

Best Practices for Novice Oocyte Retrieval: Minimizing Oocyte Loss and Temperature Related Injury

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Abstract

Surgeons new to oocyte retrieval should initially focus on patient safety and the number of oocytes retrieved. This monograph reviews the basis for minor changes in technique that may limit loss of an oocyte into the abdomen or ovarian parenchyma. More significantly, it discusses the importance of maintaining fluid flow in obtaining optimally functional oocytes. A high level of awareness of the potential for minor temperature drops during oocyte retrieval is recommended, and novice surgeons are encouraged to review the embryology outcomes of their cases as a means of assessing and refining their technique.

Keywords: Follicle entry, Follicle pressure, Oocyte damage, Oocyte loss, Oocyte retrieval, Oocyte cooling, Oocyte temperature

Introduction

Surgeons who are new to oocyte retrievals require time to master some of the mechanical and procedural techniques that more senior physicians have acquired through experience. Although it is likely that novices eventually incorporate these techniques into their practice, a discussion of the principles behind them may shorten their learning curve. These technical approaches reduce the potential for both oocyte loss and oocyte damage.

Force of Insertion

The needle should be advanced briskly and with controlled precision to minimize follicle wall distension while avoiding unnecessary trauma. The greater the distension of the follicular wall on entry, the greater the increase in intrafollicular pressure [1]. All follicles have an inherent internal pressure due to fluid accumulation. Direct measurements of this pressure in 15 mm bovine follicles have shown values to be between 4 and 8 mmHg [2]. Significant distension during entry can increase the

intrafollicular pressure to as high as 60 mmHg [2]. The kinetic force generated at the tip of the needle at entry varies with the square of the velocity (kinetic energy = $0.5 \times \text{mass} \times \text{velocity squared}$) [3]. A higher needle velocity facilitates puncture with minimal distension. To facilitate rapid entry, it may be best to hold the needle with two fingers, as one would hold a pen, rather than gripping it with the entire hand which has less fine motor control [3].

Follicle Entry

The needle should be directed along the major axis of the follicle perpendicular to the follicular wall. If the needle is inserted tangentially, the force is split into two components (tangential and perpendicular), reducing effective penetration. For example, at a 45° angle, only about 71% of the applied force contributes to puncture, meaning approximately 40% more force is required to enter the follicle than with a perpendicular approach. Once in the follicle, the needle can then be moved to aspirate remaining fluid collections as they evolve.

The negative pressure at the tip of the needle is approximately 5% of that set at the vacuum machine [1]. If the vacuum is set at 100–200 mmHg, the suction pressure at the needle tip is about 5–10 mmHg. It takes several minutes to achieve full negative pressure within the collection system [2]. Once the follicular wall is punctured, follicular fluid can either enter the needle or escape alongside the needle through the newly created elastic puncture site. If intrafollicular pressure exceeds the negative pressure within the needle, fluid may leak into the abdomen or the ovarian parenchyma. Establishing maximum vacuum pressure before entering the follicle potentially minimizes oocyte loss from this pressure differential. Slower needle insertion is associated with larger puncture sites in experimental (non-follicular) tissue [1]. The vacuum should also be maintained during withdrawal of the needle; otherwise, fluid may briefly reflux through the needle and tubing back into the partially emptied follicle because the collection tube has returned to atmospheric pressure [2].

Vacuum Maintenance

Proper timing of collection tube changes is necessary to balance vacuum pressure and intrafollicular pressure. Based on bovine experiments, changes in fluid flow likely increase granulosa cell loss from the corona radiata surrounding and protecting the oocyte [4]. Follicular fluid initially enters the needle under turbulent flow, which can cause mechanical stress on the corona radiata as the oocyte physically collides with the needle wall [3]. Although flow becomes increasingly laminar as aspiration stabilizes, shear forces still vary between the edges and center of that stream, particularly in narrower needles [3]. A drop in vacuum pressure can reintroduce turbulence, which may further increase stress on the corona radiata. Potential damage to the granulosa cells surrounding an oocyte is especially important in In Vitro Maturation (IVM) cases, where oocytes are surrounded by relatively few granulosa cells. Complete loss of granulosa cells significantly impairs these oocytes. For example, Hwang et al. [5], collected immature oocytes during cesarean section and found that if the cumulus cells were intact, 56.6 % of the oocytes progress to metaphase II, but if the cumulus was denuded, only 29.2% progressed ($p < 0.01$). Significantly, 23.1% of cumulus enclosed oocytes were visibly damaged; whereas 46.7% of cumulus denuded oocytes were visibly damaged ($p < 0.01$).

