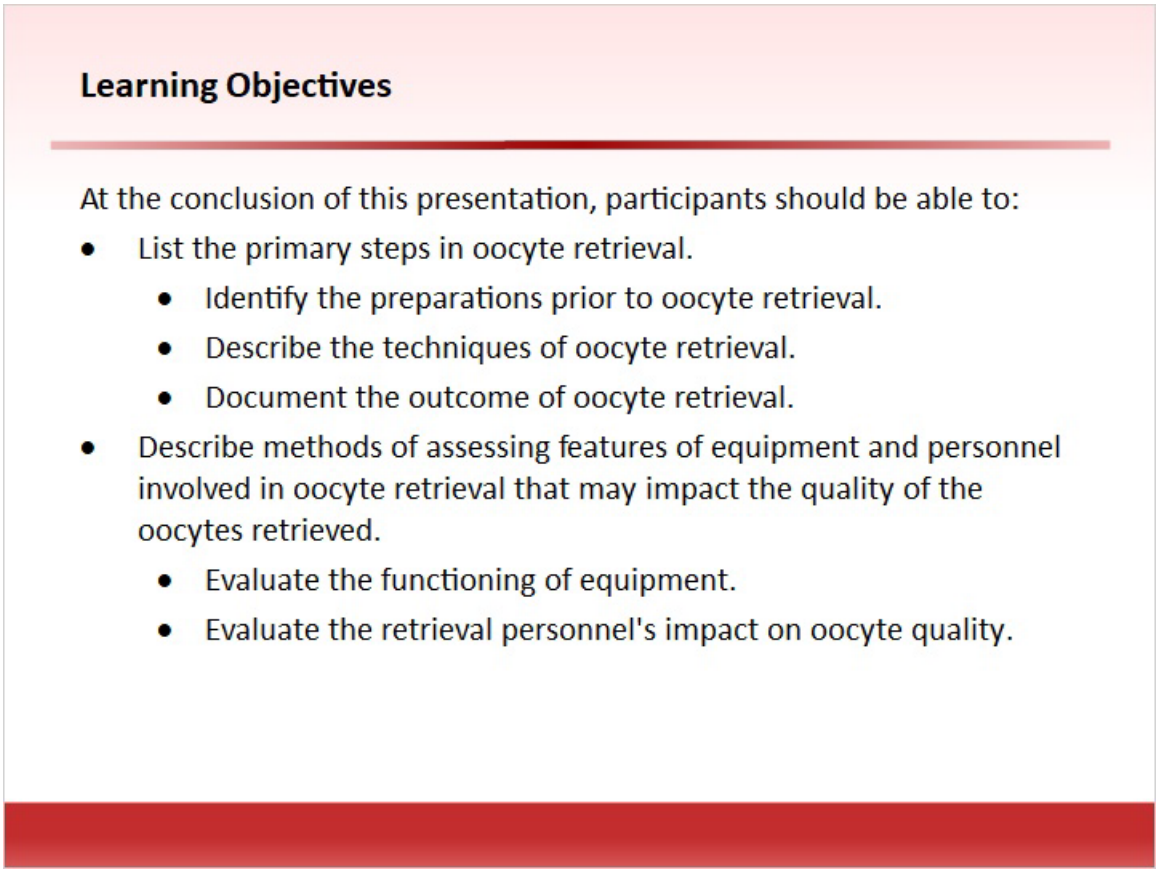
LABCC100 Lesson 15

1.1 Oocyte Retrieval for Assisted Reproductive Technology

Notes:

Welcome to the American Society for Reproductive Medicine's eLearning modules. The subject of this presentation is Oocyte Retrieval for assisted reproductive technology.

1.2 Learning Objectives

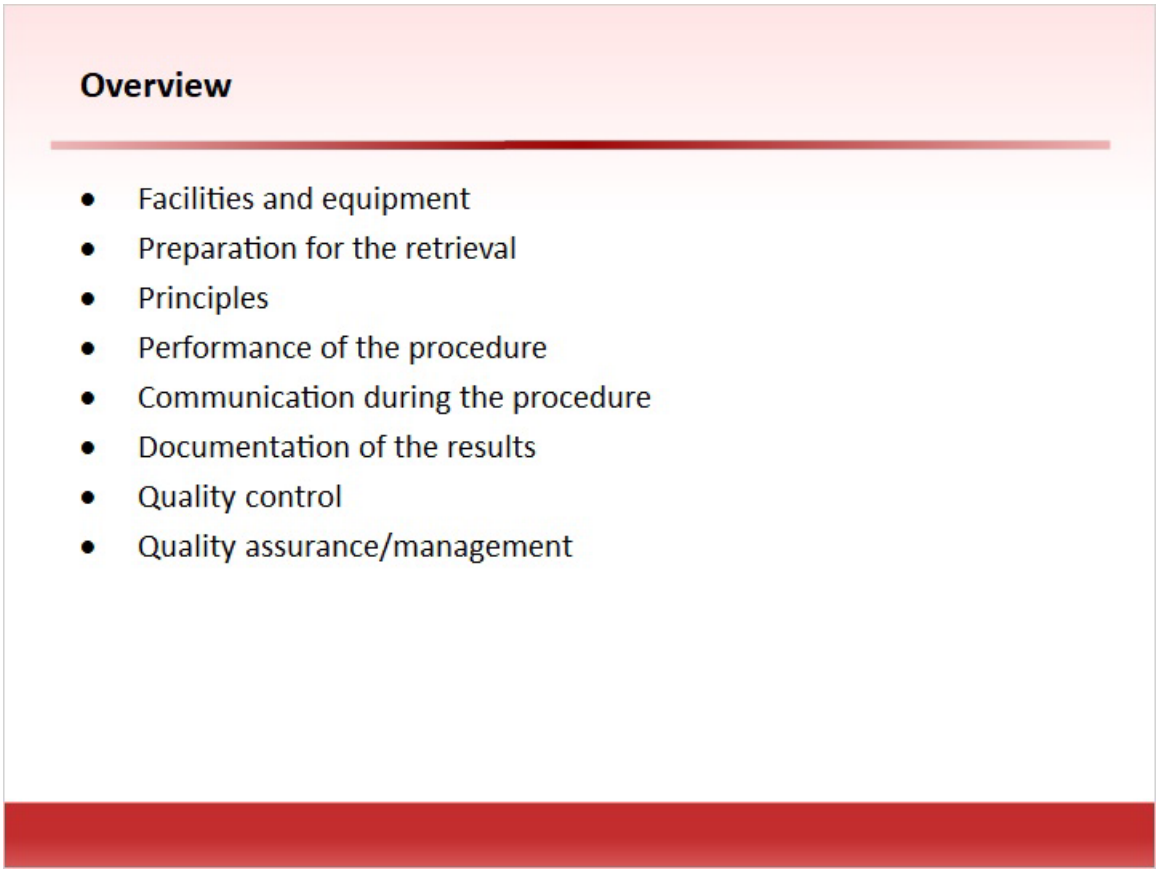


Notes:

At the conclusion of this presentation, participants should be able to:
List the primary steps in oocyte retrieval.
- Identify the preparations prior to oocyte retrieval.
- Describe the techniques of oocyte retrieval.
- Document the outcome of oocyte retrieval.

Describe methods of assessing features of equipment and personnel involved in oocyte retrieval that may impact the quality of the oocytes retrieved.
- Evaluate the functioning of equipment.
- Evaluate the retrieval personnel's impact on oocyte quality.

1.3 Overview



Notes:

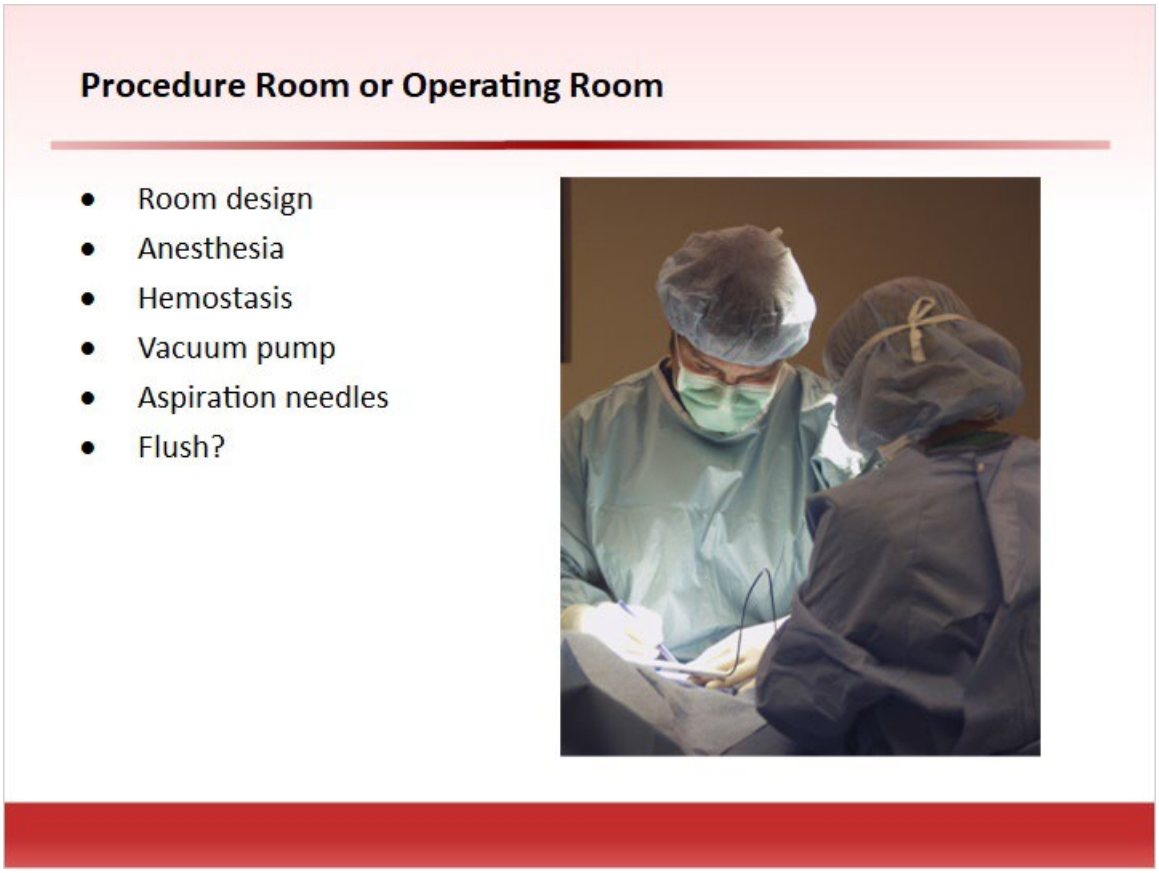
The following topics will be covered in this presentation:
The facilities required in which to perform an oocyte retrieval, including the equipment needed;
Tasks that should be accomplished prior to performance of the retrieval;
Principles that should be adhered to during performance of the retrieval;
Eight specifics steps performed during the retrieval;
Communication between the laboratory and the clinician during the retrieval;
Documentation of the results of the retrieval;
Quality control measures that must be performed prior to the retrieval to assure that equipment is functioning as intended; and
Quality assurance measures that may be helpful in assessing the performance of the retrieval.

1.4 Facilities and Equipment

Notes:

In this section the physical space and the equipment required to perform an oocyte retrieval will be discussed.

1.5 Procedure Room or Operating Room



Notes:

The procedure room should be a room designed to maintain the patient in a position that makes the physician's job easiest, while providing the patient with comfort and privacy. In addition, it should be a space with an environment that will be optimal for the collection of oocytes and maintenance of their viability from aspiration until the oocytes are in the embryology laboratory.

Most programs perform oocyte retrievals while the patient is consciously sedated. However, should there be an emergent need for laparotomy or laparoscopy, means of anesthesia should be available, either onsite, or within a short transportable distance.

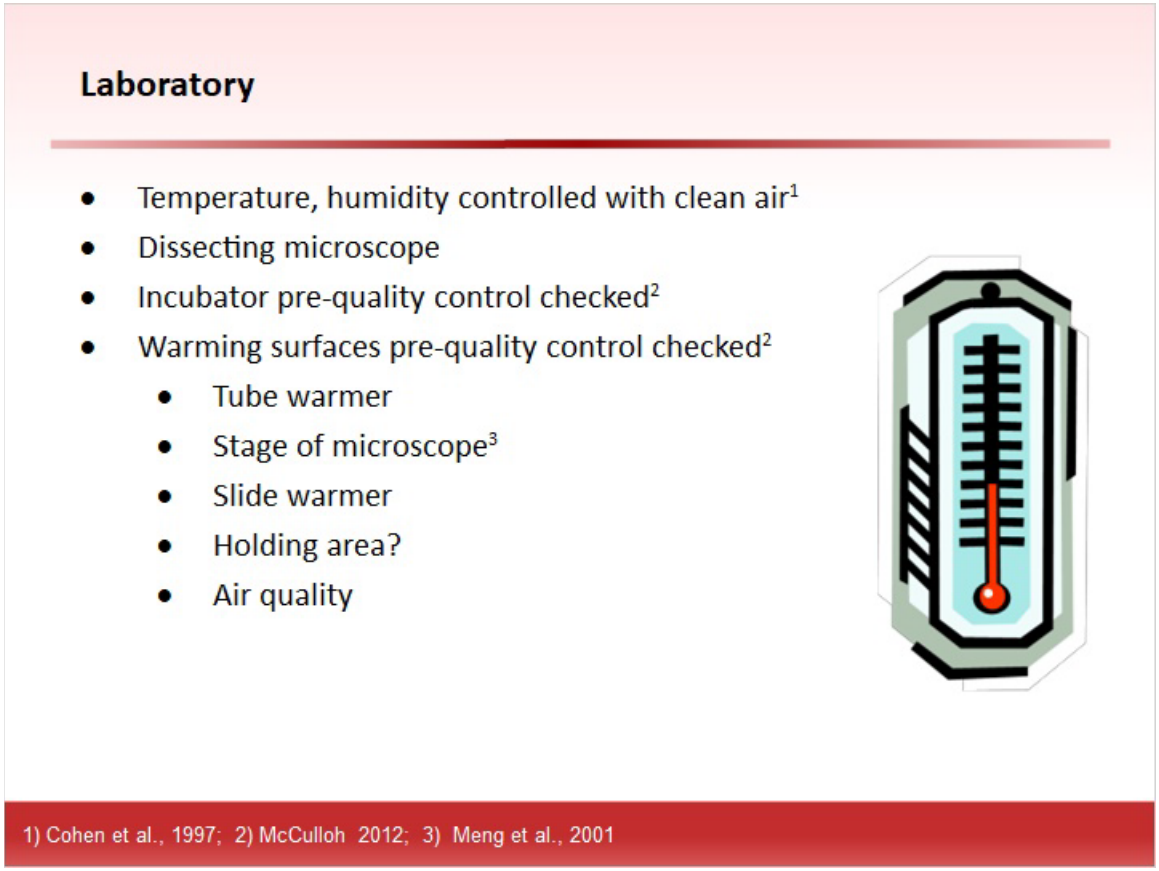
Retrievals involve the insertion of aspiration needles into the peritoneal cavity, usually through the vaginal wall, but occasionally through the abdominal wall. With repeated insertion of the aspiration needle, there is bleeding and hemostasis is commonly required after completion of the oocyte collection. The room should provide the means to accomplish this.

