retrieval by laparoscopy using a 24 cm 20-gauge needle with a 45 degree bevel. The authors concluded that an aspiration pressure of 200 mm Hg produced higher oocyte recovery than lower pressures (grouped together as pressures of 120 to 180 mm Hg). This study aspirated follicles of all sizes, but most oocytes recovered came from follicles with diameters 7 to 9 mm. The authors felt that a pressure of 200 mm Hg was better than higher pressures (operating room wall suction was estimated to be greater than 400 mm Hg) that damaged oocytes and removed surrounding cumulus cells [25].

Pressure and needle gauge likely have a different impact on expanded COCs in conventional IVF. Naked oocytes are uncommonly observed in current conventional IVF practice, but occur to some degree with CCOCs with any needle size or pressure. Fry et al. separately looked at the 468 more developed bovine follicles between 5 and 10 mm that they aspirated [18]. Recovery rate and the proportion that were good quality oocytes using various needle gauges and pressures remained the same as their findings for follicles in the 2 to 4 mm size range. The highest oocyte recovery occurred with the mid range pressures (50 and 70 mmHg) with the 17-gauge needle. With eight groups being evaluated, conclusions were based on much less data than some bovine studies. The quantity of data for 10 to 15 mm follicles with even more cumulus cells was inadequate for evaluation.

Aspiration pressure, fluid velocity and flow rate

Although IVF and IVM researchers frequently report the aspiration pressures used in their methodologies, this information can be misleading or, at least, not easily reproducible. The pressure at the exit of the aspiration device is different from the pressure experienced by the oocyte at the needle tip. Needle gauge, length of needle, connecting tube gauge, length of connecting tube, size of the collection tube and size of the vacuum reservoir in the pump all play a role in determining the pressure experienced within the needle from the aspiration device [17]. As demonstrated by Horne et al. using bovine ovaries, Hagen-Poiseullie's law (for steady flow though pipes) does not adequately predict flow and pressure in the more complex system required for oocyte aspiration. However, it does highlight the most important variables. Poiseullie's law says that increasing the length of the tube decreases pressure at the end in proportion to the percentage that the length was increased. More importantly, decreasing the interior diameter of the needle or tube decreases the pressure by the fourth power of the decreased percentage of the diameter (that is, decreasing the diameter of pipes by 10 % results in decreasing the pressure to 65.6 % of what it was). Since aspiration needle sets commonly vary from one another with respect to their components in addition to needle gauge and length, aspiration techniques would be more easily reproducible if papers cited the fluid flow rate as well as the needle gauge in the aspiration needle set used. The flow rate is an expression of the velocity of fluid at the tip of the needle, but is easier to measure and adjust. The flow rate commonly used for IVF with a Wallace 17-gauge needle using a Craft suction unit (Rocket Medical plc., Watford. Herts, England) aspiration pump set at 100 mm Hg is 0.42 ml/sec (aspirates 10 ml of water in 24 seconds). A common flow rate used for IVM using the same needle but with the pump set at 50 mm Hg is 0.12 ml/sec. It takes about 0.5 s after pressure is applied before 75 % of the maximal pressure is attained and about 5 s before the maximal pressure is reached [12]. Since the aspiration of a follicle usually takes less than 5 s, we measured flow by recording the time it takes to aspirate 10 ml from the onset of suction.

The average velocity of the fluid moving in the needle does not completely explain the forces acting on the oocyte during aspiration. At the pressures commonly used for IVF aspiration, fluid moves within the needle in a laminar flow pattern

