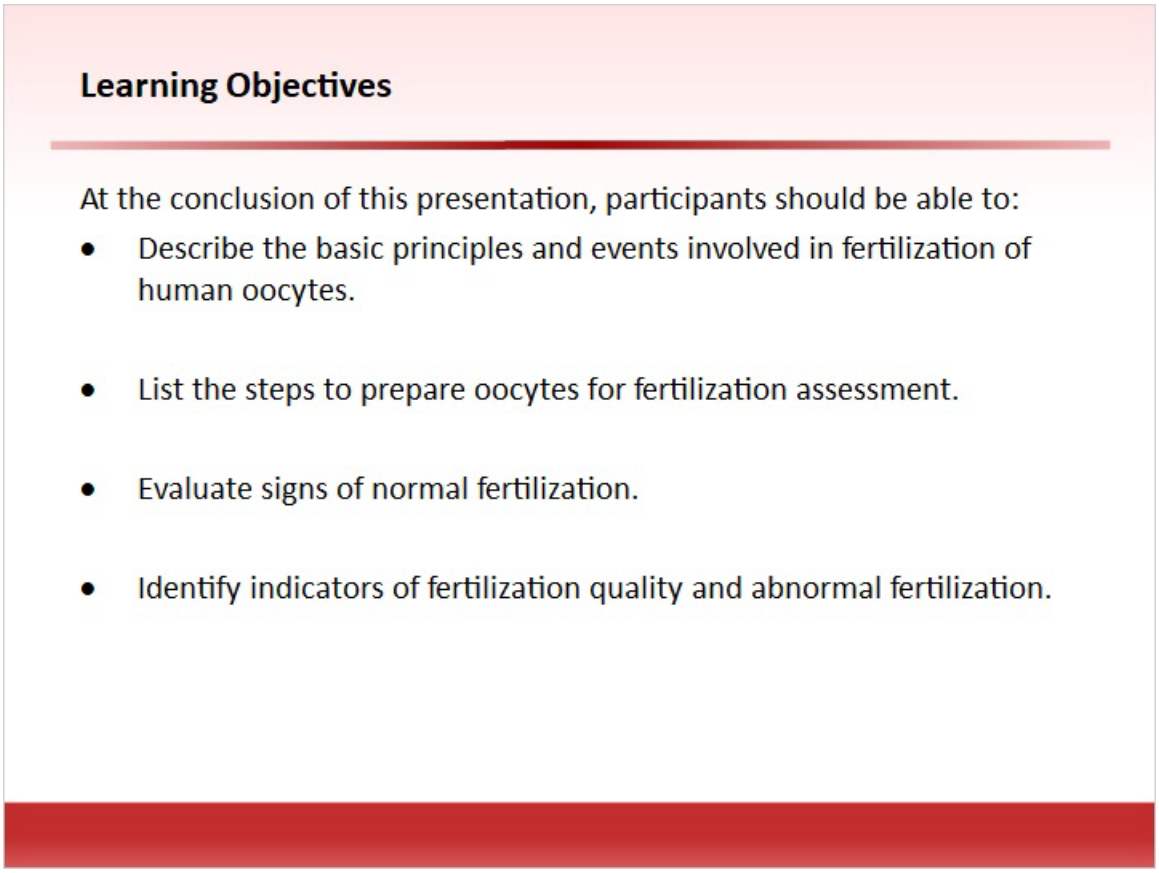
LABCC100 Lesson 19

1.1 Changeover Day: Fertilization Assessment

Notes:

Welcome to the American Society for Reproductive Medicine's eLearning modules. The subject of this presentation is Changeover Day: Fertilization Assessment.