Aspiration of follicular fluid requires the insertion of an aspiration needle into the

graafian follicles of the woman's ovaries and application of suction to draw the follicular fluid out of each follicle and deposit it into a tube for examination. The suction may be provided by a vacuum pump or by syringes and the suction method must be available in the room at the time of the procedure.

Some programs prefer to flush fluid into the follicles after aspiration. This fluid is typically culture medium or a simple isotonic salt solution. There should be a mechanism in the procedure room throughout the duration of oocyte retrieval to maintain the flushing medium at the appropriate pH, by equilibration with CO_2 if necessary, and at body temperature.

1.6 Laboratory



Notes:

The embryology laboratory should be a room with controlled temperature and humidity so that sensitive-temperature controlled devices such as incubators and warming surfaces do not undergo fluctuations or unusual offsets due to the ambient conditions in the lab. The room should be located conveniently to the operating room or procedure room to minimize exposure of follicular aspirates to

uncontrolled environmental conditions during transit to the laboratory.

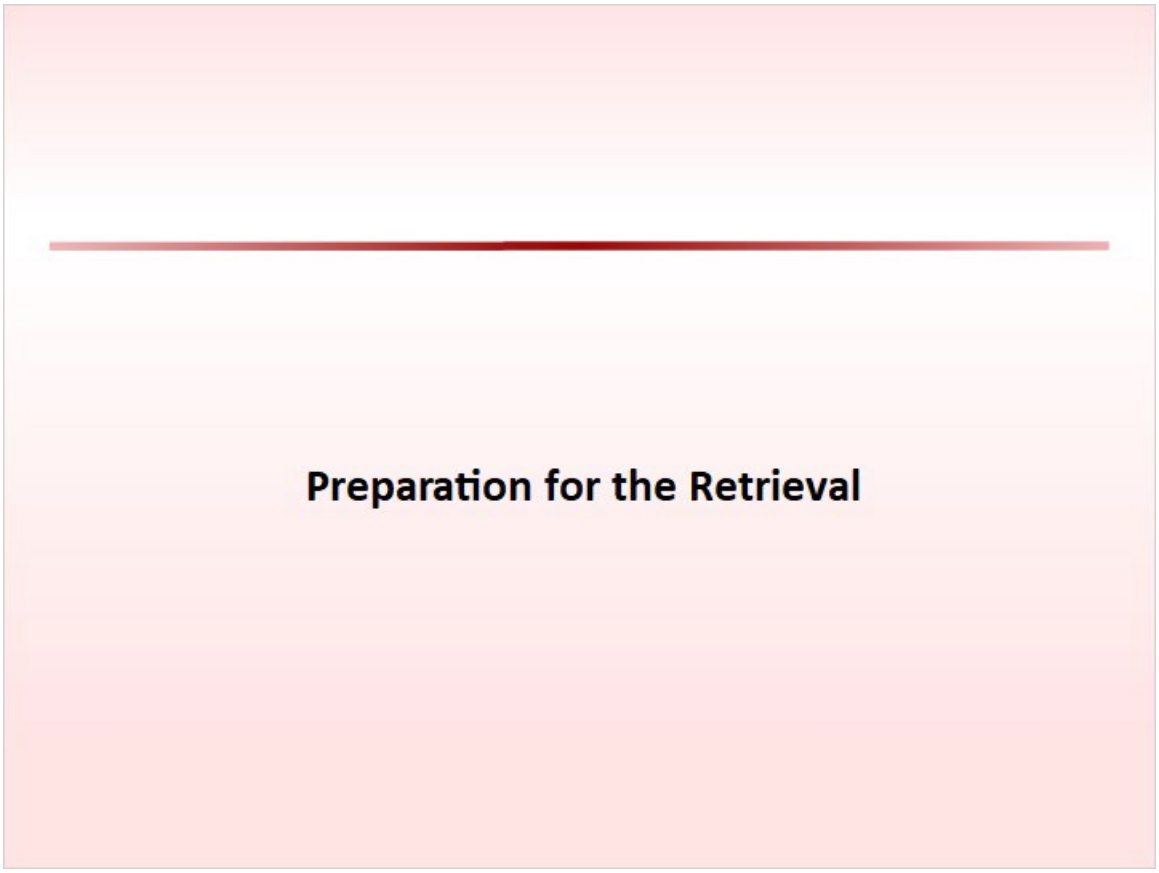
Many programs locate the embryology laboratory immediately adjacent to the procedure room. Other programs prefer to use a re-engineered pediatric incubator with a temperature, humidity, and CO_2-controlled interior, and fitted with a dissecting microscope. This incubator may be wheeled into the procedure room, and the laboratory portion of oocyte retrieval can be performed by a technician working with the follicular aspirates and identified oocytes while they are maintained in the incubator's controlled conditions.

The embryology laboratory where oocytes will ultimately reside should be equipped with incubators that have undergone quality control to assure that their temperature, gas concentrations, and humidity are within tolerable ranges specified by the laboratory. Whereas appropriate pH and osmolality of media have been documented and targets for these values are well known, the ideal temperature for embryo culture has not really been established (McCulloh, 2012). Generally, laboratories rely upon the notion that the ideal culture temperature to mimic body temperature is 37.0º C. Temperature is considered quite important, at least in part, due to the temperature sensitivity of the meiotic spindles (Meng et al., 2001).

The embryology laboratory should have sufficient equipment to perform the embryology tasks anticipated, such as dissecting microscope(s) for examination of cumulus masses, corona radiata, and oocytes, and warmed surfaces (stage of microscope, tube warmers, large surfaces for dishes in which follicular aspirates are examined), should be preventatively maintained and checked frequently as a quality-control exercise.

Air quality in the laboratory has been the subject of great concern during recent decades (Cohen et al., 1987). It is notable that gametes and embryos in culture do not have the advantage of organ systems in the body to help detoxify their environment (immune system, lungs to limit exposure to environmental gases, liver to remove toxins from the body fluids). Therefore, similar systems should be employed to limit the gametes' and embryos' exposure to airborne agents while maintained in vitro. Systems such as HEPA and carbon filtration have been created to remove particulates and minimize the presence of volatile organic compounds. Minimization of redox reagents using potassium permanganate is another technique. Systems like these should be considered, especially in the typical urban setting of an IVF facility.

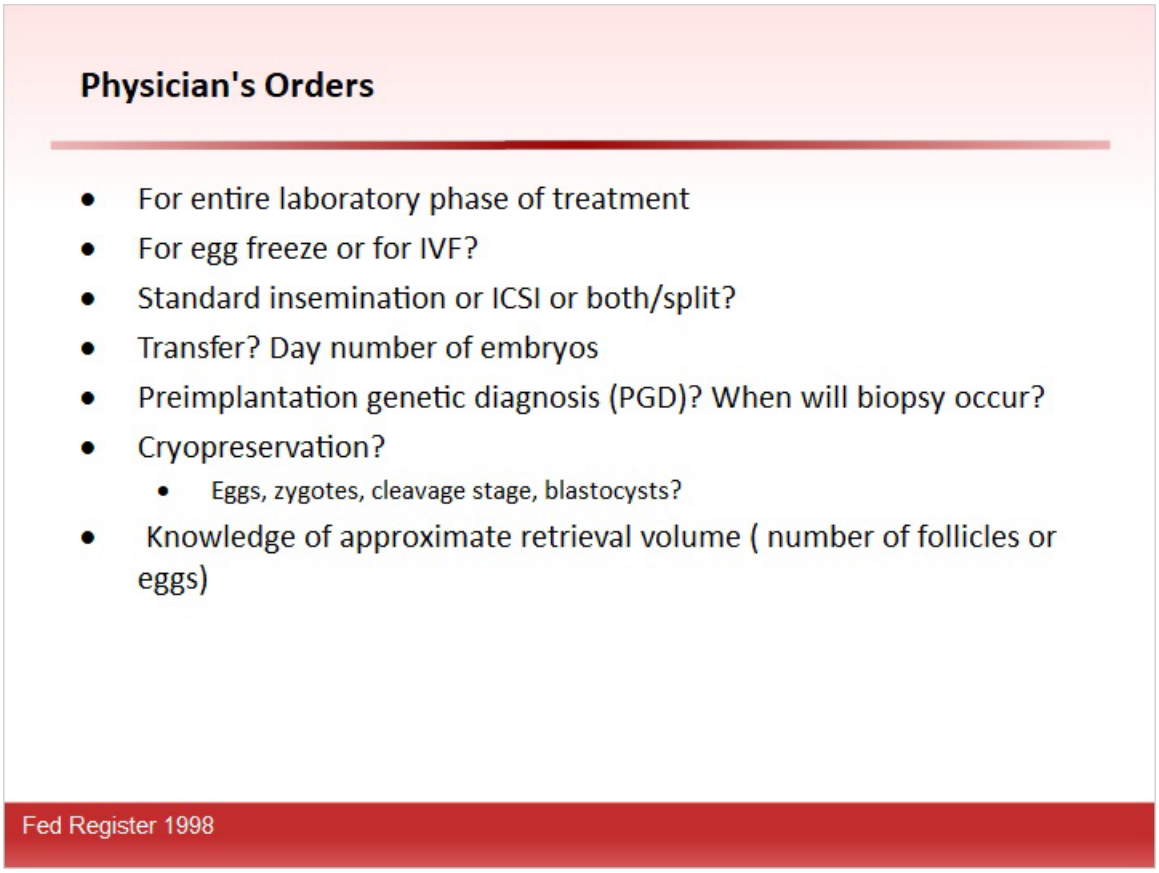
1.7 Preparation for the Retrieval



Notes:

Several tasks that are important to perform in preparation for oocyte retrieval include: obtaining written orders from the physician, indicating what procedures are to be performed following oocyte retrieval; preparing the culture dishes and equilibrating them; setting up the area for oocyte retrieval procedure; and having some idea of the magnitude of the procedure so that the technician may be mentally prepared for what is expected.

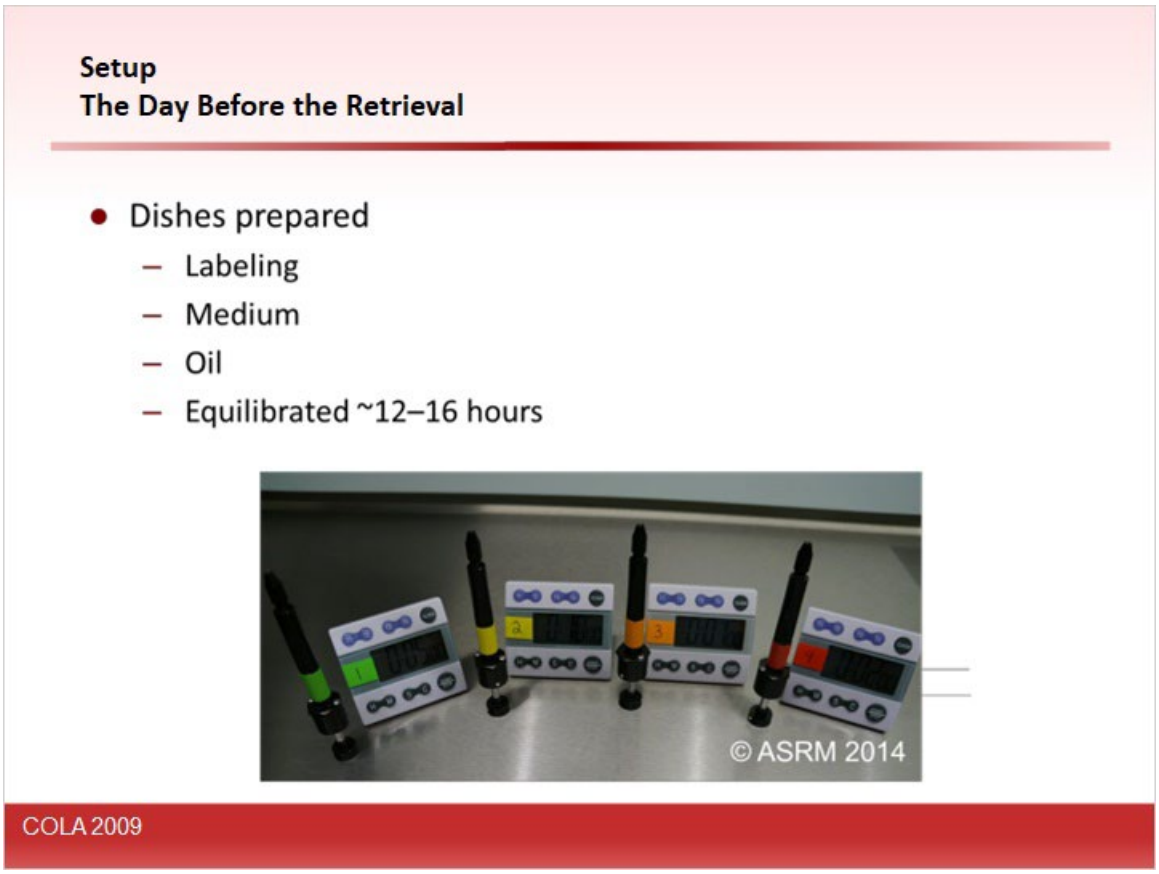
1.8 Physician's Orders



Notes:

Physician's orders should be written unambiguously so that the laboratory knows what procedures must be performed with the oocytes retrieved. Written orders may be maintained on paper or electronically and are generally expected to be provided on requisitions furnished by the laboratory. Not knowing the orders prior to the retrieval may lead to confusion and delays in processing or even inappropriate treatments of the oocytes. The orders should include the intent of oocyte retrieval for oocyte freezing or for fertilization; whether insemination will be via standard co-incubation of the oocytes with sperm or via intracytoplasmic sperm injection (ICSI) or whether some oocytes will have standard insemination and some ICSI; whether the patient is intending to undergo transfer (replacement) of fresh embryos from this retrieval or whether the embryos are all intended for cryopreservation; and at what stage should cryopreservation be performed. Finally, it is extremely helpful for the technician performing the retrieval to have some idea of the number of oocytes anticipated. It allows the technician to be emotionally prepared for a long retrieval with many oocytes or for a short retrieval with few oocytes and possibly more extensive examination to find the small number(s) of oocytes expected.

1.9 Setup



Notes:

Some of the materials necessary for oocyte retrieval require preparation well ahead of time. These generally include those materials involving the use of culture medium that must be equilibrated to attain appropriate temperature and pH (through equilibration with elevated levels of CO_2 inside the incubator). Dishes for culture are generally prepared the day before oocyte retrieval so that the culture medium can equilibrate overnight (12-16 hours).