[17] (Fig. 2). Even at these pressures, since the COC occupies a significant fraction of the diameter of the needle different shear stress forces are acting on different parts of the COC. Laminar flow within a needle has a parabolic distribution of velocities with fluid along the inner wall of the needle moving slowest (due to friction) and fluid in the center of the needle moving fastest. In a 17-gauge needle, a typical COC will occupy more than 25 % of the diameter of the needle. The shear stress force acting on the COC is the force component perpendicular to the flow, which varies related to how far the COC is from the center of the needle (Fig. 3). Different velocities of layers of laminar flow moving fluid may be visualized as the opening a collapsing telescope. The magnitude of the shear stress that a COC may experience thus depends on the velocity of the fluid and the diameter of the needle [26, 27]. Increasing either the velocity of the fluid (by increasing aspiration pressure) or increasing the diameter of the needle increases the shear forces on the COC. The impact of shear stress forces on the oocyte during its transit in the needle may also depend on characteristics of the cumulus mass. An oocyte with compact cumulus may require higher or lower shear forces in order to strip the cumulus cells from the oocyte than an oocyte with an expanded cumulus. Thus a program that uses priming with FSH that leads to more oocytes with expanded cumulus may benefit from a different optimal pressure aspiration procedure than a program that does not use priming. On a positive note, healthier COCs may be harder to strip than degenerating COCs [17].

Stripping cumulus cells from the oocyte likely occurs either during passage through the needle, as the oocyte enters the needle, or as the oocyte leaves the follicle wall. As noted above, shear stress forces on the cumulus oocyte complex (COC) increase with increased diameter of the needle or increased velocity of the fluid in the tube. The theory of flow dynamics suggests that with sufficient velocity, fluid flow in the needle can change from laminar flow to turbulent flow, which exposes the COC to more severe randomly directed forces (since the Reynolds number is proportional to the velocity and the Reynolds number predicts when flow becomes turbulent). However, most flow rates commonly used for IVF and IVM (Table 2) are likely to keep flow within the needle laminar [17]. For example, flow in a 16-gauge needle begins to become non-laminar when vacuum is increased to 375 mmHg.

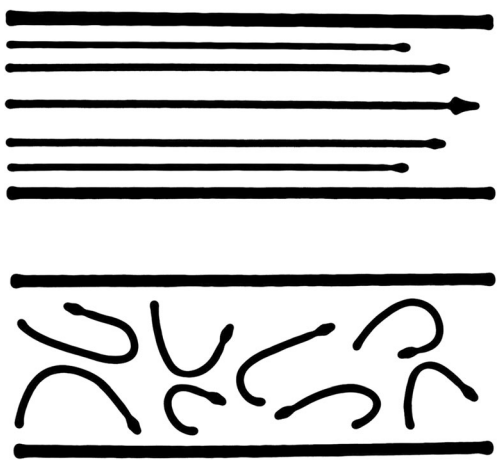

Fig. 2 The upper frame illustrates laminar flow within the needle. Fluid has the highest velocity in the center of the needle as indicated by the thicker longer arrows. Differences in the velocity of moving fluid in the needle decrease in a parabolic pattern to a zero velocity at the needle wall. The lower frame illustrates turbulent flow. The magnitude and direction of flow at any point in the needle is not predictable. Turbulent flow causes mixing of the fluid

The CCOC is embedded in the wall of the follicle with physical connections between granulosa cells and the zona of the oocyte. Aspiration of oocytes that become naked before entry into the needle would require that the cumulus cells remain attached to the theca interna layer of a follicle after the oocyte is detached. The forces exerted on the cumulus and on the oocyte while in the wall of the follicle will be similar suggesting that loss of the cumulus covering is more likely to occur during other parts of the aspiration process where different force vectors are exerted on different parts of the cumulus mass.

The entry of the CCOC into the beveled tip of the needle generally involves a change of direction for the CCOC while it undergoes rapid changes in velocity (Fig. 4). This will result in turbulent flow before entry into the needle and for the first few millimeters of flow inside the needle until laminar flow is established. This period of turbulent flow exerts stronger forces on the CCOC that are differently directed and at times causes the CCOC to hit the walls and bevel of the needle. The strength of these forces increases with increased velocity (increased flow rate) due to increased pressure. Rapid radial movement of the needle (twisting) or vigorous flushing, as some surgeons do, may also increase the magnitude of the force exerted on the CCOC. Consideration of the forces acting on CCOCs due to flow characteristics provides a unifying explanation of why CCOCs are denuded more frequently with a short rather than a long bevel needle, why there is a linear relationship between increased pressure and loss of cumulus cells (Fig. 1), and why the impact of increasing pressure has more impact with thicker rather than thinner needles [26, 27]. Increasing pressure increases volume of flow and the velocity of the fluid much more rapidly in a larger than in a smaller gauge needle. Turbulent flow occurs over a longer distance in long bevel compared to short bevel needles. The turbulence on entry into the needle exerts randomly directed forces on the CCOC, which with increased velocity, may overcome the adherence of the cumulus cells to the oocyte.