Collection Speed and Oocyte Cooling

The speed of the oocyte retrieval process is critically important for minimizing oocyte cooling. Although some degree of cooling is unavoidable during every retrieval procedure, both the magnitude of the temperature decrease, and the duration of exposure should be minimized. Even relatively small temperature reductions could disrupt the meiotic spindle through depolymerization of its microtubules.

Wang et al. evaluated human *in vitro*-matured metaphase II oocytes exposed to varying degrees of cooling and assessed spindle integrity using polarized light microscopy. They found that a temperature reduction of only 4°C for five minutes resulted in near-complete disappearance of the spindle signal, as evidenced by a marked decrease in retardance, a surrogate measure of microtubule density. Although all five oocytes in this group subsequently demonstrated spindle recovery after rewarming, recovery rates declined sharply with greater temperature reductions. Only 2 of 5 oocytes recovered after cooling to 28°C, and none recovered after cooling to 25°C. In one experiment, spindle disassembly was observed within 90 seconds of cooling. Furthermore, even when spindle reassembly occurred following more severe cooling, the density of microtubules remained lower than baseline, suggesting that recovery may be incomplete [6].

The clinical significance of spindle integrity was highlighted by Garcia-Oro et al. who demonstrated that oocytes with a visible meiotic spindle had higher fertilization rates and lower rates of abnormal fertilization than oocytes in which a spindle could not be visualized. Among transferred embryos, implantation rates were 32.9% when the originating oocyte exhibited a visible spindle, compared with only 8.8% when no spindle was detected. In addition, implantation rates correlated positively with spindle retardance, indicating that greater microtubule density was associated with improved reproductive outcomes [7].

Pickering et al. used surplus human oocytes to investigate the effects of cooling duration on spindle and chromosome integrity. Exposure of oocytes to room temperature for either 10 or 30 minutes resulted in spindle abnormalities in the vast majority of oocytes, even after rewarming. Chromosome dispersal was also commonly observed, particularly following longer periods of cooling, and individual chromosomes were occasionally found displaced from the metaphase plate [8].

The degree of cooling experienced by oocytes in the Pickering et al. study was not precisely specified. The investigators placed dishes containing oocytes in approximately 100 µL of medium under oil at room temperature for predetermined intervals, meaning that the temperature of the medium likely remained appreciably higher than ambient conditions [8]. Almeida and Bolton examined this issue more directly by exposing human oocytes to a room temperature of 23°C for 2, 10, and 30 minutes while suspended in 50 µL droplets of medium under oil. At the end of these exposure periods, the measured temperatures of the culture medium were approximately 32°C, 27°C, and 25°C, respectively. Using anti- α -tubulin immunostaining, parthenogenetic activation, and cytogenetic analyses, the investigators demonstrated progressive spindle disruption, chromosome dispersal, reduced rates of oocyte activation, and increased chromosomal abnormalities with greater degrees and durations of cooling [9].

The increased prevalence of meiotic spindle abnormalities in oocytes from older women raises the possibility that their oocytes are particularly vulnerable to mild or moderate cooling [6,10,11]. This possible problem has not been evaluated using human oocytes. However, after cooling-induced spindle depolymerization, successful recovery requires both reassembly of the microtubule spindle and precise chromosome realignment. These processes are safeguarded by the Spindle Assembly Checkpoint (SAC), which ensures proper chromosome attachment before cell division proceeds. Evidence from a knockout mouse model reported by Blengini et al. indicates that SAC function declines with maternal age, providing a biologically plausible mechanism by which aging oocytes may be less capable of recovering from temperature-induced spindle disruption and, consequently, may be at greater risk for chromosome segregation errors [12].