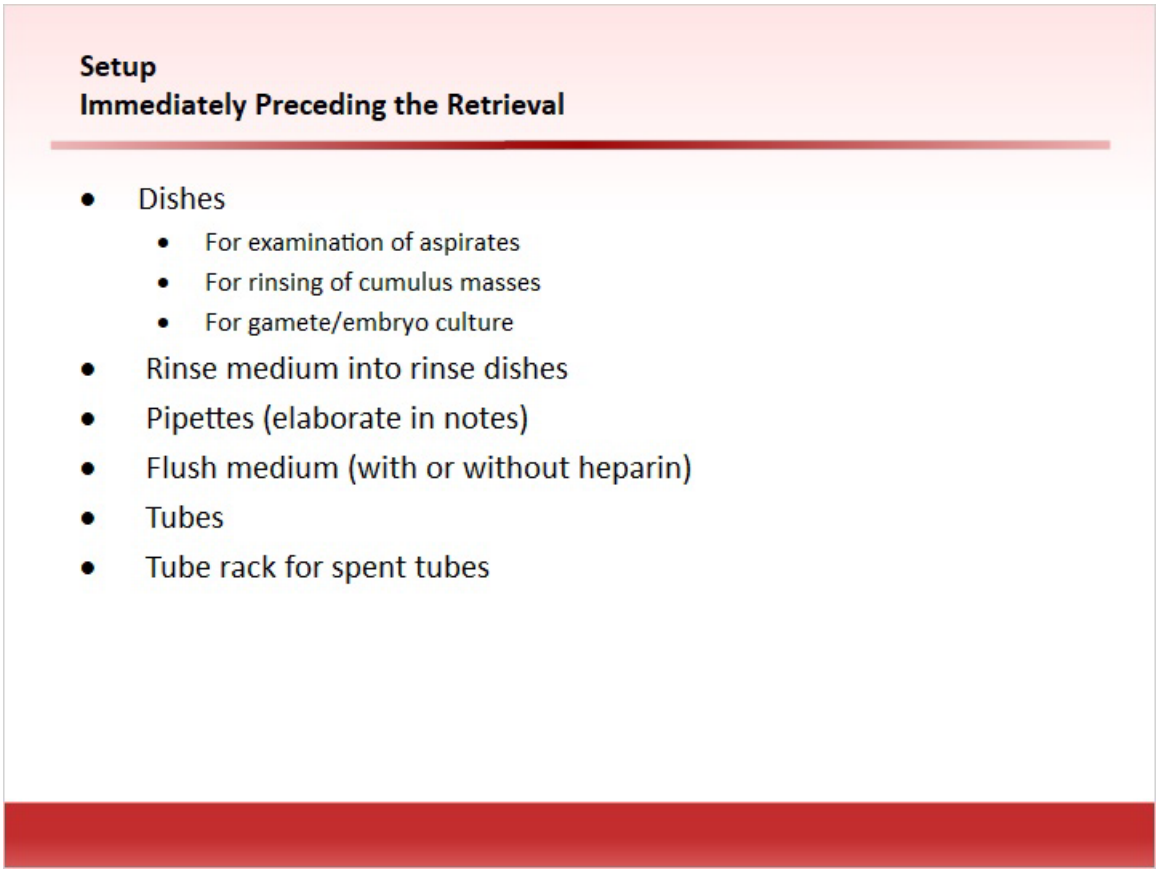
Prior to putting medium in the dish, it should be labeled unambiguously with at least 2 unique identifiers for the patient. It is common to use the patient's name on the dish but since names can be similar, especially in programs with larger numbers of procedures, it is important to use an additional unique identifier on the dish so that the dish identifier may be compared to and matched with the patient's identification immediately prior to oocyte retrieval. Some programs use a truly unique identifier, such as a unique medical record number maintained for each patient at the facility. Other

programs may use a patient's social security number or driver's license number. However not all patients have these. Dates of birth are not really unique, but the combination of patient name and date of birth improves uniqueness over the name or the date of birth alone. Regardless of the method used, care must be taken to avoid having two patients with similar identifiers in the same proximity during the laboratory process.

Further steps may be taken to ensure that patients' gametes are appropriately associated. Many programs use a color-coding system to match sperm to oocytes and to match cultures to paperwork throughout laboratory procedures. When color coding (use of colored tape on cultureware and paperwork), the designation of color requires some thought to avoid matching another patient's color throughout their time in the lab. The color designation must be made before dish preparation so that the color is applied to the cultureware prior to equilibration.

Once the dishes have been labeled appropriately, medium should be introduced into the dishes in volumes specified by laboratory policy. Today, most programs cover media in dishes with a layer of oil that creates a diffusion barrier between the medium and environmental conditions. This layer of oil serves to slow temperature fluctuations and variations in CO_2 levels that affect pH when incubator doors are opened or dishes are removed from the incubators. The use of oil to slow diffusion of heat and gases is advantageous only after the dishes have been equilibrated. However, one disadvantage is that the oil layer also necessitates a longer equilibration period to attain desired conditions prior to oocyte retrieval. Therefore, it is quite common to equilibrate dishes 12-16 hours before oocyte retrieval.

1.10 Setup



Notes:

The retrieval technician ensures that everything is immediately available (within arm's reach) prior to the beginning of the retrieval. These items should be assembled near the dissecting microscope used for oocyte retrieval.

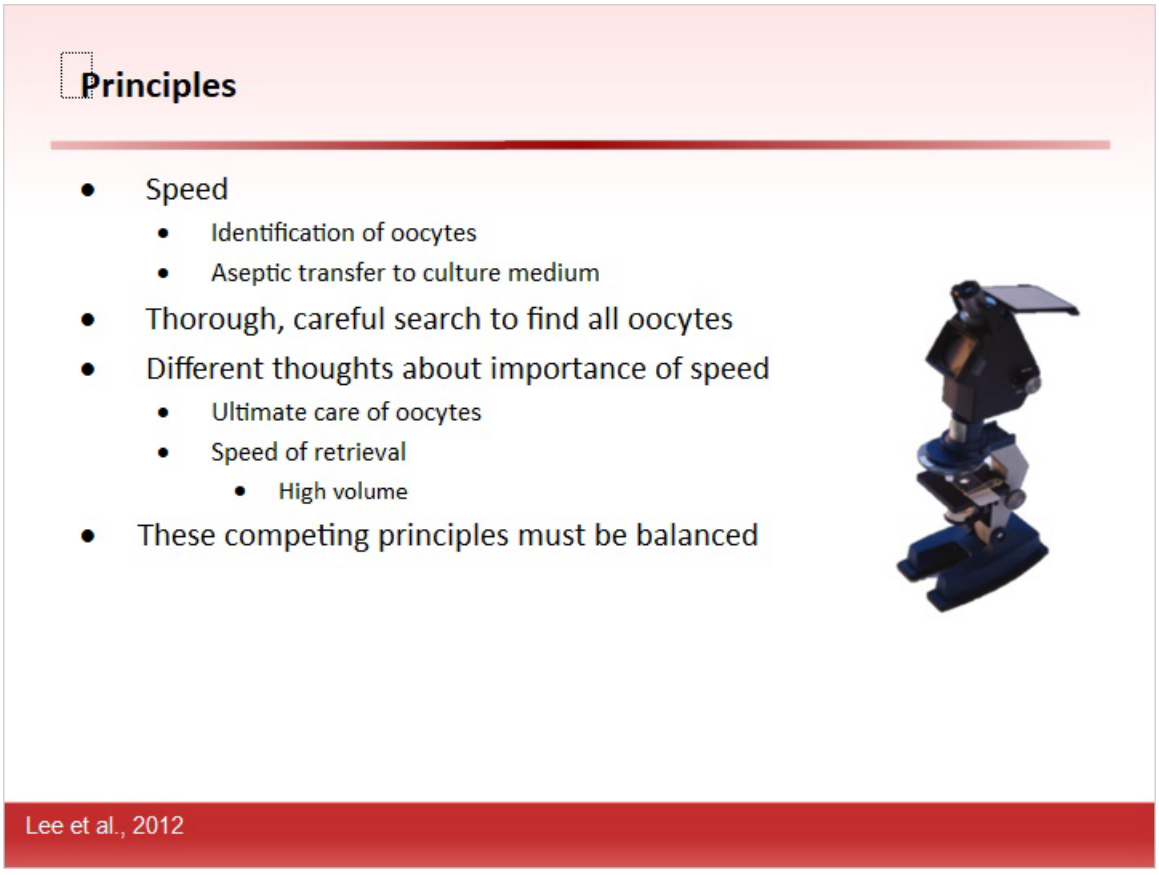
During the retrieval, the technician will need:

- A tube warmer to hold tubes of aspirates until they are examined
- Dishes for examination of the follicular aspirates
- Dishes with some type of warmed, isotonic, pH-appropriate medium for rinsing cumulus masses (this could be culture medium or a simpler version buffered with HEPES or MOPS buffer for use in room air)
- Culture dishes, pre-equilibrated
- Pipettes for moving the cumulus masses from dish to dish (these could be large stripper tips or sterile Pasteur pipettes or plastic transfer pipettes)

- A rack for emptied aspirate tubes

In some facilities the tubes used for aspiration are maintained in the laboratory and presented to the clinical team in the procedure room prior to the retrieval. If the facility flushes follicles during oocyte retrievals, flushing medium that has been pre-equilibrated should be provided to the clinical team in the procedure room. Inclusion of an anticoagulant such as heparin in the flushing medium varies from facility to facility. While some prefer to use an anticoagulant, others avoid using any additional additives that require further quality control and whose influence on fertilization and embryonic development may be unclear. Heparin, generally, is not necessary.

1.11 Principles



Notes:

It is important that personnel performing oocyte retrieval are aware of their facility's specific principles for oocyte retrievals so that expectations are met

during the procedure.

Most facilities have some expectation that aspirates will be examined with some particular speed. In some programs, this speed is dictated by the speed at which aspirates are accumulated by the clinical team. In others, the speed of aspiration is dictated by the speed at which the aspirates can be examined in the laboratory. Regardless of the specific policy, aspirates should be identified quickly enough that the aspirated fluid (often containing considerable blood) does not clot before it is examined.

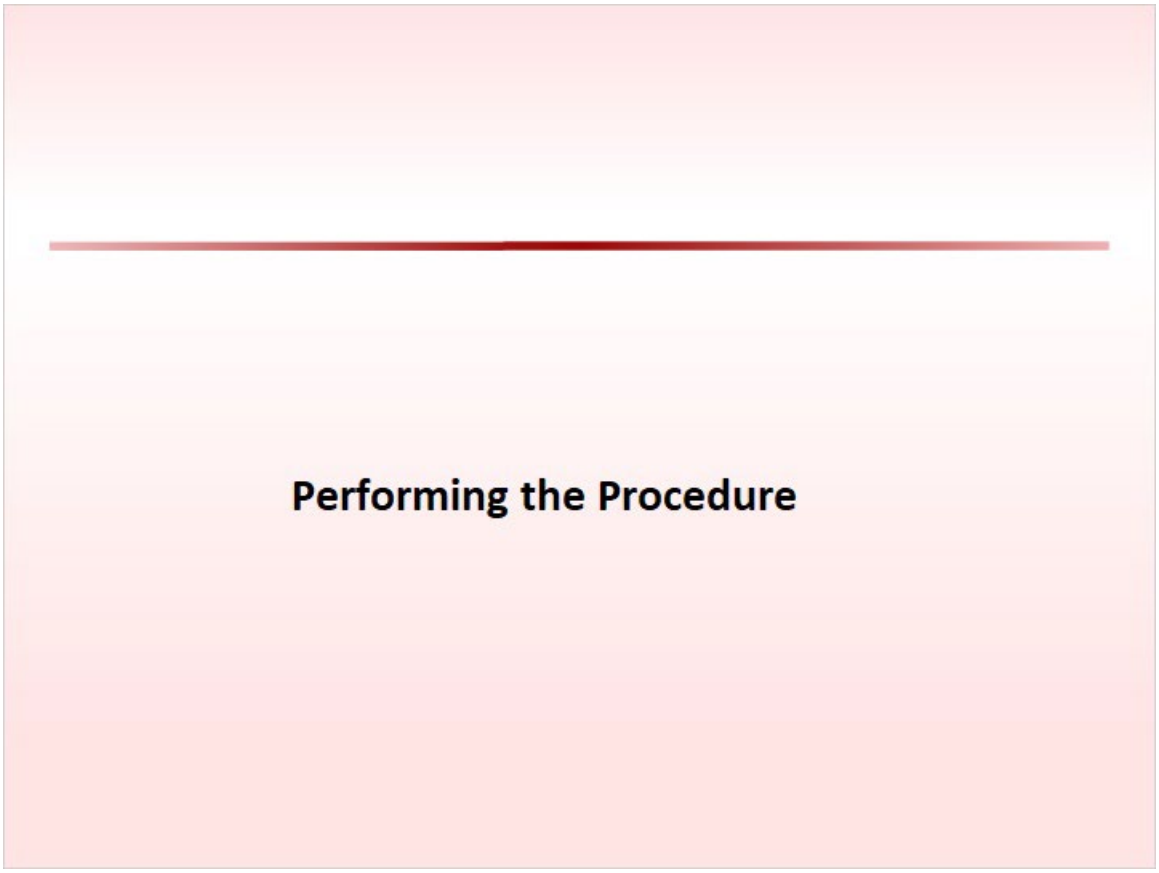
The most basic principles are that

1. Oocytes are found and moved to medium quickly and aseptically so as not to contaminate the culture medium with exogenous pathogens.
2. The aspirates should be examined completely enough to assure that all oocytes are found.

In general, the time of examination is when the oocytes are exposed to adverse conditions. Uncontrolled conditions may affect oocytes/embryos in culture (Lee et al., 2012), so the time between aspiration, identification, and placement in the incubator should minimize adverse exposure of the oocytes to uncontrolled environmental conditions.

The desire to minimize exposure is at odds with the desire to be complete and not miss any oocytes. Due to this dichotomy, the technician performing the retrieval must balance both speed and completeness.

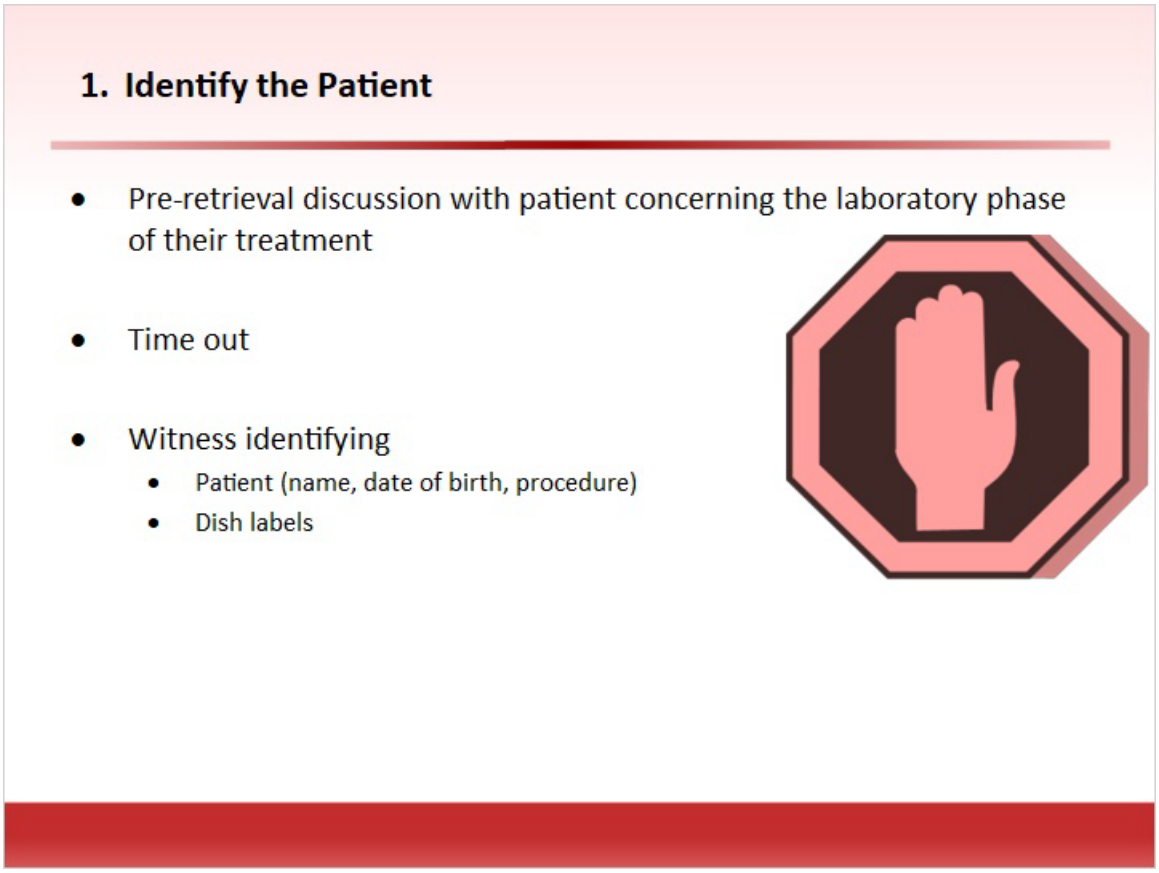
1.12 Performing the Procedure



Notes:

In this section, oocyte retrieval process has been subdivided into 8 steps. Each is described in detail.