1.2 Learning Objectives



Notes:

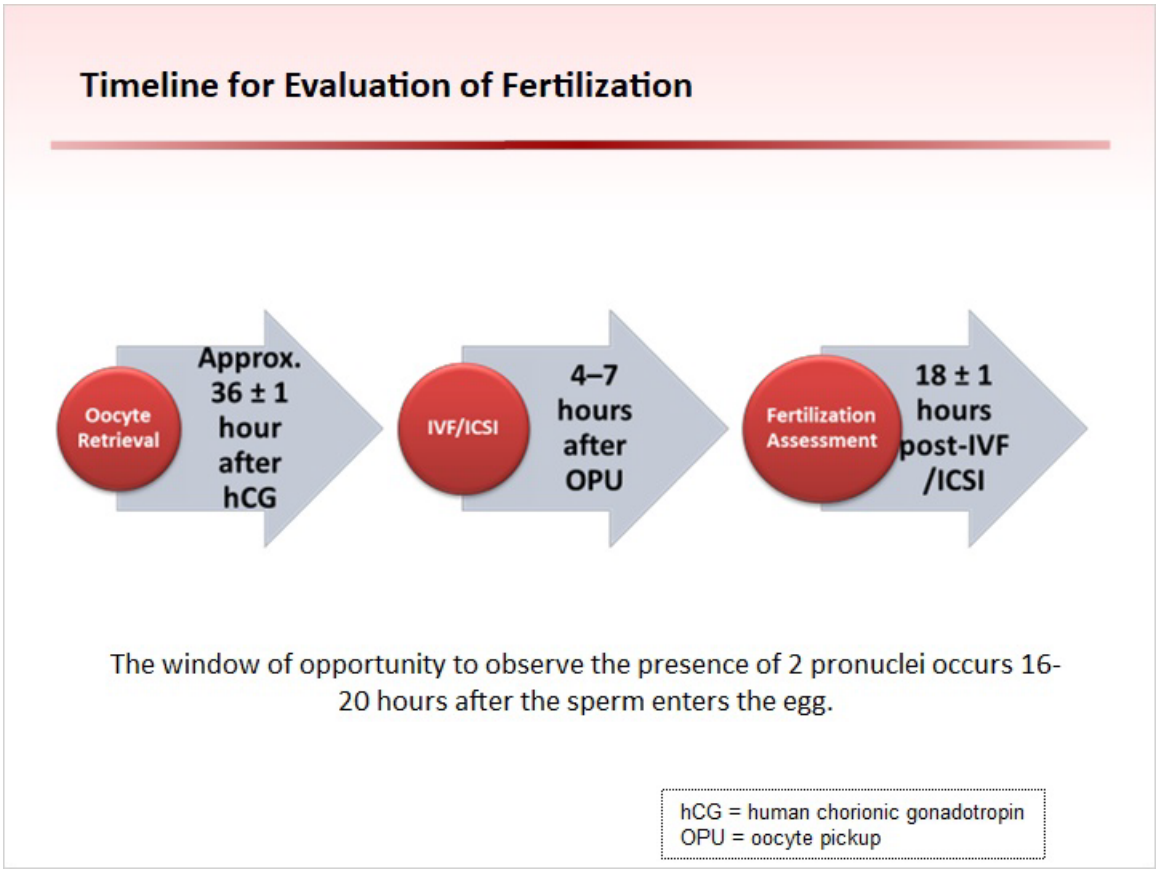
At the conclusion of this presentation, participants should be able to:
Describe the basic principles and events involved in fertilization of human oocytes.

List the steps to prepare oocytes for fertilization assessment.

Evaluate signs of normal fertilization.

Identify indicators of fertilization quality and abnormal fertilization.