Flushing

Since oocytes are embedded in a granulosa cell matrix with IVM instead of free floating in follicular fluid, the data on

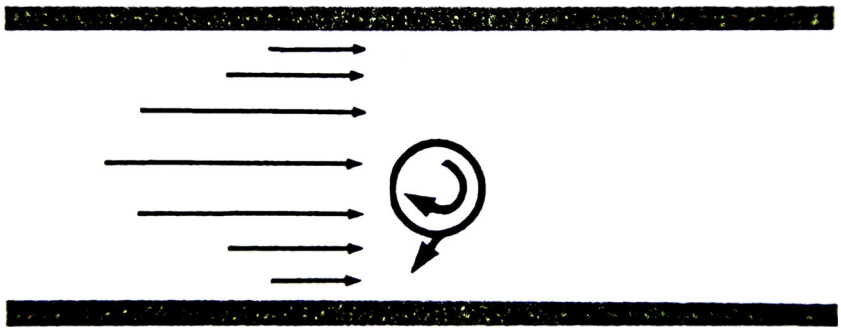

Fig. 3 Because of its size, as the oocyte moves through the needle, it lies in the path of fluids moving at different velocities. The diameter of the cumulus oocyte complex is at least 25 % of a 17-gauge needle. The oocyte cannot move straight through the needle, it must bump up against its sides

flushing in conventional IVF does not apply. For conventional IVF, several meta-analysis studies show that there is no difference in oocyte recovery with and without flushing [28–30].

Given the small volume of fluid in antral follicles and the large dead space in most single lumen needles, flushing antral follicles using single lumen needle makes no sense (Tables 1 and 3). A double lumen needle or a pseudo-double lumen needle (Steiner-Tan) is required for flushing. If a double lumen needle is used, the diameter of the aspiration channel is reduced, and pressure at the aspiration machine needs to be increased to maintain the desired velocity at the tip.

Creating turbulence within the follicle by flushing is potentially another approach to freeing the oocyte. Such turbulence could also enhance factors leading to damage of the CCOC. A benefit of flushing is that placement of the needle tip in the center of the follicle is easy to see when the follicle gently inflates and deflates like a balloon. A theoretical problem created by flushing is inadvertently pushing the oocyte out of the follicle. The needle tip may have tracked through the posterior follicle wall or may have created a large opening in the anterior follicle wall. Fluid can easily be injected into the ovary outside of the follicle, theoretically loosing an oocyte from the follicle and also leading to impaired visualization.

Available data suggests that flushing is unlikely to increase oocyte yield in IVM cycles. Fry et al. looked at flushing with a 17-gauge double lumen needle and compared it to a single lumen needle. They aspirated 1500 follicles from discarded bovine ovaries, using 50 mm Hg of pressure with and without flushing, and found no difference in the oocyte recovery rate [18].

Rose and Laky compared use of the 19-gauge Cook immature oocyte aspiration needle without flushing to the Steiner-Tan needle with flushing and found no difference in the number of oocytes retrieved [12]. Flushing was preferred for improved oocyte handling. The large volume of flush used prevented most clots from forming and enabled oocyte identification using conventional IVF methods (without needing a filter to strain the aspirate or heparin in the media to prevent clot formation). This was viewed as a significant benefit of the Steiner-Tan needle.

There is some concern that the standard approach in IVM of removing the needle to flush it and having to pass it into the vagina and ovary several times makes an IVM retrieval more traumatic than a conventional IVF retrieval [13]. Flushing enables the surgeon to leave the needle in the ovary and not remove it until all follicles have been aspirated.