1.13 1. Identify the Patient



Notes:

Maintaining the identity of all specimens is one of the tasks that patients take for granted in ART. They never anticipate that there could be misidentification. However, few are aware of the extensive efforts that are employed to ensure the identity of specimens is maintained at all times.

Prior to the patient losing the ability to communicate during the induction of conscious sedation, she should be involved in the identification process. Patients are required to undergo identification in most hospitals and wear some type of identification, often a wrist band with the patient's name and unique identifiers. This type of identification is a convenient way to check the patient's identity and compare it with the identification on both the paperwork and dishes before the retrieval begins.

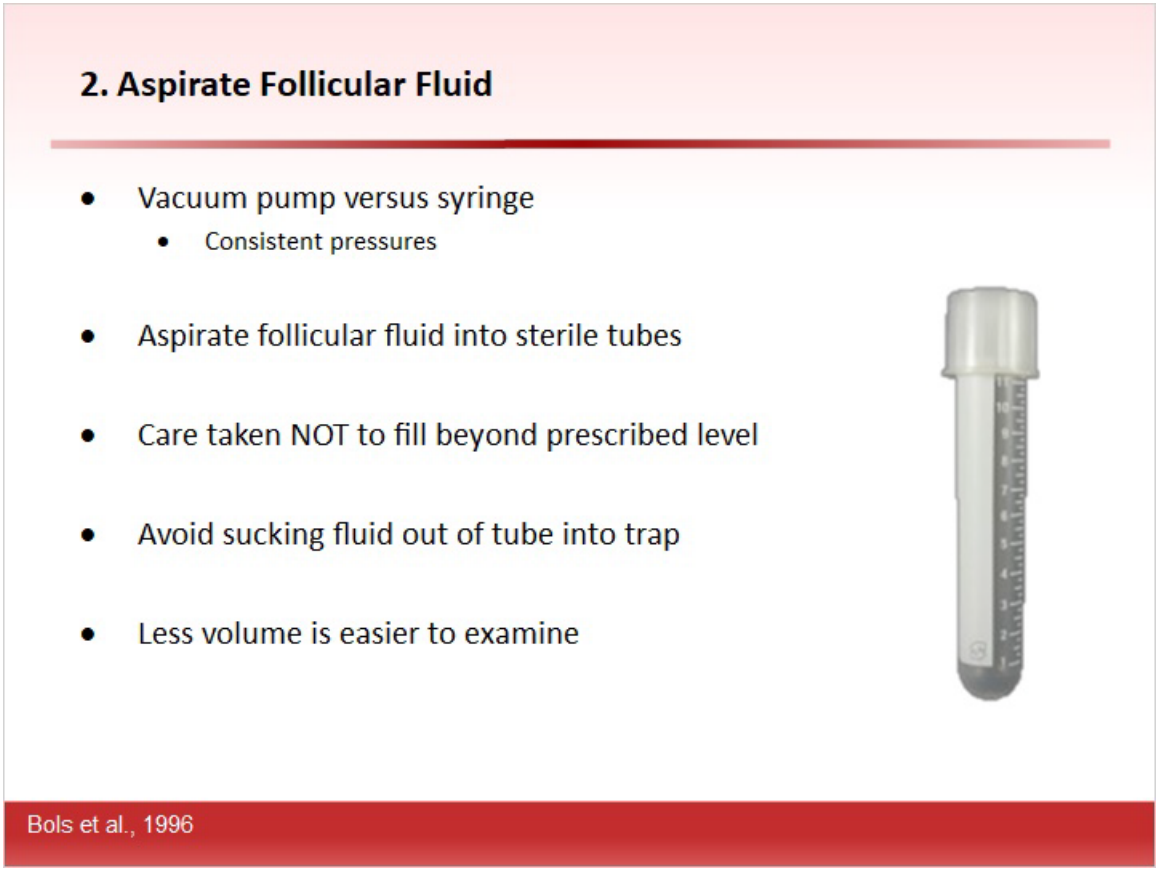
With a patient undergoing anesthesia, it has become more and more prevalent in health care facilities for the clinical team to perform an assessment (a “time-out”) during which all parties in the procedure room stop what they are doing and participate in identifying the patient and stating the nature of the procedure to be performed. Identifiers typically include the patient name, date of birth, and

procedure. This is done while the patient is still competent to agree or disagree so that the patient can also affirm her identity and the reason for being there. This is an ideal time for laboratory personnel to confirm the patient's identity and the identifying inscriptions on the dishes to be used for the patient.

As a further check, most programs now employ a laboratory witness to confirm the identity of the patient and the names on the dishes; thereby performing a double-check of the identifiers on the dishes confirming that it is the same as the patient's identifiers.

Use of these multiple checks has 2 purposes: 1) to ensure that the patient's identity is known and associated with all of her oocytes, and 2) to assure the patient that multiple steps are taken to ensure that her oocytes and embryos are identified in all procedures by multiple individuals at each crucial step.

1.14 2. Aspirate Follicular Fluid



Notes:

Following patient identification and induction of sufficient sedation to maintain

patient comfort, the vagina is cleansed, the ultrasound probe is draped with sterile covers, and the ultrasound-guided aspiration needle is inserted through the vaginal wall and into a follicle.

Suction is applied to the needle either by application of regulated vacuum pressure from a vacuum pump or by using a syringe to apply suction to the aspiration needle.

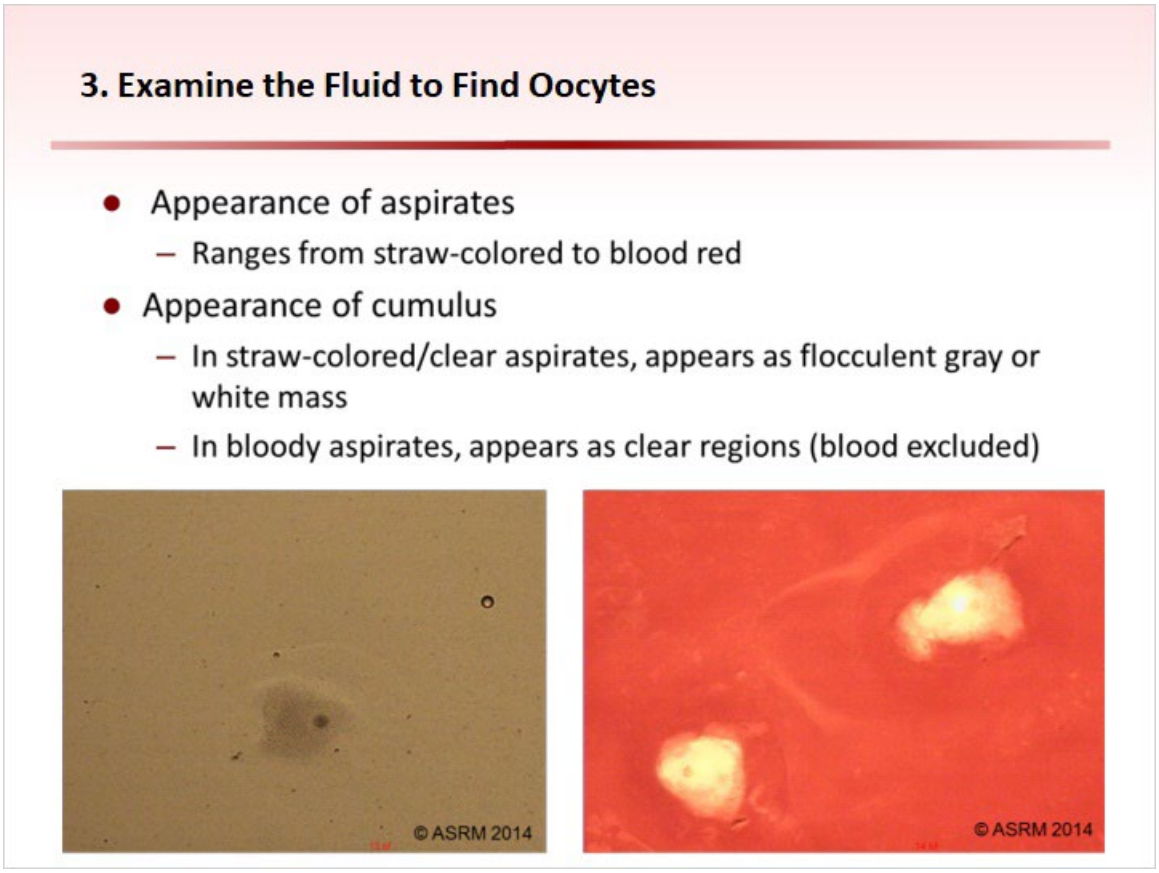
The vacuum pump is an ideal way to maintain constant suction in a repeatable way from follicle to follicle and from patient to patient. The use of a vacuum pump requires that the pump undergo quality-control checks to confirm the displayed vacuum pressure. In addition, the vacuum pressure displayed during the retrieval should be checked frequently to assure that it does not deviate from predetermined values.

The use of a syringe, while less expensive in the short run, may be less regulated and less consistent from follicle to follicle and patient to patient. High suction pressures may affect outcome (Bols et al., 1996).

Follicular fluid aspirated by suction is collected in sterile culture tubes so that it may be delivered to the laboratory for examination. During the aspiration procedure, care must be taken to avoid overfilling the culture tubes. If tubes are overfilled, the follicular fluid inside the tubes can be aspirated into the vacuum trap between the culture tube and the vacuum pump. Further, it is easier to find oocytes in bloody dishes when the fluid depth is shallow (less volume) rather than deep (more volume). Therefore, some embryologists prefer to limit the amount of follicular fluid aspirated into culture tubes to approximately 5 ml less than the tube capacity when there is a risk of aspirating fluid from the culture tube into the vacuum trap.

Culture tubes, once filled to the desired level, are delivered to the technician performing oocyte retrieval for examination.

1.15 3. Examine the Fluid to Find Oocytes



Notes:

Once the technician receives the aspirated follicular fluid in culture tubes from the procedure room, it may be maintained in a tube warmer for a brief time until it is examined.

For examination, the aspirate is carefully poured from the culture tube into a sterile Petri dish on or near the stage of the dissecting microscope. Transmitted light is reflected from a mirror under the stage set to give a slightly oblique illumination that results in the technician's preferred optical contrast.

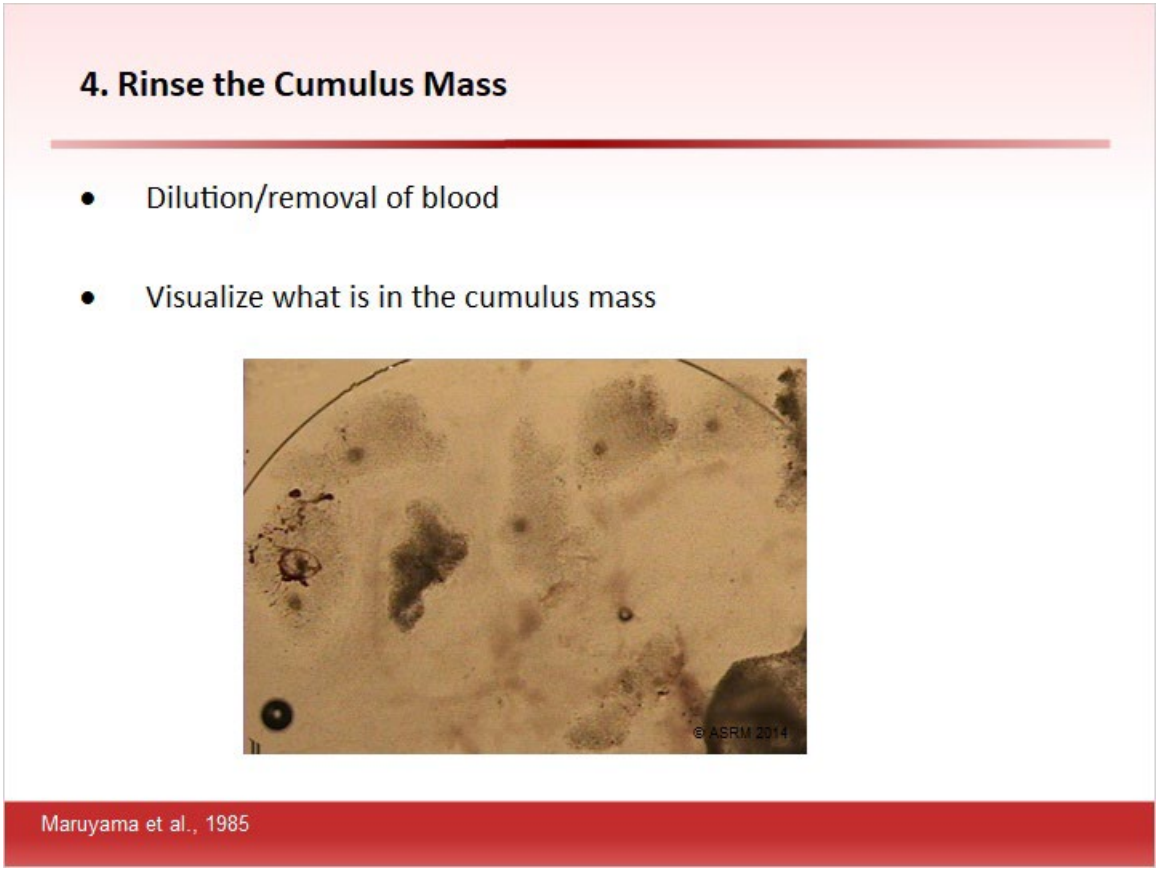
Without using the dissecting microscope, observations may be made. The color of the aspirate is obvious. It may range from clear, straw-colored, or amber solution to pinkish-tinged, to red, and nearly opaque, all depending of the amount of blood mixed with the fluid. When flushing is performed, aspirated fluid may even be quite clear (nearly as clear as water). Often it is easy to identify some suspected cumulus-oocyte complexes (cumulus masses) without the aid of the dissecting microscope.