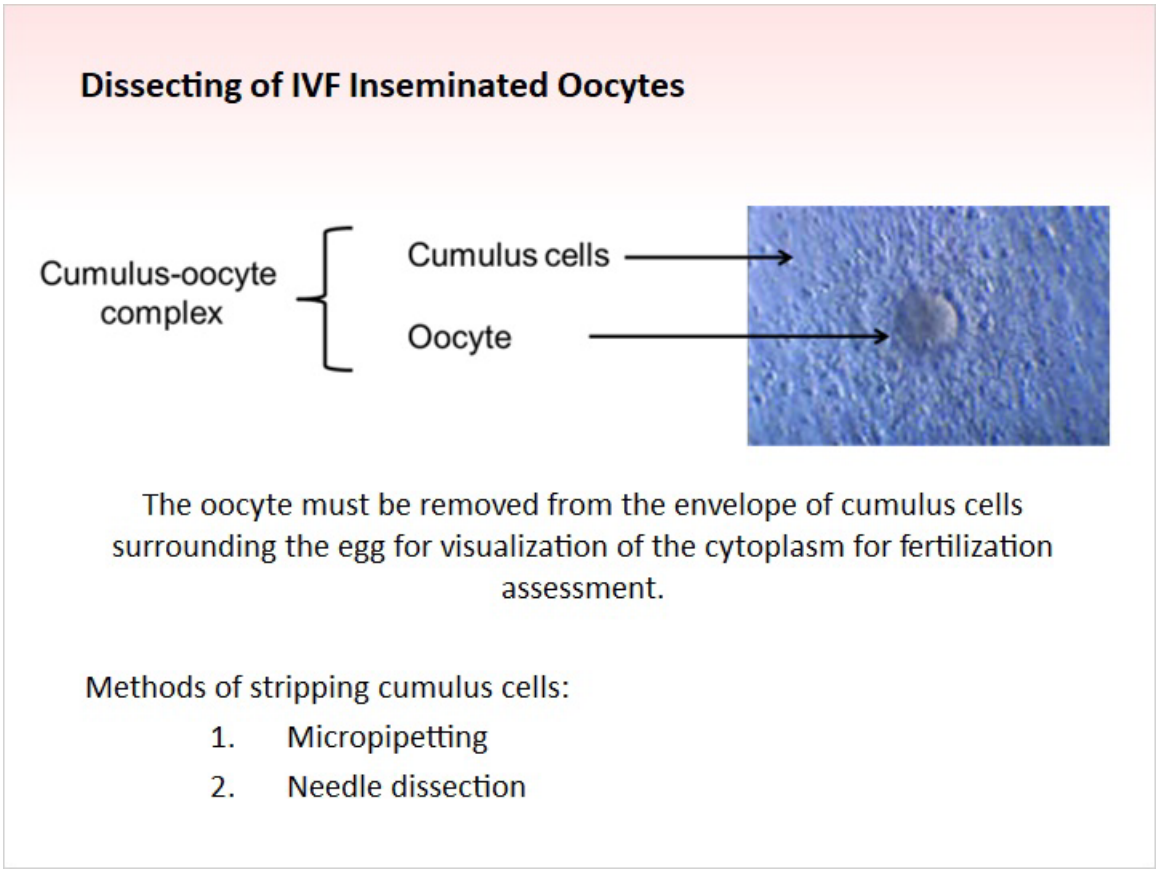
1.3 Timeline for Evaluation of Fertilization



Notes:

The oocyte pickup (OPU) or retrieval is scheduled approximated 36 hours after the hCG trigger. Typically, the IVF/ICSI procedure is completed 4-7 hours after retrieval. Oocytes inseminated for conventional IVF are prepared for fertilization assessment and evaluation approximately 16-20 hours post-insemination (hpi). With time-lapse technology it has been observed that pronuclear formation in zygotes arising from IVF occurs approximately 1 hour after those produced by ICSI. As a result, most protocols will state that fertilization assessment will take place 18±1 hpi.