Retrieval technique

Needle insertions into biological tissues can be viewed as having several phases. The first involves a boundary displacement in which the tissue is displaced or tented without being punctured. The tissue boundary deflects under the load applied to the needle. If the object being entered is not fixed (like the ovary), the force applied to the needle can be transferred into motion of that object increasing displacement without resulting in puncture. Sufficient force applied perpendicular to the boundary and the strain energy stored in the tissue from deformation result in a puncture event as the boundary is breached. Insufficient axial vector force increases tissue distortion. As the needle crosses the boundary, the load at the needle point decreases and a planar crack is created in the follicle wall. The crack size and shape depends on the needle

Fig. 4 As the cumulus oocyte complex enters the needle it experiences a period of turbulent flow and is pulled in random directions. The diagram is proportional for a 17-gauge needle with a 3 mm bevel tip

used. The crack is enlarged as the tip fully enters the follicle [11]. A larger crack, which will result from a higher degree bevel angle, is less desirable since it increases the potential of an oocyte being lost from the follicle [17].

Multiple experimental studies show that the puncture force required decreases as the insertion velocity of the needle increases and that there is less tissue displacement when the velocity of needle insertion increases [11, 31]. This is partly due to the fact that the kinetic energy at the tip increases with square of the velocity (kinetic energy=0.5 x mass x velocity squared). Thus a 25 % increase in needle velocity should increase the energy available at the tip to induce puncture by 50 %.

These observations suggest that it is desirable to insert a needle into the follicle with as much velocity as safely possible. This provides physical science support for a preference of Dr. J-H Lim on how the retrieval needle should be held during IVM retrievals (personal communication). Dr. Lim believes that the best way to undertake retrieval is to grasp the needle with the surgeon's fingertips (like a pencil) and advance it using an "overhand motion". Advancing the needle using the muscles of the fingers and wrist increases the velocity of needle insertion while maintaining control. The alternative and more common aspiration approach is to keep the table lower and advance the needle with an "underhand" motion using the muscles of the wrist and forearm. The larger follicles of conventional IVF may make either approach reasonable, but more control in needle placement is desired to retrieve oocytes from smaller follicles.

Many surgeons pass the needle into a follicle and aspirate without moving the needle. Others advocate that the needle should be rotated and moved up and back slightly. For conventional IVF, Dahl et al. showed that rotating the needle in a follicle during aspiration increased the number of oocytes obtained [32]. Some surgeons believe that this needle motion causes scraping or curetting of the wall of the follicle. It is not clear what the basis is for this interpretation. One clear benefit of gentle needle rotation is decreasing the likelihood of the needle lumen becoming prematurely blocked by a collapsing follicle wall or large debris. Rotation and axial movement may also help keep the tip of the needle inside the follicle. Needle movement is likely to make the needle's ultrasound markings easier to see and thus it will enhance the surgeon's visualization. Finally, rotating the needle may enhance penetration into a follicle that has only partially been entered and whose wall stretches under the slow advancement of the needle.

It is also desirable to apply vacuum pressure just before entering each follicle. There is an internal pressure in a follicle, which is dependent on the follicle's diameter, shape, and location in the ovary. This pressure also increases with increased distortion of the follicle by pressure from the needle prior to puncture. Some fluid (possibly containing an oocyte) may escape between the outside wall of the needle and the hole created by the puncture into the ovary. This fluid loss is

Table 4 Advantages and disadvantages of choices related to transvaginal oocyte recovery

Issue	Advantages	Disadvantages	Comments
Short bevel	Stays inside follicle Less damage to cumulus than long bevel	More force required for entry Deforms follicle more on entry	
Long bevel	Sharper Easier to enter follicle accurately	Easier to damage cumulus with higher pressures	3 mm bevel with or without diamond cut is common
Thin needle	Less dead space Less damage to cumulus as pressure increased Less pain for patient	Bends easily Harder to direct Lower oocyte recovery rate (with same pressure)	May decrease bleeding compared to larger needle
Thick needle	Easier to direct Higher oocyte recovery rate	More damage to cumulus using lower pressures	
Low pressure	Less damage to cumulus	Procedure takes longer Lower oocyte recovery	
High pressure	Higher oocyte recovery rate Shorter procedure	More cumulus damage as pressure increases Very high pressure may damage the oocyte directly	
Stable needle after entry	Less turbulence at tip	Lumen may be obstructed prematurely	
Twist needle after entry	Higher oocyte recovery May enhance penetration in stretchy follicle walls	Possible damage to cumulus Creates larger hole in follicle wall	The vigor used with twisting may have different impacts
Overhand entry	Increases velocity of puncture Decreases deformation of follicle wall by needle Increases accuracy of entry	Awkward in some patients	
Underhand entry	More natural Table can be lower Easier to twist needle	Less accurate entry	
Flushing	Useful to demonstrate needle in follicle Large volume flush with Steiner-Tan needle simplifies IVM	Not shown to increase oocyte recovery Can push fluid and oocytes outside follicle	Cannot use single lumen needles