Most of the examination for cumulus masses is performed with the aid of the dissecting microscope. The appearance of cumulus oocyte complexes masses varies with the appearance of the surrounding aspirated fluid. When the aspirated fluid is clear or

amber, cumulus masses appear as flocculent grey or white clumps of cells, slightly darker than the surrounding fluid. When the aspirated fluid is quite red (from accompanying blood), the cumulus masses appear as small clearings in the fluid. In shallow volumes of fluid the clear masses (excluding the blood) are quite obvious. However, as the depth of the red fluid increases, smaller masses of cells are less obvious. Therefore, it is advantageous to examine follicular aspirates in volumes that are not too deep. The depth of aspirates can be adjusted, to a degree, by slightly elevating an edge of the Petri dish and examining the shallower (more elevated) portion of the dish.

In order to perform this initial examination of the dish to identify suspect cumulus masses, the dish may be rocked slightly or swirled and moved under the microscope to assure that the entire aspirate is examined. When suspected cumulus masses are identified, they are picked up with a pipette for transport to a rinse dish.

1.16 4. Rinse the Cumulus Mass

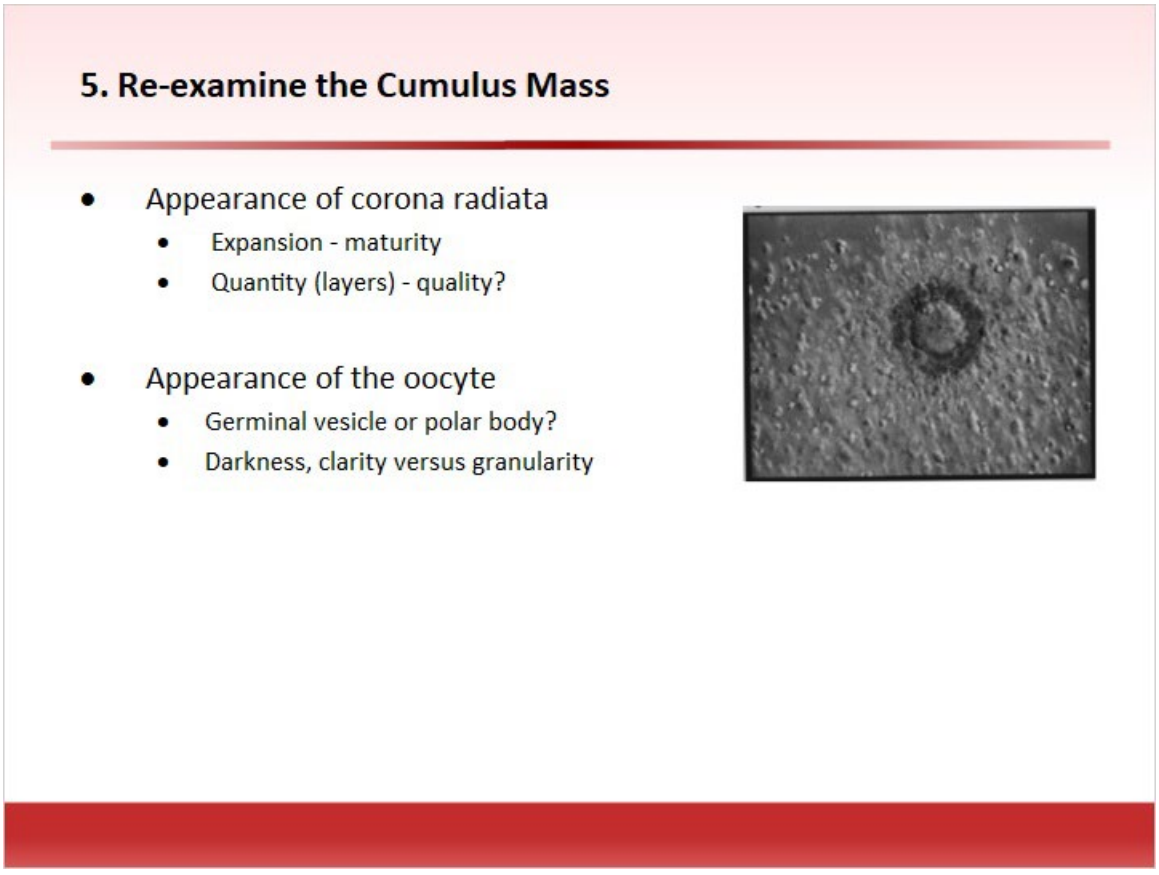


Notes:

A separate dish or well of rinse solution should be maintained near the stage of

the dissecting microscope. When suspected cumulus masses are found they should be introduced to the rinse solution to effectively remove any blood from the solution near the mass.
Removal of the red blood cells serves two functions:
1) Some components of blood have been attributed with the ability to interfere with fertilization (Maruyama et al., 1985). Thus blood should be limited to minimize its effect on sperm-egg interactions.
2) Blood obscures vision of the cumulus mass. Thus, the removal of (or significant dilution of) blood in the aspirate makes for easier examination of the cumulus mass for description of the corona radiata and presence of an oocyte.
Once the cumulus mass has been rinsed, it is re-examined.

1.17 5. Re-examine the Cumulus Mass



Notes:

While the cumulus mass is in the rinse solution, it can be examined (or re-examined) to make these observations:
1)What does the corona radiata look like?
- How many layers of corona radiata cells?

- How expanded is the corona radiata layer?

2)What does the oocyte look like?

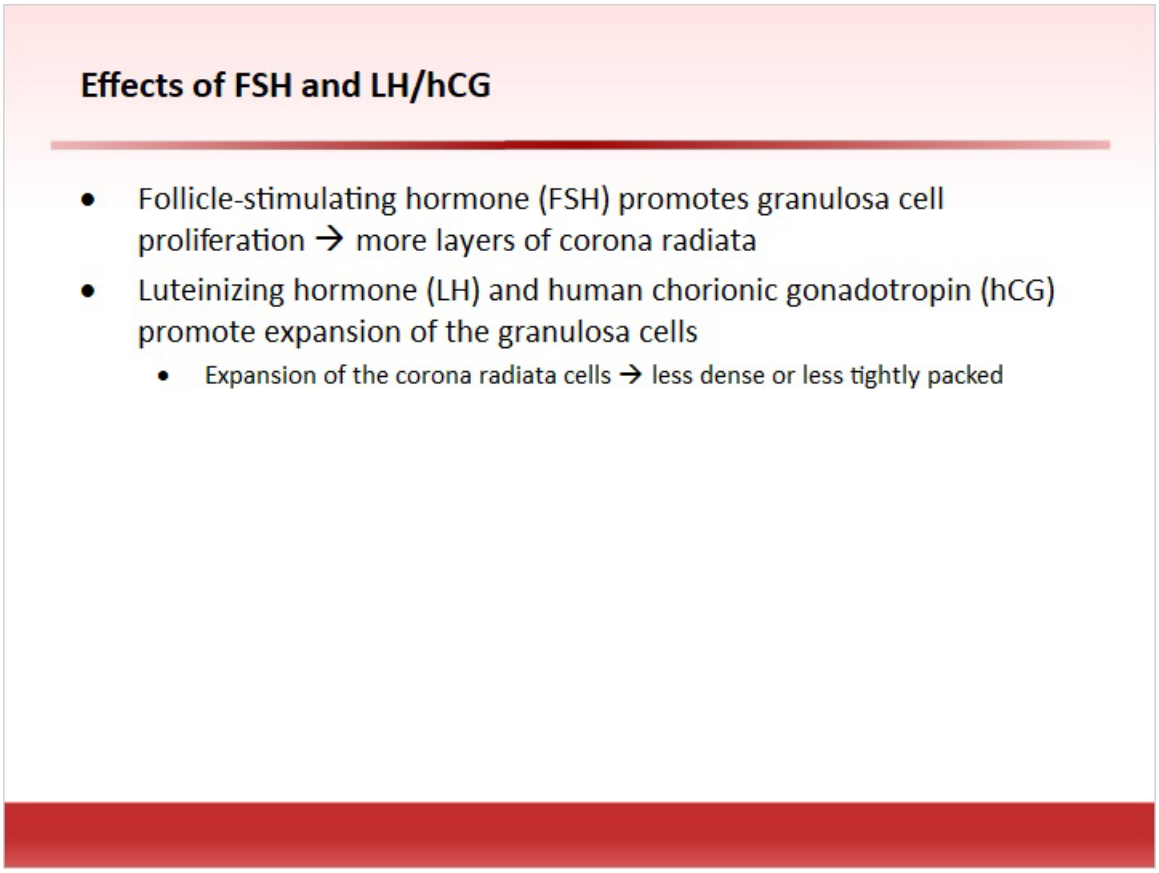
- Can you see the oocyte to document its presence?
- Can you see a polar body to document the oocyte's maturity?
- Can you see a germinal vesicle to document immaturity?
- Is the cytoplasm clear or granular?

Examination of the corona radiata can help to define the conditions under which the oocyte was prepared for retrieval.

Examination of the oocyte, itself, is rather difficult. Some programs have used a simple procedure to gain a better look at the oocyte. The oocyte is moved in a small drop of medium to the bottom of a dry Petri dish. Suddenly jarring the dish, laterally, will spread the drop over a larger surface area of the dish, thereby making the medium much less deep and actually flattening the cumulus oocyte complex. Through flattening, the oocyte may be visualized briefly to ascertain the presence of a polar body or a germinal vesicle and clarity or granularity before quickly removing the cumulus mass and placing it in a culture dish. This examination should occur rapidly to avoid desiccation of the oocyte in the small drop of medium with a high ratio of surface area to volume.

Once re-examined, cumulus masses with oocytes are transferred to culture medium, where they will remain until the next step in their processing occurs. The presence of an oocyte and its status are documented.

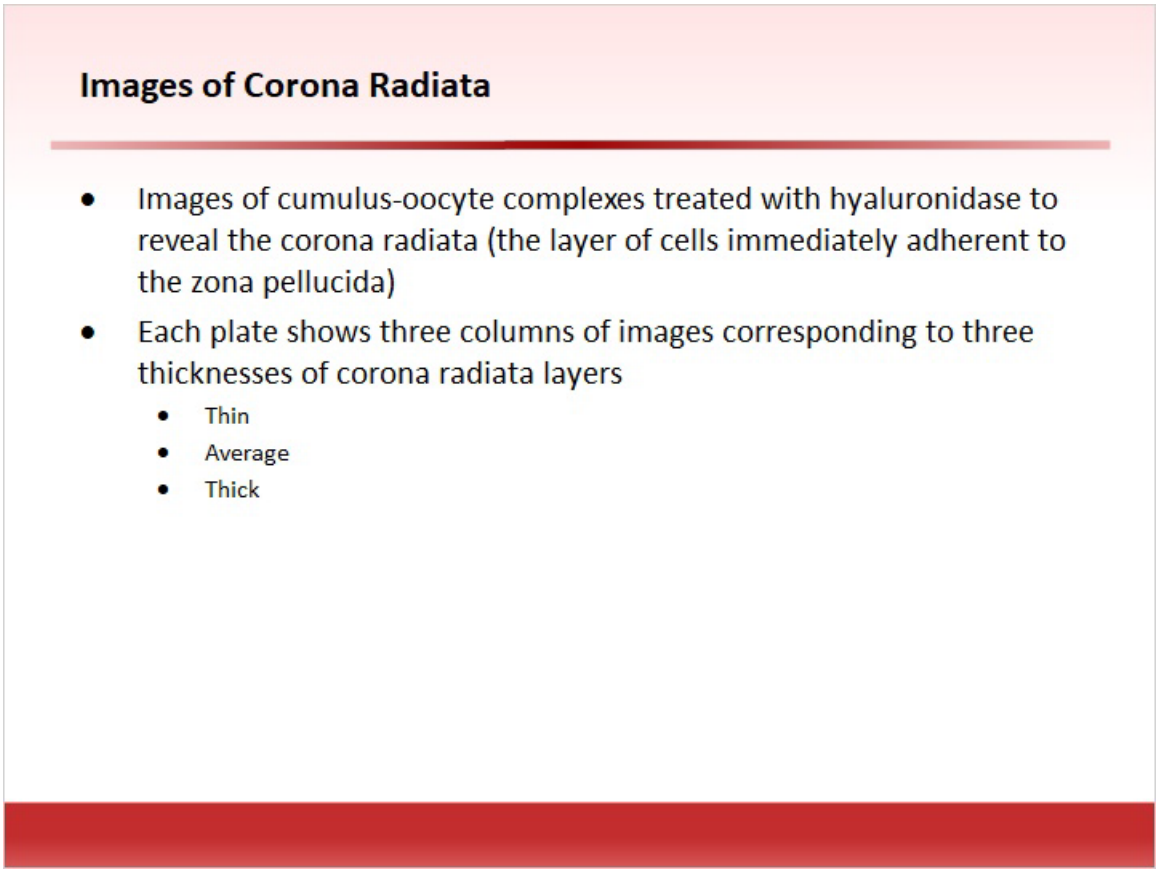
1.18 Effects of FSH and LH/hCG



Notes:

The role of follicle-stimulating hormone (FSH) in folliculogenesis is to promote granulosa cell proliferation. Therefore, a good response to FSH would result in more corona radiata cell proliferation leading to more layers of corona radiata. On the other hand, luteinizing hormone (LH) promotes expansion of the granulosa cells so that the anticipated response to LH or to human chorionic gonadotropin (hCG, that mimics LH) will be expansion of the corona radiata cells, making them less dense or less tightly packed.

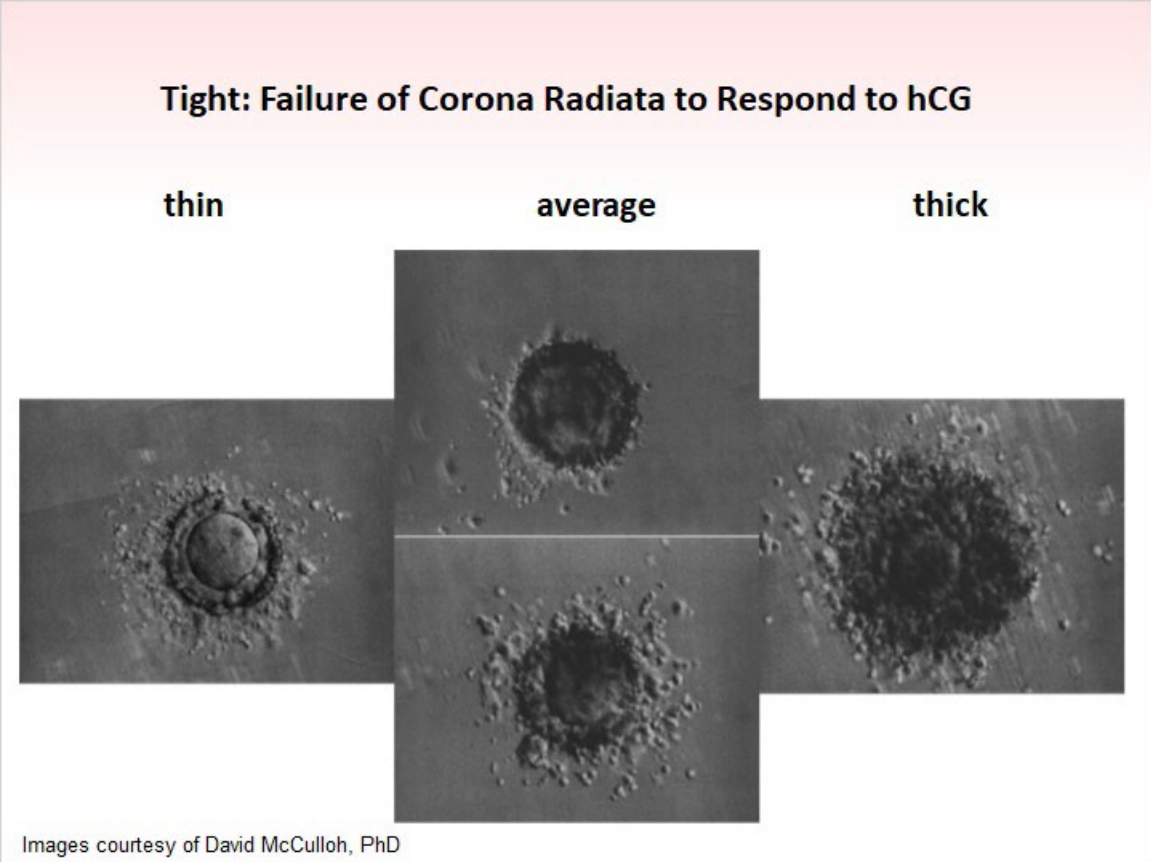
1.19 Images of Corona Radiata



Notes:

The next slides will show cumulus-oocyte complexes that were treated with hyaluronidase to disperse much of the cumulus oophorus and reveal the corona radiata, adherent to the zona pellucida (the clear glycoprotein coat surrounding each oocyte).
In some of the examples that follow, polar bodies are visible, but usually they are not.
It is difficult to determine whether germinal vesicles are present or whether the cytoplasm is dark, granular or clear when corona radiata cells are present.
These images confirm the difficulty of viewing the oocytes.