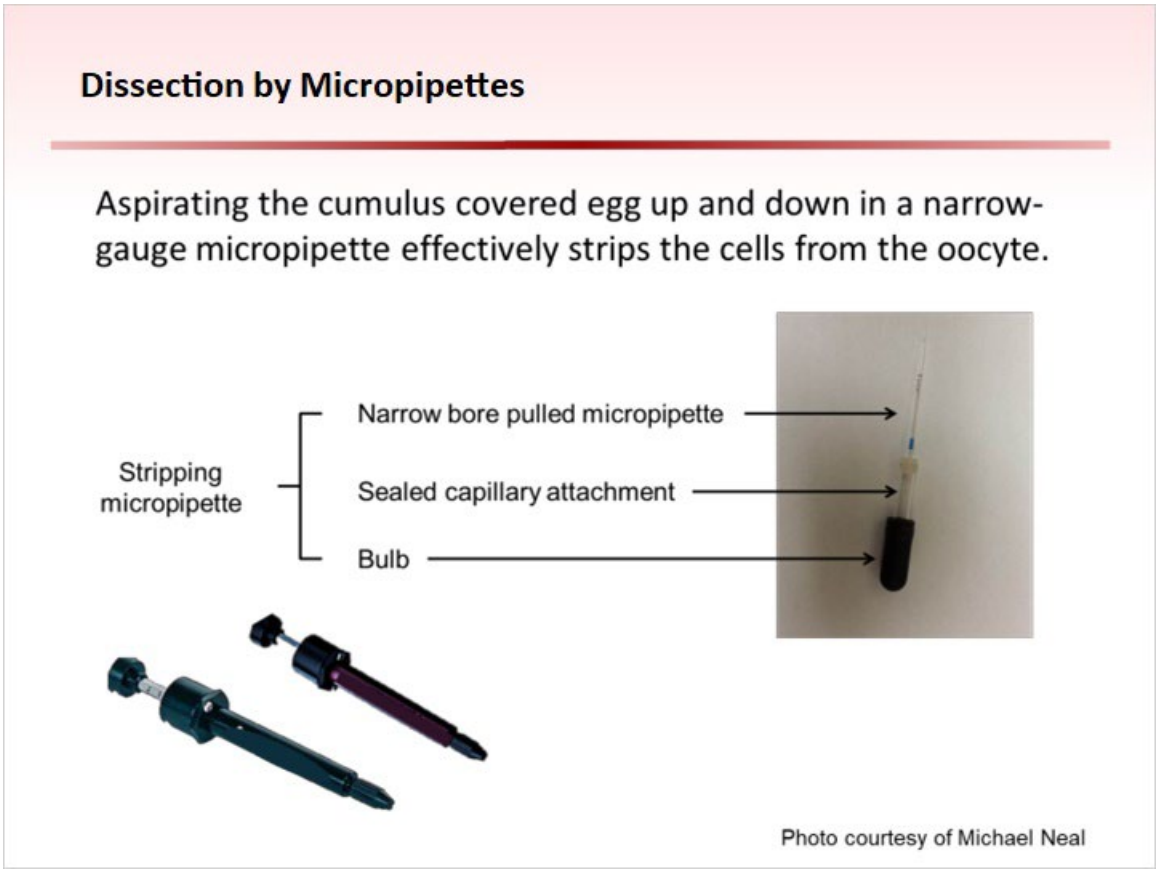
1.4 Dissecting of IVF Inseminated Oocytes



Notes:

Oocytes inseminated with sperm for conventional in vitro fertilization (IVF) normally are covered with a layer of dispersed coronal and cumulus cells at the time of fertilization assessment. These cells must be carefully removed to allow for appropriate visualization of the cell cytoplasm and to properly assess for signs of fertilization. There are 2 common methods of stripping cumulus cells away from the oocyte: micropipetting and needle dissection. The chosen method is partly by personal preference. However, sometimes the characteristics of the cumulus-oocyte complex may dictate that one or both methods be employed to successfully release the oocyte from the cumulus envelope of cells. Regardless of the technique, this procedure must be carried out efficiently with precision so as to not physically damage the egg or cause a change in the pH, temperature, or osmolarity of the media. Since cumulus cells are stripped away from the oocyte prior to insemination by intracytoplasmic sperm injection (ICSI), this step is not required for zygotes derived from ICSI.