The impact of decreased temperature in a human clinical setting on fertilization, embryo development, and implantation is difficult to directly study. Crane et al. [13] looked at the interval from oocyte aspiration to placement of the oocyte in the laboratory incubator and found that elapsed times were longer in nonfertilized compared to fertilized oocytes. Although this study did not directly look at temperature, temperature drops are a potential consequence of longer time intervals between ovary and incubator. Pollard et al. [14] looked at bovine blastocyst production using oocytes from slaughterhouse ovaries collected at various temperatures for use in IVF. They found both decreased blastocyst production and fewer cells in the blastocysts produced from oocytes collected at lower temperatures.

To recapitulate, cooling may cause oocyte damage with more severe cooling or prolonged duration of the temperature drops leading to unreparable depolymerized spindles, reduced fertilization rates, slower cleavage/embryo cellularity, decreased blastocyst formation, decreased implantation rates, and increased aneuploidy.

Significant oocyte cooling during retrieval most likely commonly takes place in the needle and collection tubing [8]. The internal volume of the 33 cm needle and 75 cm aspiration tubing is typically about 1.2 mL, meaning that fluid from a medium-sized follicle will remain in the tubing until displaced by subsequent aspiration [3]. The collection tubing is thin-walled, has a small diameter (approximately 1 mm), and has a large surface area (generally 55–65 cm²), which allows rapid heat loss. Calculated temperature losses using published heat transfer coefficients for small plastic tubing applied to Newton's law of cooling suggest a temperature loss over time to be approximately 3.6°C after 30 seconds, 6.2°C after 60 seconds, 8.2°C after 90 seconds, 9.7°C after 120 seconds and 11.6°C after 180 seconds (For calculations and specific assumptions see **Supplementary Material**). These calculated estimates were consistent with the temperature drop findings in a simulated retrieval at our laboratory using

a Wallace oocyte recovery set with a 17 g 33 cm needle with 750 mm transfer tubing (ONS 1733-500- 750, Cooper Surgical, Trumbull, CT). Thus, once the fluid in the transfer tubing has been static for 180 seconds, tubal fluid can almost reach room temperature. If there is a delay in fluid flow, for example, when repositioning the needle to access a difficult follicle, it is preferable to flush the system and resume with an empty needle. Such delays are easy to overlook amid the many considerations being processed by the surgeon's mind. Short delays may be damaging, but as noted above, within 120 seconds, up to 50% of oocytes have been reported to fail to recover properly after re-warming, potentially leading to chromosomal loss. Therefore, minimizing both the degree and duration of cooling is essential. Unintended brief interruptions in the aspiration procedure may be more important to patient outcome than any other action that the surgeon does or does not undertake during the retrieval.

Surgeon-Specific Effects

The novice may assume that when aneuploidy occurs or the patient has no or few euploid blastocysts, it is because of the patient's underlying pathology or perhaps because of problems in the embryology laboratory. They should consider whether aspects of their retrieval technique, including oocyte cooling, may have contributed to the outcome. Surgeons do not provide an identical prognosis for individual patients. We compared the oocyte collection rate, fertilization rate, and blastulation rate among several experienced surgeons over a three-year interval and found statistically significant differences [15]. The differences were not clinically important, but they illustrated that there are subtle differences in surgical techniques possibly related to minor variations in managing oocyte cooling.

A developing surgeon can examine the embryology records and determine if their outcomes meet the norms for oocytes with optimal morphology, absence of degenerated oocytes, fertilization and blastulation rates within their practice using the same ovulation induction protocols and embryologists. If this data is not available, norms obtained from published literature could be pursued.

Conclusion

With the best of intentions, a surgeon may fail to optimize the retrieval of oocytes because of minor variation in technique. They should have a heightened awareness that oocyte cooling always occurs during retrieval and try to minimize its impact by limiting both temperature drops and the duration of those drops. Optimizing performance to achieve this goal is likely one of the more difficult challenges in developing good techniques. Unless retrieval-related damage is specifically assessed, its effects may remain unrecognized and be incorrectly attributed to patient or laboratory factors.

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Author contributions

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Ethics approval

This manuscript did not involve human or animal subjects and ethics committee approval was not required.

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