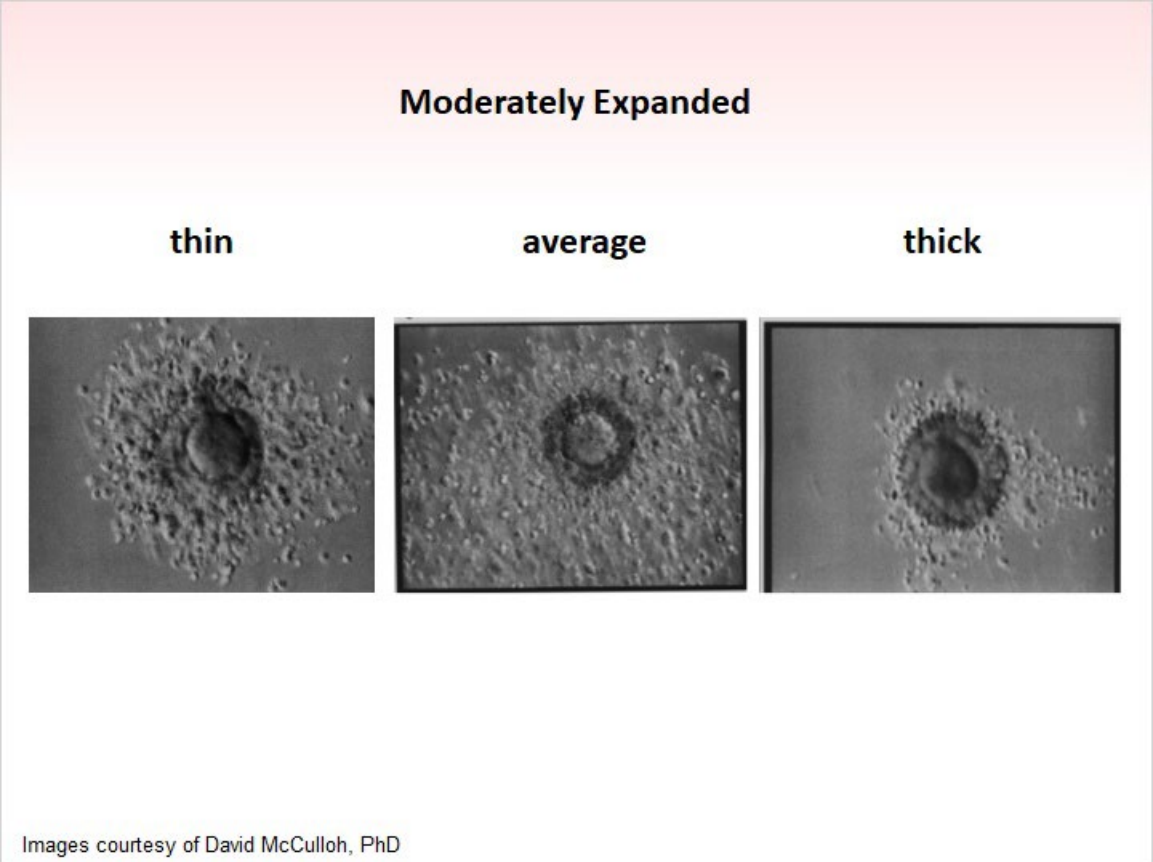
1.20 Tight: Failure of Corona Radiata to Respond to hCG



Notes:

This plate shows several oocytes with tightly packed corona radiata. In the left column there is a thin layer of corona radiata and in the right column there is a thick layer of corona radiata. The thickness of these layers is indicative of the number of cells and layers of cells in the corona radiata in response to administered FSH. Since each of these oocytes has a tightly packed corona radiata it can be inferred that the response to hCG was less than adequate. One would expect that an oocytes contained within this corona radiata would be immature; however, the oocyte in the left (thin) column suggests the presence of polar body-like material.

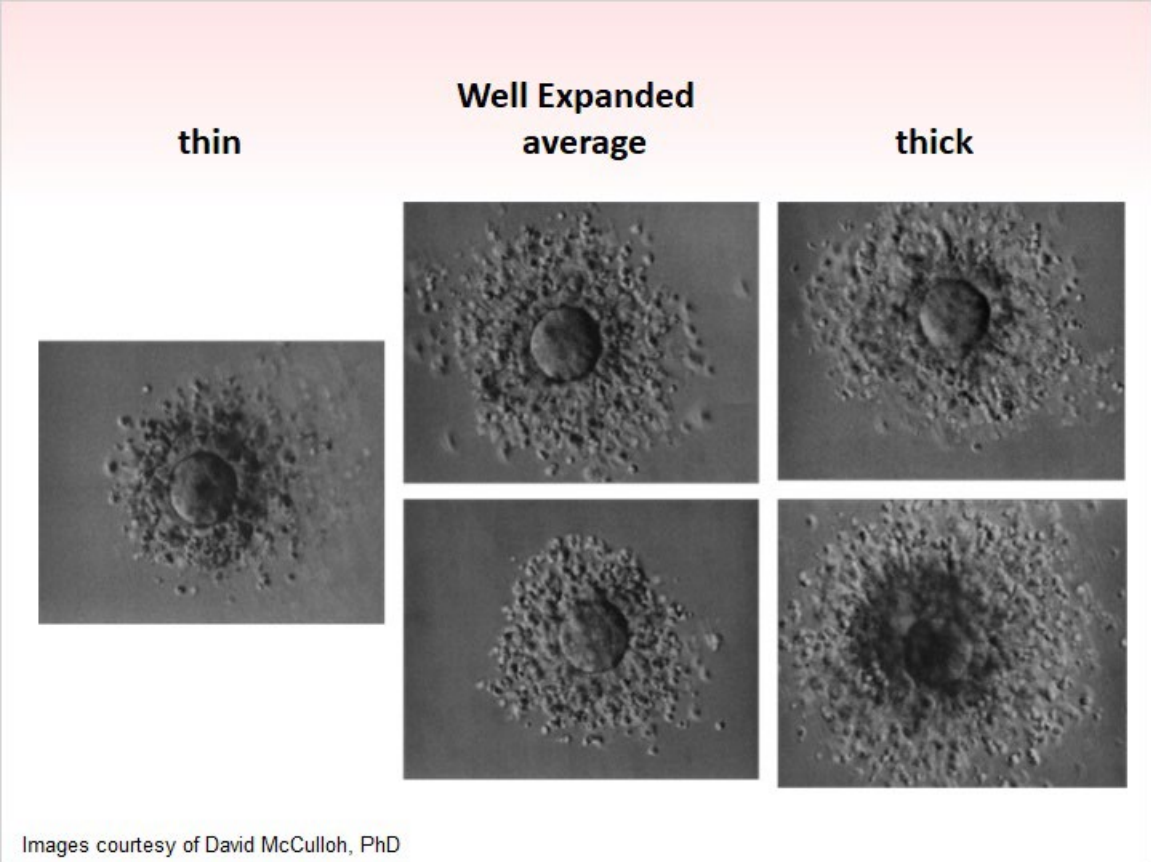
1.21 Moderately Expanded



Notes:

Three examples of oocytes with moderately expanded corona radiata are shown here. The left oocyte has a thin layer of corona radiata while the right oocyte has a thick layer of corona radiata. The thicker corona radiata in the right image suggests a more robust response to the application of FSH during stimulation. From the moderate expansion of the all three coronae radiatae, it can be inferred that these coronae radiatae responded to the application of hCG. These figures are quite typical of oocytes retrieved from patients undergoing controlled ovarian stimulation with FSH and human menopausal gonadotropin (hMG) for assisted reproduction. Most of these oocytes had undergone the first meiotic division and extrusion of a polar body.

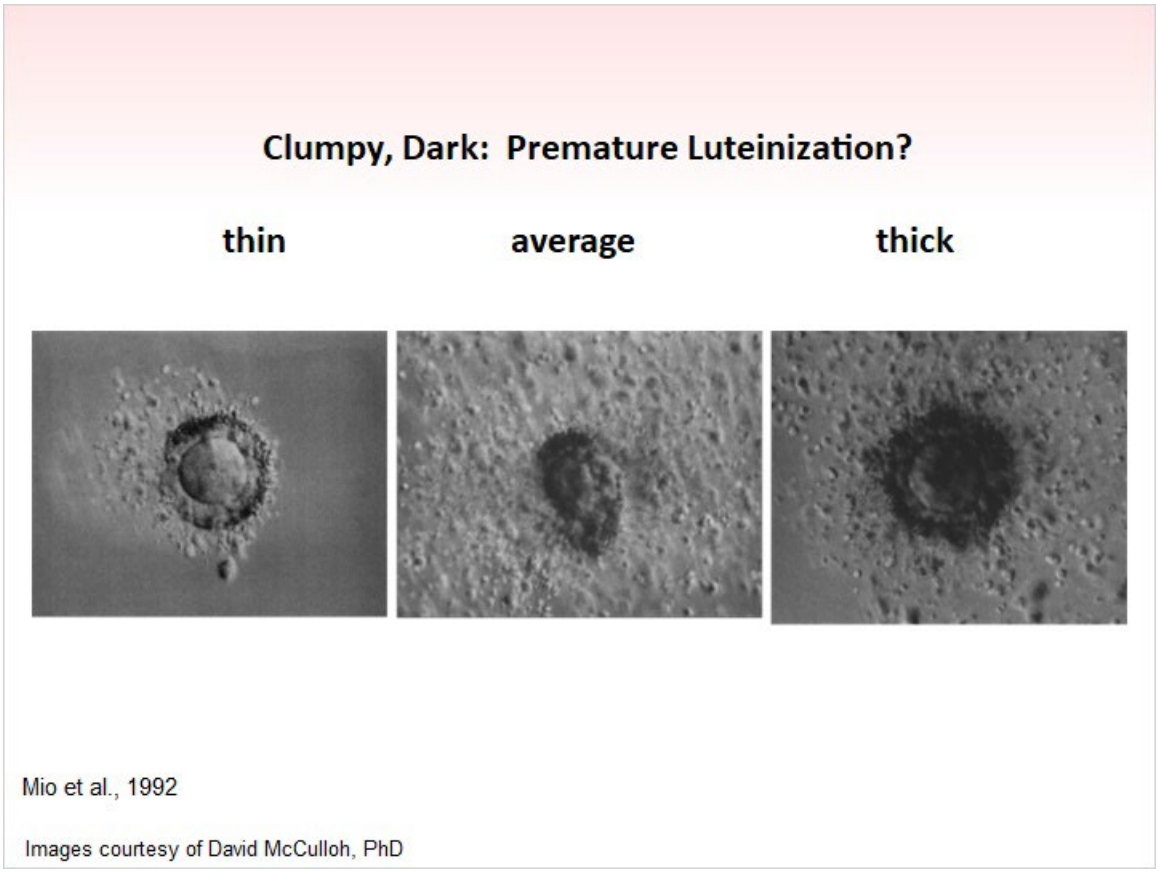
1.22 Well Expanded



Notes:

These 5 images are of oocytes with well-expanded corona radiata. On the left is an oocyte with well-expanded but sparse (thin) corona radiata. The two oocytes in the right column (thick) have well expanded, plentiful (thick) coronae radiatae. This is inferred to have occurred due to a good response to administered FSH during controlled ovarian stimulation; whereas the sparse corona radiata is more likely due to a poor response. The extreme expansion is indicative of a robust response of the corona radiata to administration of hCG. The two oocytes with thick coronae radiatae are similar to oocytes that are obtained from women during natural cycles (although the ones shown here were generated during ovarian stimulation).

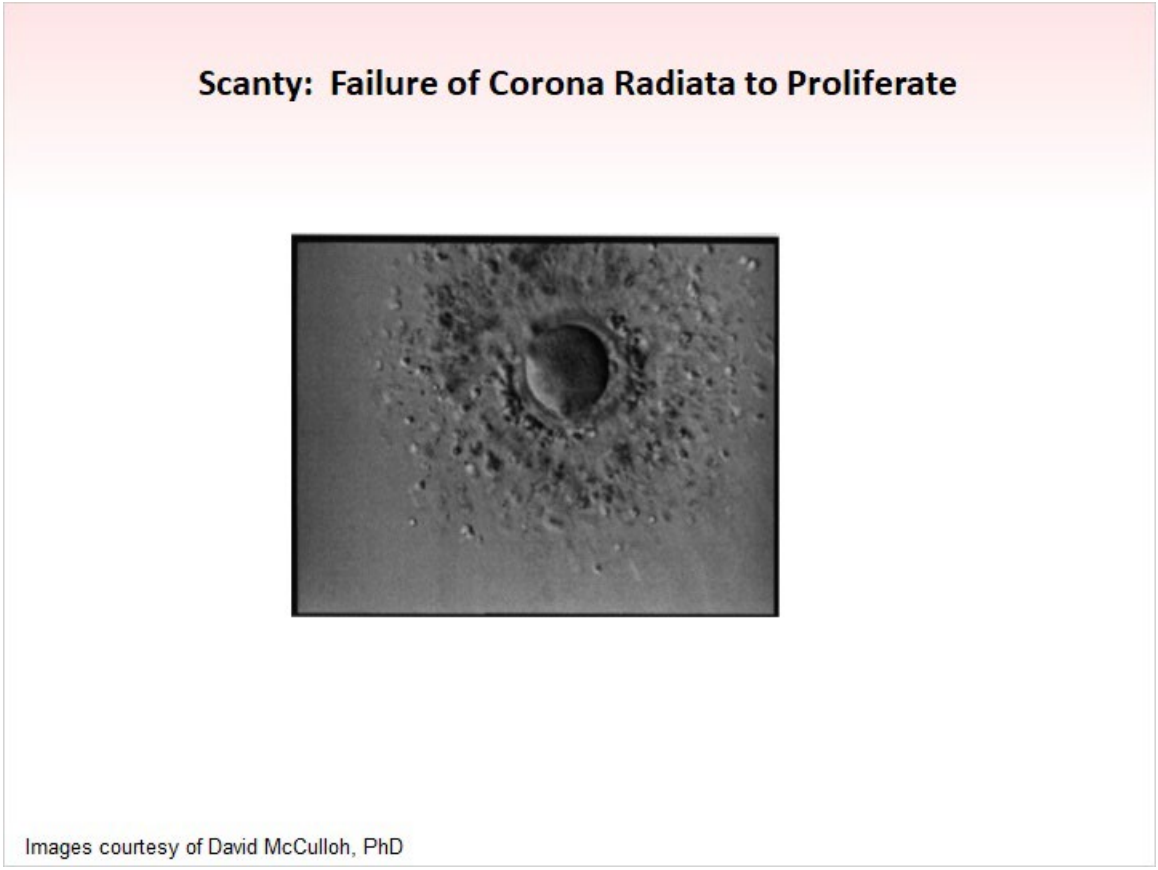
1.23 Clumpy, Dark: Premature Luteinization?