1.5 Dissection by Micropipettes

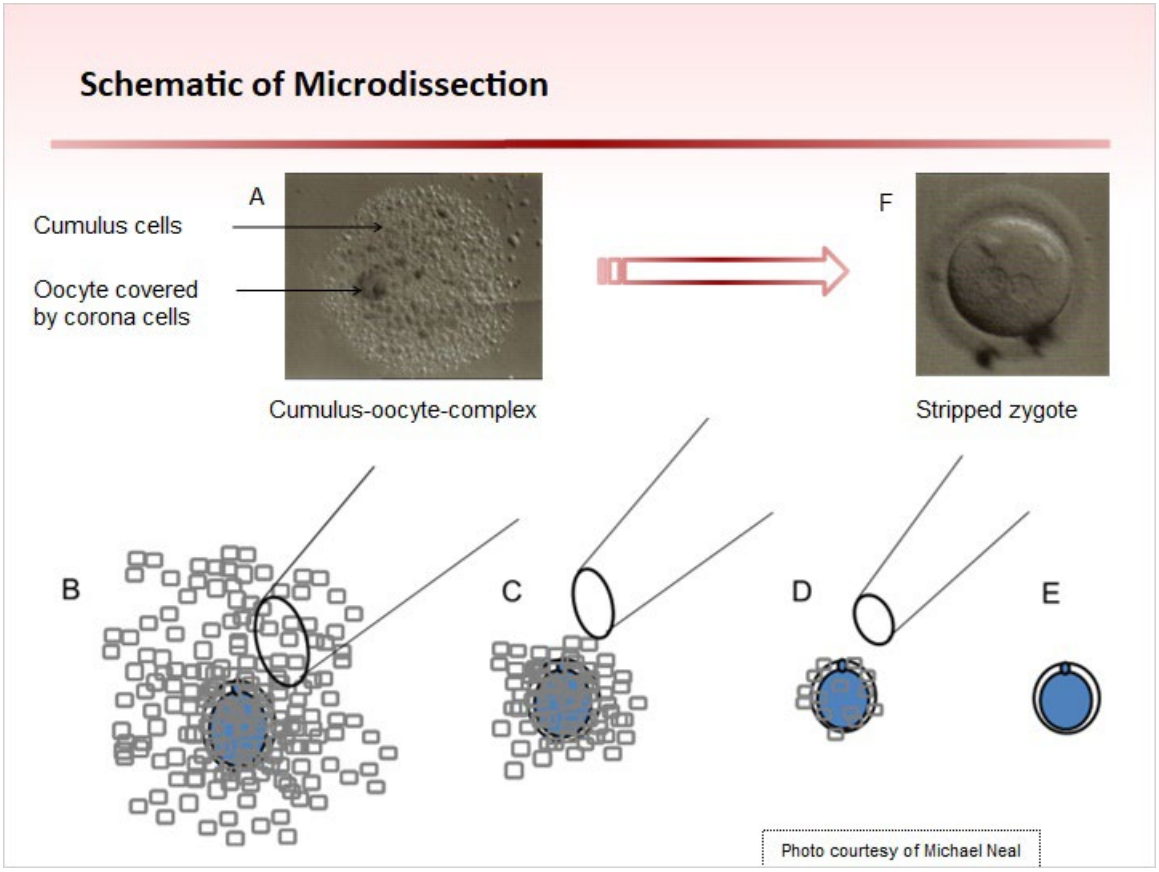


Notes:

Cumulus cells can be removed by gently aspirating the oocyte up and down with micropipettes. These narrow-gauged pipettes can be made by pulling a fine glass pipette over an open flame (Bunsen, butane, or spirit burner). In addition, pulled pipettes are commercially available from suppliers of IVF consumable materials. These micropipettes are attached to a bulb or holder. Select a micropipette with a diameter that is slightly larger than the oocyte. Commercial micropipettes are available from 75-600 µm (inner diameter). Start with a micropipette that will accommodate the cumulus-oocyte complex (approximately 150-175 µm), then work down to approximately 100 µm, which is about the diameter of the average oocyte. Keep in mind that adjusting the size of the micropipette is important because of variation in oocyte size. A micropipette that is too small will damage the oocyte. To strip cumulus from the oocytes, begin by aspirating a portion of media into the shaft of the micropipette. Then place the mouth of the micropipette over or beside the egg and gently aspirate it into the shaft. If the oocyte with intact cumulus cells does not easily enter the pipette, then a pipette with a larger diameter must be used. Conversely, if the diameter is too large then it will not effectively strip the cumulus cells away from the egg. With the appropriately sized micropipette, continue to gently aspirate and expel the egg through the pipette until

sufficient cumulus cells have been stripped away to allow clear visualization of the cell cytoplasm to allow for assessment of fertilization.