driven by the pressure on the fluid in the follicle prior to puncture and much of the fluid loss could likely be avoided by applying the vacuum pressure prior to entering the follicle [17, 25].

After fluid has been aspirated from a follicle and the aspiration pressure has been released, it is possible for negative pressure to pull an oocyte back into a follicle. This would require a tight seal around the needle by the follicle wall. After vacuum pressure is no longer applied, the system reverts to atmospheric pressure. Negative pressure in the follicle could cause a small amount of back flow into the follicle. If the oocyte is in the last portion of fluid in the needle, it theoretically could be pulled back into the follicle [17].

Pulling the needle out of the ovary and vagina while continuing to apply vacuum pressure to the collection system, causes a massive spike of pressure within the needle. The pressure is high enough that the fluid in the needle will experience high speed turbulent flow instead of lower speed laminar flow. If an oocyte is in the fluid remaining in the needle, it will experience severe shear stress forces in route to the collecting tube with an increased possibility of being damaged [17].

Conclusion

As this paper illustrates, Trounson et al. identified many of the important details that effect follicle aspiration for IVM. The issue they highlighted as most important, using a lower pressure during transvaginal retrieval than for IVF, has been shown to be important based on multiple bovine studies and a closer look at the mechanics underlying the aspiration process. However, the situation is more complex than their original paper suggested. Lower aspiration pressures did not increase oocyte recovery. Lower aspiration pressures decreased damage to CCOCs by the aspiration process. A better way to view Trounson et al's advice for IVM oocyte recovery is that is it is necessary to balance the many issues that impact the oocyte recovery process and that this balance is likely different for IVM than for conventional IVF.

Oocyte aspiration involves stripping off a delicate complex structure from the follicle wall located differently within the ovary for different follicles and transporting it over a very long distance (compared to its size of the oocyte) in a poorly controlled and possibly damaging journey. Since the entire aspiration set up has an impact on what the COC experiences at the tip of the needle, more information needs to be supplied to make one IVM program's approach to oocyte recovery reproducible for another program. The primary issues that need to be specified are flow rate and needle gauge.

Most programs could likely improve their recovery rate by increasing their needle size and/or increasing the aspiration pressure used, but this may impair the overall quality of the oocytes recovered. The optimal aspiration pressure for human IVM has not been determined experimentally, but clearly it is related to the needle gauge used as well as the fluid flow rate it produces. Currently the literature on IVM sheds limited light on these issues.

Selection of an optimal technique for immature oocyte aspiration is further complicated by a program's approach to patient management. Thicker needles are more traumatic and cause more pain and bleeding than thinner needles, but likely could provide higher oocyte recovery rates. Table 4 summarizes the advantages and disadvantages of making different choices for the IVM retrieval. Controlled high velocity entry of the needle into follicles, gentle rotation of a needle to prevent premature blockage of the lumen and a greater awareness of when aspiration pressure should be applied have no obvious disadvantages. For other issues, there is little specific clinical data to aid in the surgeon's selection. The pressure/fluid flow that is used has to be balanced to limit damage to CCOCs and also to compensate for the decreased yield due to the using a thinner needle. Most choices will have to be made on another basis.

Because of limited human clinical studies, this review has not been able to conclude that a specific aspiration pressure or needle gauge is optimal. As originally suggested by Trounson, et al., lower aspiration pressures are advantageous for obtaining high quality oocytes. The best clinical decision to balance the effect of the retrieval on the patient and on both oocyte recovery and quality very much depends on the specifics of the particular IVM program in which it is used. The primary objective of this paper has been to emphasize this interaction of needle choice, aspiration pressure, and patient issues.

Acknowledgement The author wishes to thank Barbara Ward for the illustrations produced for this paper.

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