Notes:

These oocytes have an appearance that is associated with endocrine events that occur with different frequencies in different programs. In some patients during controlled ovarian stimulation, progesterone levels elevate abruptly from early follicular values and plateau at higher follicular levels prior to the administration of hCG. This premature elevation of progesterone has been referred to as premature luteinization. Luteinization is the transformation of granulosa cells from proliferating cells to transformed granulosa cells that have changed their predominant steroidogenesis from estrogen-producing to progesterone-producing. During this transition the granulosa cells change from bright and refractile cells to dark and less refractile cells. The dark clumps of cells in the coronae radiatae of the left and center oocytes at roughly 11:00 and 5:00 are reminiscent of this follicular-to-luteal transformation. The right oocyte is surrounded nearly entirely by dark cells similar to those in the clumps of the other two oocytes. This darkness is seen in patients for whom progesterone levels have risen prior to the administration of hCG (Mio et al., 1992). It is thought that this dark appearance of corona radiata cells is a sign of premature luteinization within the follicle from which the oocyte came.

1.24 Scanty: Failure of Corona Radiata to Proliferate



Notes:

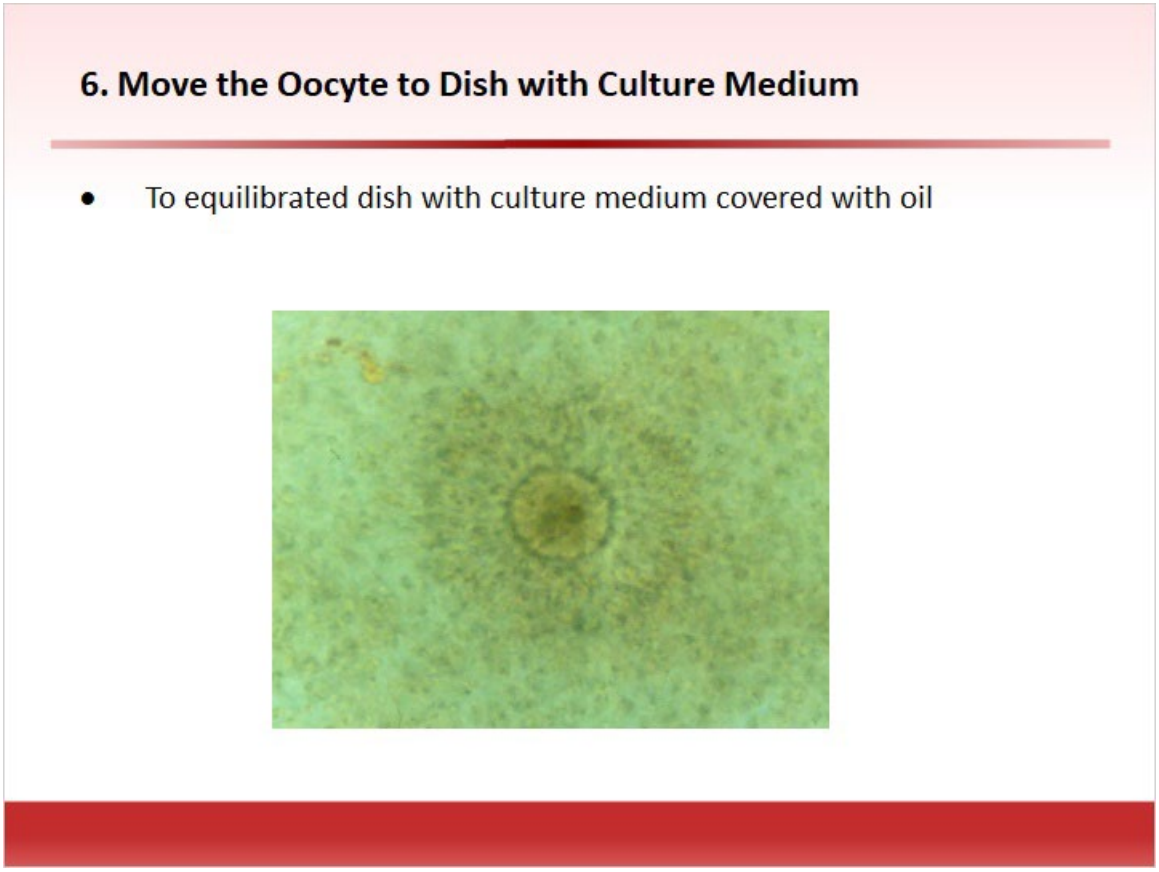
Shown here is an oocyte surrounded by very few corona radiata cells. This appearance suggests that the follicle responded poorly to administration of FSH. With the cellular projections of corona radiata cells through the zona pellucida during folliculogenesis and the presence of gap junctional communication between the corona radiata cells and the oocyte, it is believed that intercellular communication is important for oocyte development and that this communication helps to establish a better quality oocyte. The presence of more corona radiata cells surrounding the oocyte is thought to be indicative of an oocyte with more communication from its surrounding corona radiata cells and that it may be a better candidate for development as a good embryo.

While the amount of corona *expansion* may be a correlate of maturity for the oocyte, some believe that the *quantity* of corona radiata surrounding the oocyte may be a correlate of oocyte quality, affecting its ability to develop into a more attractive embryo after fertilization.

While the premises of these interpretations are not well substantiated, these images provide something that may provide a basis for discussion in the future.

In addition, the observations about corona radiata thickness may provide some insights that could help embryologists to provide feedback to physicians about how the stimulation progressed.

1.25 6. Move the Oocyte to Dish with Culture Medium

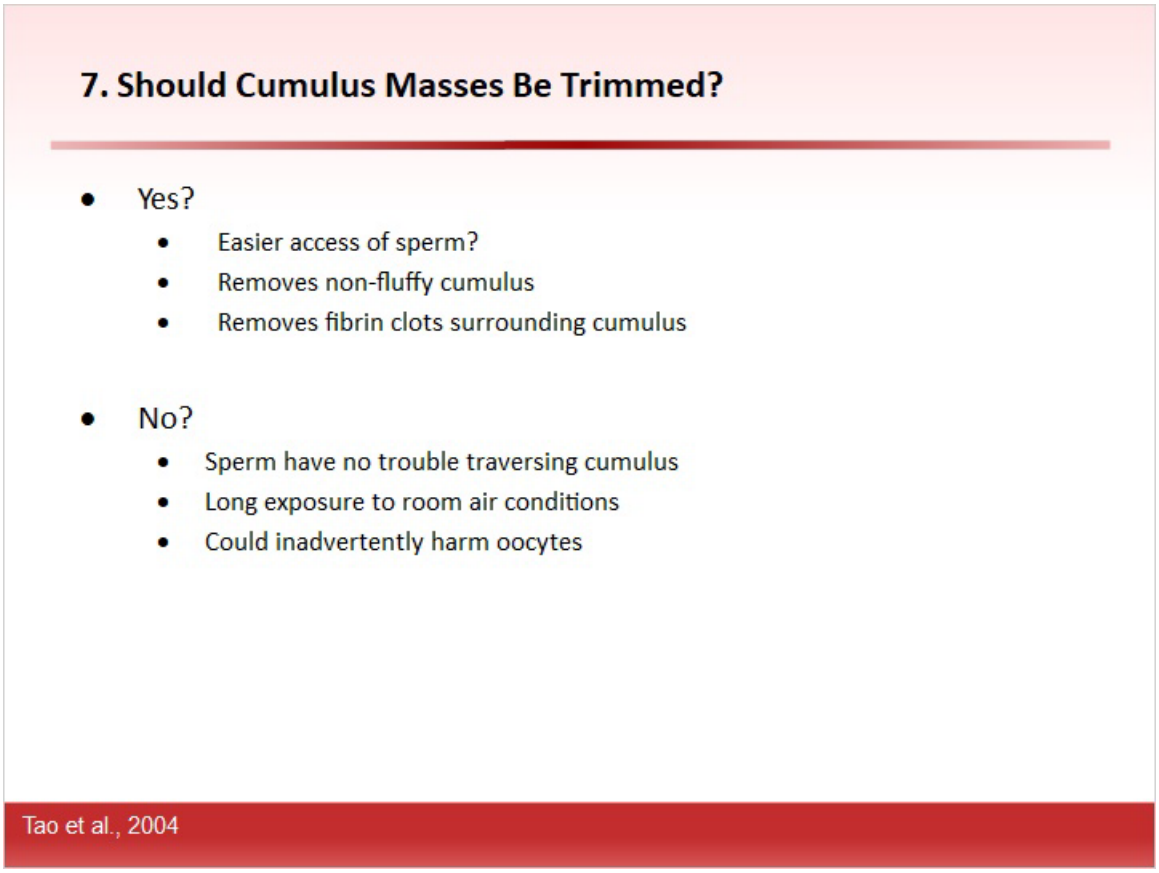


Notes:

Once the cumulus masses with oocytes have been identified, they are moved to equilibrated culture medium. This is the medium in which the oocytes will be inseminated or from which they will be removed for stripping in preparation for intracytoplasmic sperm injection.
The equilibrated dish should be maintained in incubation conditions with the appropriate temperature, humidity, and gas concentrations. This will ensure that the oocytes are maintained in the conditions defined by the laboratory's policies as soon as possible after identification. Incubator conditions may be mimicked by placement on a warming surface with humidified mixed gas flow surrounding the dish(es) and using an inverted funnel or a gassed bell jar, or a specially designed benchtop temporary incubator.
If organ culture dishes or multi-well dishes are used for culture, one well can be

designated as a rinse well. Cumulus masses may be rinsed in culture medium, quickly, prior to placing the masses in culture. This provides an added rinse to avoid dilution of culture medium with rinse medium and provides an additional rinse to further diminish the amount of blood in the final culture.

1.26 7. Should Cumulus Masses Be Trimmed?

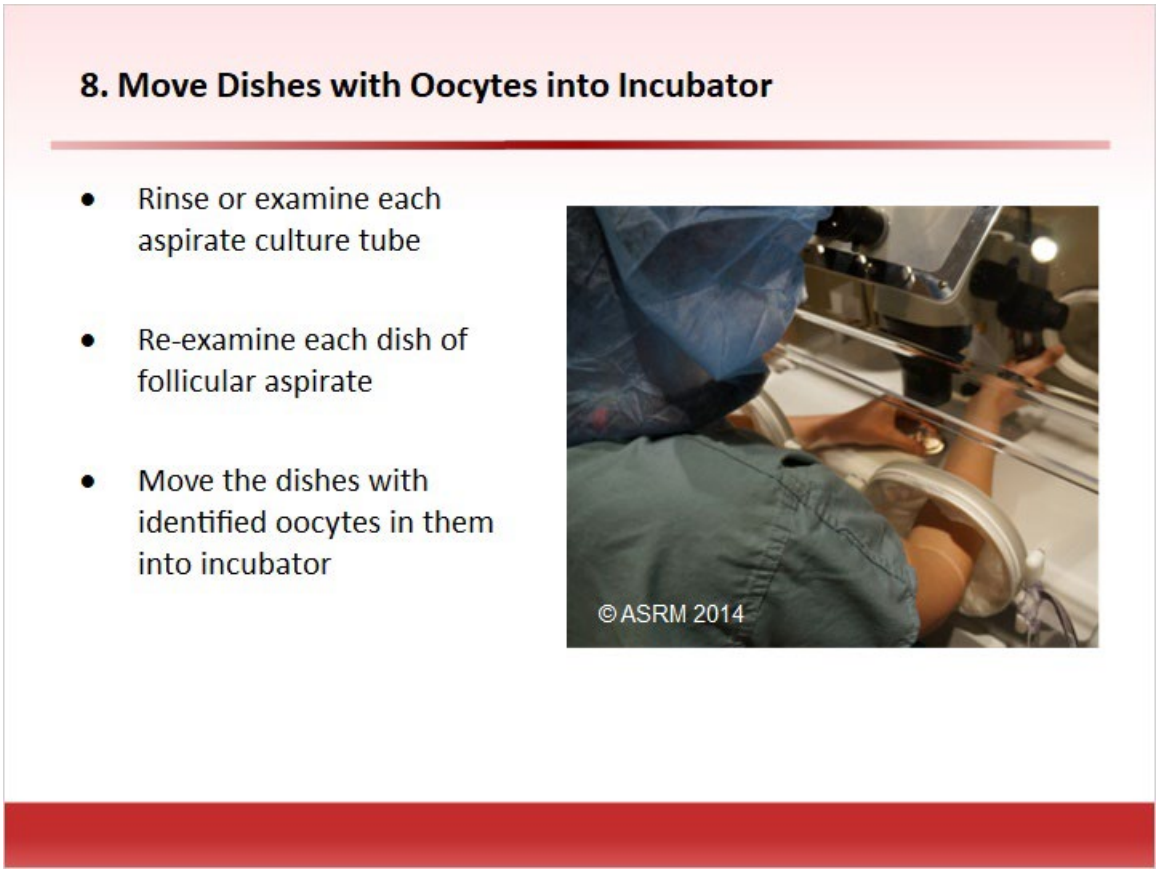


Notes:

While removal of oocyte investments in preparation for ICSI is considered necessary prior to the procedure, there is little consensus on whether reducing the amount of cumulus oophorus by cutting it away with needles following oocyte retrieval is either crucial or beneficial prior to standard in vitro insemination via co-incubation of oocytes with sperm. In some programs, this procedure is performed routinely and prior to placement of oocytes in culture medium after the retrieval.
One could speculate that reducing the amount of cumulus oophorus by trimming could provide easier access for sperm. It could remove non-granulosa cell tissues that may adhere to the cumulus from the culture. Further, trimming the cumulus may remove or disturb fibrin clots from the surface of the cumulus that

are both difficult to see and might interfere with sperm access to the oocyte. Alternatively, one could speculate that trimming is not necessary and may be harmful (Tao et al., 2004) since normal sperm have little difficulty traversing the cumulus oophorus, since trimming requires extended time and exposure of cumulus masses and oocytes to non-incubator conditions, and therefore could inadvertently harm oocytes either through exposure or by accidental needle-induced damage.