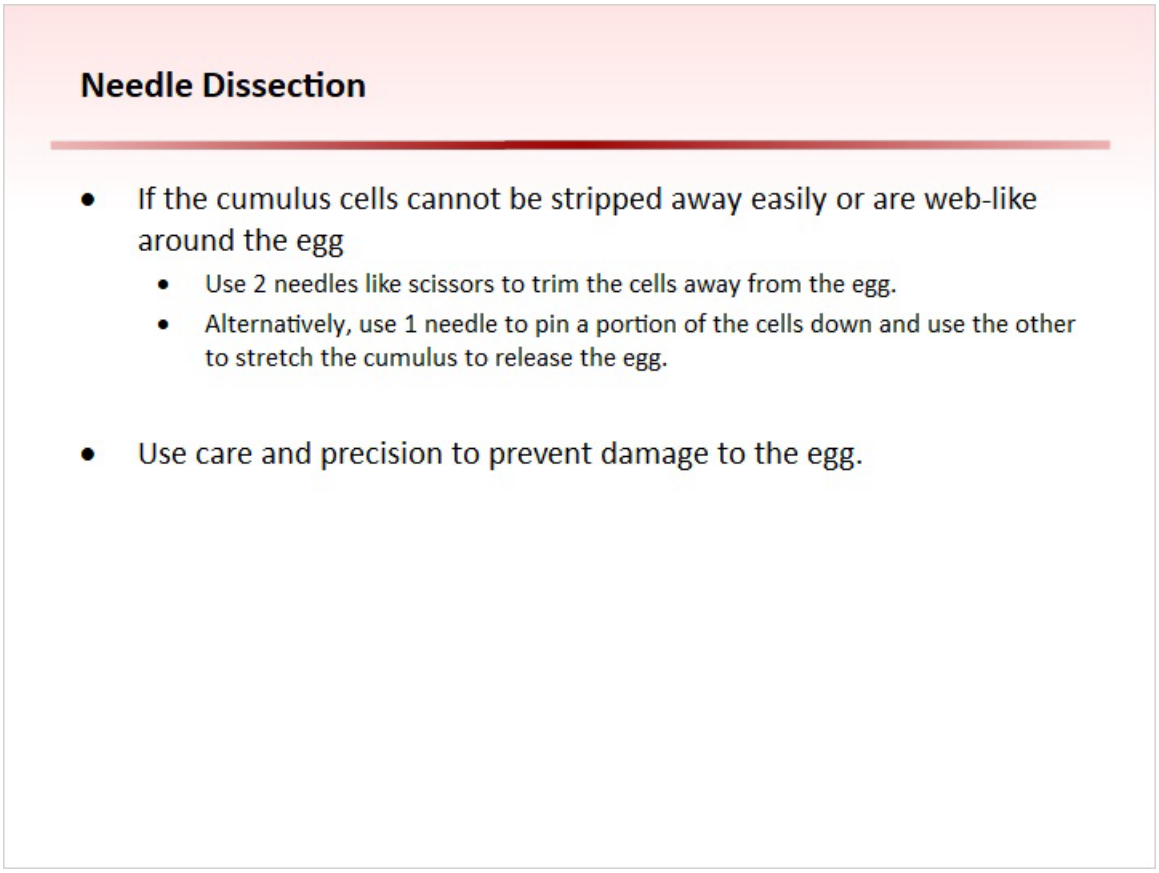
1.6 Schematic of Microdissection



Notes:

The cumulus-oocyte complex (COC) after IVF insemination has a web-like appearance as shown here. Beginning with a large-bore pipette, the COC is aspirated up and down to remove the outer layer of cumulus cells. Progressively smaller-bore pipettes are selected to remove more cells until the majority of cumulus cells have been stripped away. Then fertilization assessment of the presumed zygote can occur.

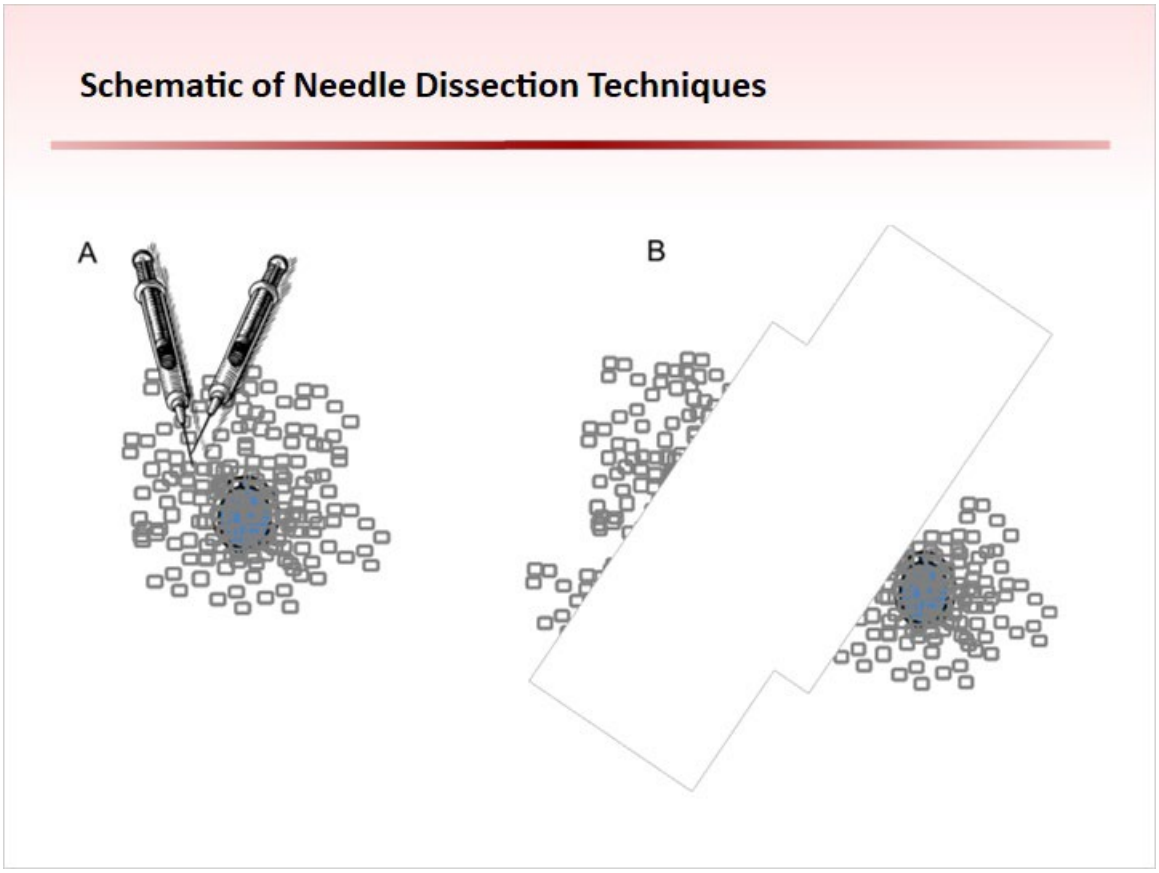
1.7 Needle Dissection



Notes:

If the cumulus cells cannot be stripped away easily with micropipetting, then needle dissection may be required. Sometimes the cells will appear to be web-like and stick together, making it challenging to strip them away from the egg. To tease the oocyte away from the cumulus-oocyte complex 2, 26-gauge needles attached to 1 mL syringes can be used. While visualizing under the dissecting microscope, use 1 of the needles as a guide and pin down a portion of the cellular debris containing the oocyte. Use the second needle in scissor-like fashion to trim away the cells. Alternatively, with one section of cumulus held in place by the initial needle, the second needle can be used to stretch the cumulus. Often this will allow the eggs to break free from the web of cellular debris. With either method, care must be taken to ensure that the tip of the needle does not touch and/or penetrate the zona pellucida and damage the oocyte.