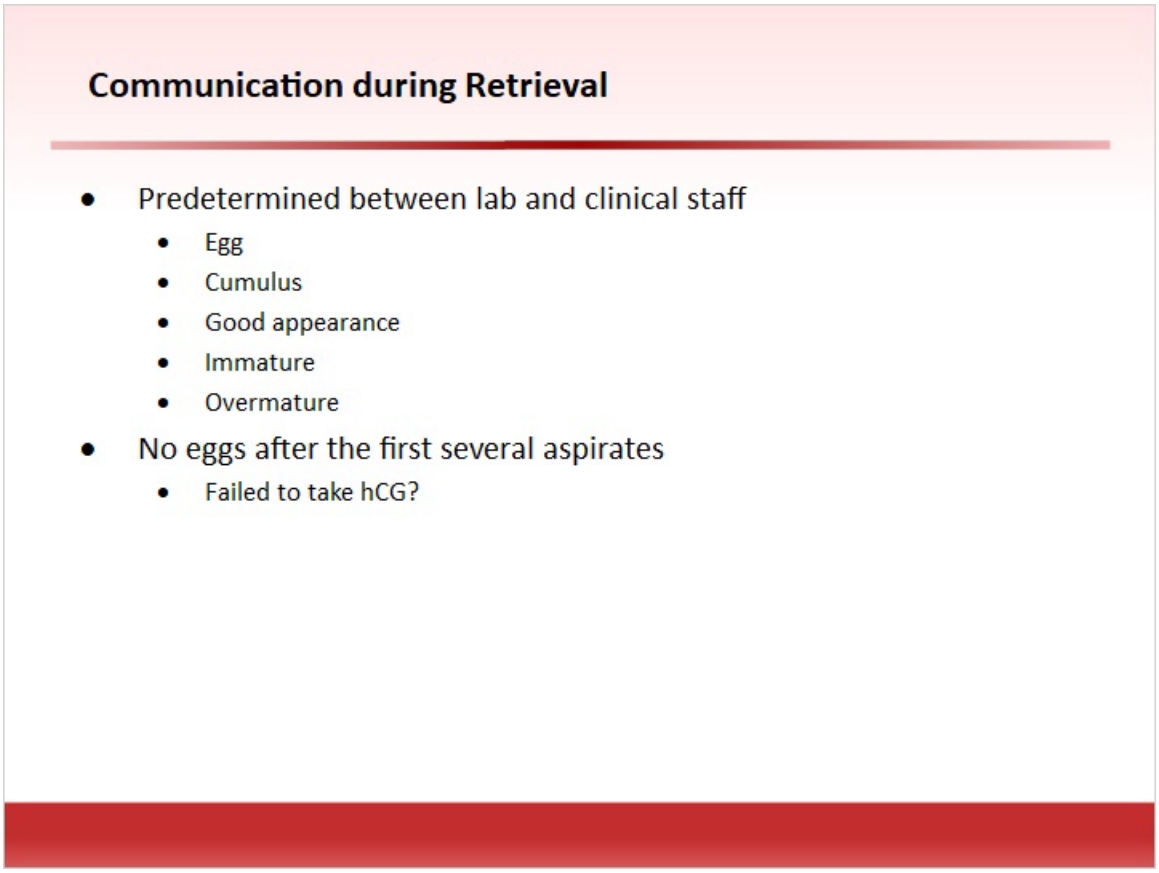
1.27 8. Move Dishes with Oocytes into Incubator



Notes:

Once oocyte retrieval is completed (having examined and re-examined all dishes of aspirates and having rinsed and re-examined all cumulus masses containing oocytes), the dish(es) containing the identified oocytes should be moved to an incubator verified to have the desired temperature, humidity, and gas concentrations.

1.28 Communication during Retrieval

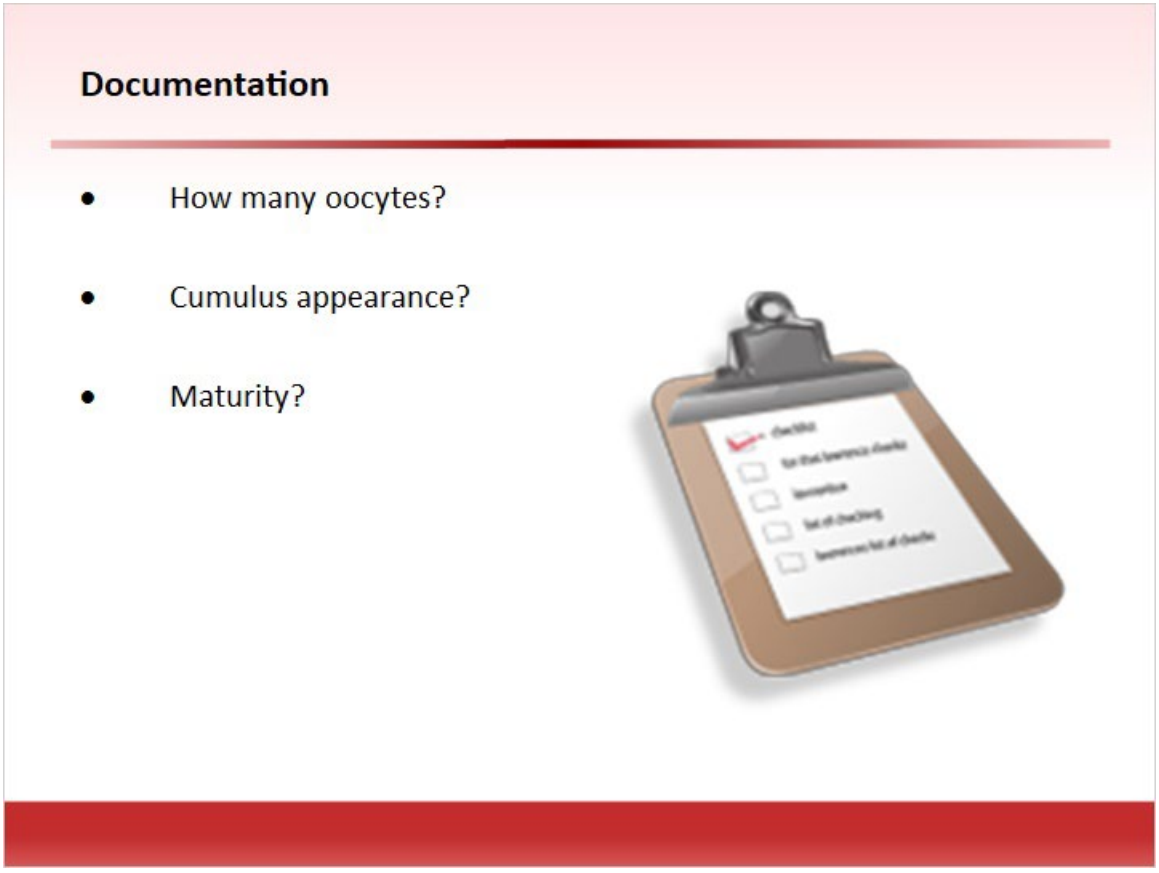


Notes:

Communication between the clinician performing the aspiration of follicles and the technician performing the examination and identification of oocytes is quite varied from facility to facility.
In some facilities, the clinician performing the aspiration prefers to have feedback that the retrieval is resulting in the accumulation of oocytes during the procedure. In other facilities, the clinician prefers to know after the retrieval how many oocytes were found. If the clinician will alter the approach to aspiration, then the clinician may desire to be to be informed of the progress of oocyte count during the retrieval.
Most of the specific details are more easily presented in the form of a written report following completion of the procedure. In particular, specifics of the corona radiata's appearance would not alter the performance of the retrieval and is probably best reported following the procedure.
Under some circumstances, it is appropriate to communicate the progress of oocyte identification during the procedure. If no oocytes are identified with the first several aspirates, it is possible that the retrieval could be halted.
Occasionally, patients fail to administer the triggering injection of hCG or GnRH agonist and the final maturation and ovulation are not initiated. Rapid testing for

hCG or LH can be performed on patient blood (or follicular aspirates) to determine if the ovulatory injection was administered before progressing with the retrieval.
If rapid testing indicates that the injection was not administered, the expectation for a standard retrieval is that no oocytes will be recovered. In this case, the retrieval can be halted, the patient can have the ovulatory injection administered and retrieval can be attempted again later.
If rapid testing indicates that the injection was administered, the retrieval may be continued and some oocytes may be retrieved. Some programs verify that the ovulatory injection has been administered by drawing blood the morning following the ovulatory injection (one day prior to oocyte retrieval) and performing determinations of the hCG or LH level to confirm appropriate administration of the injection.

1.29 Documentation



Notes:

Following completion of oocyte retrieval, written (or electronic) documentation of the results of oocyte retrieval should be performed.

The most direct and valuable information to be provided is the number of oocytes found. Further information concerning the appearance of the cumulus oophorus or the corona radiata may not directly represent the maturity or quality of the oocytes. Such descriptive data should be interpreted with caution.
Information about the presence or absence of polar bodies and germinal vesicles is also somewhat preliminary immediately following oocyte retrieval since polar bodies are not necessarily in an orientation to be seen and cytoplasmic structures may be difficult to visualize during oocyte retrieval. In addition, the completion of the first meiotic division (resulting in extrusion of the first polar body) occurs near the time of oocyte retrieval and may occur in the laboratory after the retrieval. Therefore, these data should be provided and interpreted with caution.
If ICSI is ordered, then information concerning oocyte maturity is far more accurate and useful following the preparations for ICSI procedures than immediately following oocyte retrieval.

1.30 Quality Control

Quality Control

- Incubators
- Media
- Dishes
- Personnel
- Heating block
- Vacuum pump
- Other factors (humidity, optimum temperature, CO_2, O_2, and pH)

McCulloh, 2012
Lee et al., 2012
Lane et al., 2008
Johnson and Gardner, 2011
Vermilyea et al., 2014

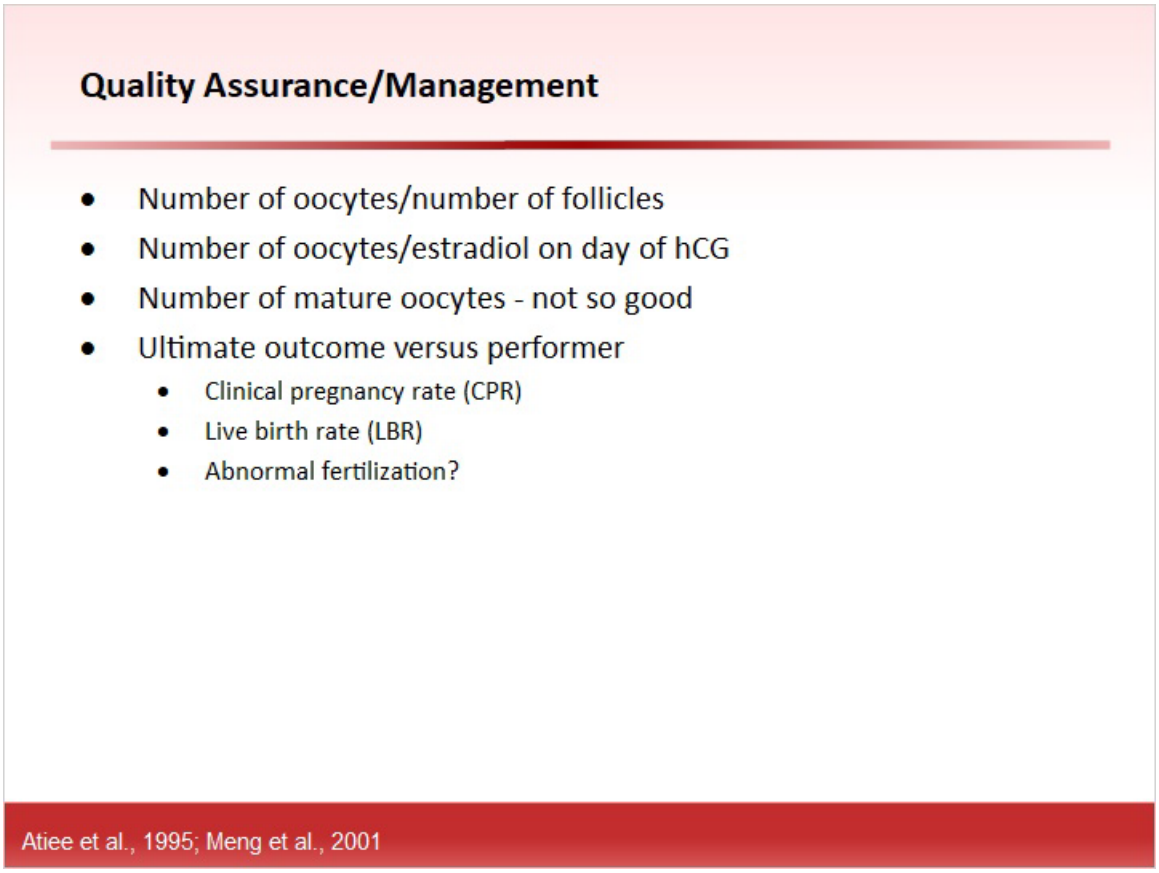
Notes:

All of the environmental conditions and equipment functions should be documented as within tolerable levels at least daily. Any equipment for which intolerable values are found should be corrected and should not be used until proper function can be documented. Small fluctuations of incubator conditions have been associated with increased incidence of spontaneous pregnancy losses weeks after the incubator exposure (McCulloh, 2012). Therefore, it is imperative to maintain conditions within predetermined “tolerable limits” for all rooms and equipment at all times.

It is equally important to assure that all plasticware and media are capable of supporting optimal gamete and embryo culture conditions. Further, records of personnel competence are illustrative of the technician's ability to perform procedures consistently without harm.

Recent publications suggest that some factors previously not considered important may have the ability to affect embryonic development (Lee et al., 2012), implantation (Lane et al.,2008; Johnson and Gardner, 2011), and fetal development (McCulloh, 2012). Therefore, embryos may be susceptible to more harm than was previously believed. Further consideration will be needed to understand the observation that humidity of the culture environment may (McCulloh, 2012) or may not (Vermilyea et al., 2014) be important for embryo culture. It is also interesting to note that there are not really any data on the optimum temperature for embryo culture whereas there have been studies to compare culture under different CO_2, O_2, and pH conditions.

1.31 Quality Assurance/Management



Notes:

It is good practice to develop methods for comparing personnel competency for all procedures. Unfortunately, many of outcome parameters are extremely dependent on multiple factors that are out of our control in the laboratory. However, with sufficient numbers, it may be assumed that inter-patient variables may become rather uniform for each technician.
In order to assess competency, it may be possible to determine the average percentage of follicles that result in retrieved oocytes. If the number of follicles is determined by clinicians prior to retrieval, then the number of oocytes retrieved can be compared with the number of follicles (#oocytes/#follicles) and the average yield can be determined for each oocyte retrieval technician. If significant differences are found, then attempts could be made to improve the performance of technicians with poorer yield.
In addition, it is possible to determine the incidence of clinical pregnancy per patient retrieved by each technician. After consideration for patient age and embryos transferred, it may be possible to obtain a fair comparison that could suggest whether a particular technician is better or poorer at performing harmless oocyte retrievals. Similar comparisons might be possible using incidence of abnormal fertilization or incidence of aneuploidy compared by

grouping patients according to the retrieval technician since there may be controversy about the effects of temperature on oocytes (Atiee et al., 1995 versus Meng et al., 2001).

1.32 Thank you!

Notes:

Thank you for participating in this educational activity.