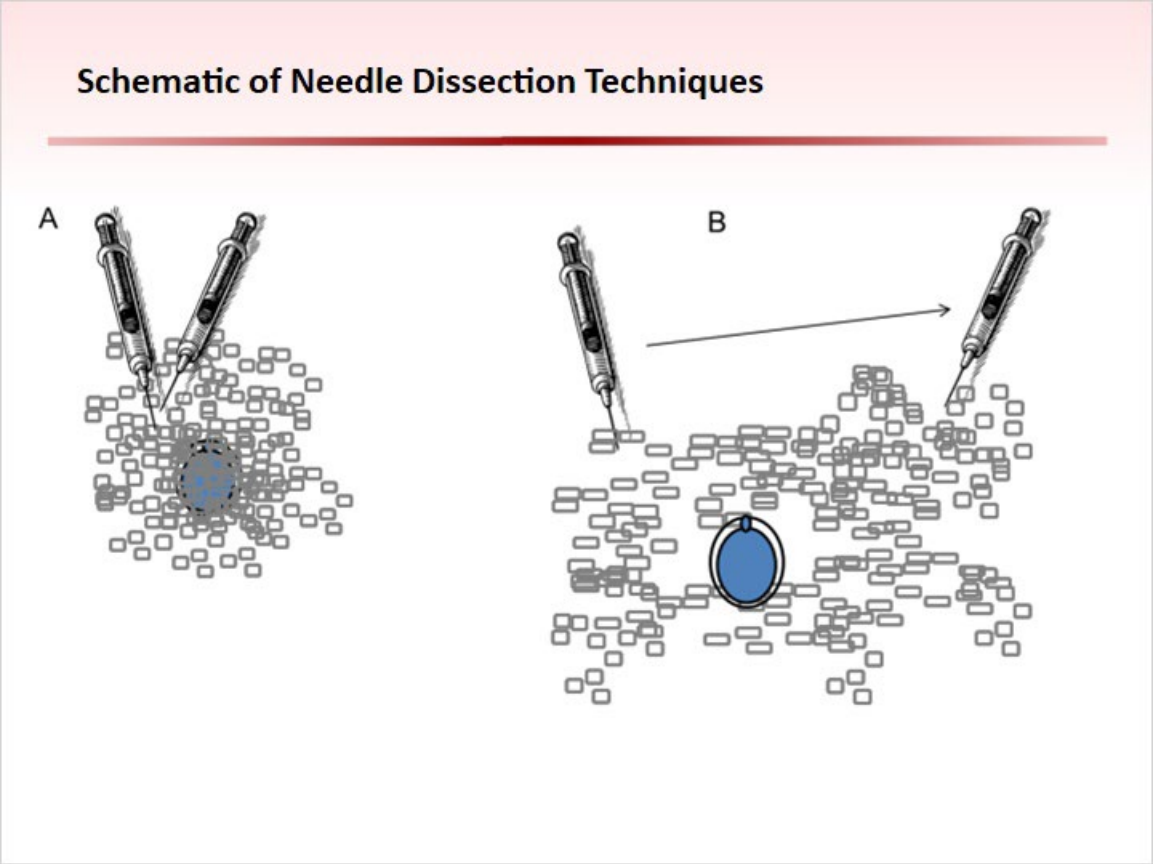
1.8 Schematic of Needle Dissection Techniques



Notes:

Two fine-gauge needles can be used in a scissor-like fashion to trim the cumulus cells away from the oocyte prior to fertilization assessment.

1.9 Schematic of Needle Dissection Techniques



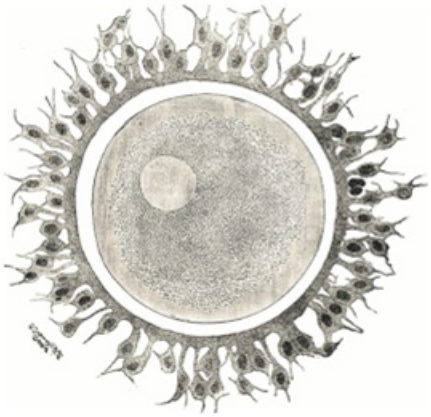
Notes:

One fine-gauge needle can be used to pin down one section of the COC and the other can be used to stretch the cumulus cells, allowing the oocyte to be freed from the cumulus mass.

1.10 Definitions Related to Fertilization

Definitions Related to Fertilization

- **Cortical granule clearing or cortical reaction**
  - Once the sperm has penetrated the zona pellucida, the cortical reaction occurs.
  - Cortical granules are released by exocytosis to the zona pellucida.
  - This causes structural modifications in the glycol-proteins in the zona pellucida, rendering it impermeable to other spermatozoa.

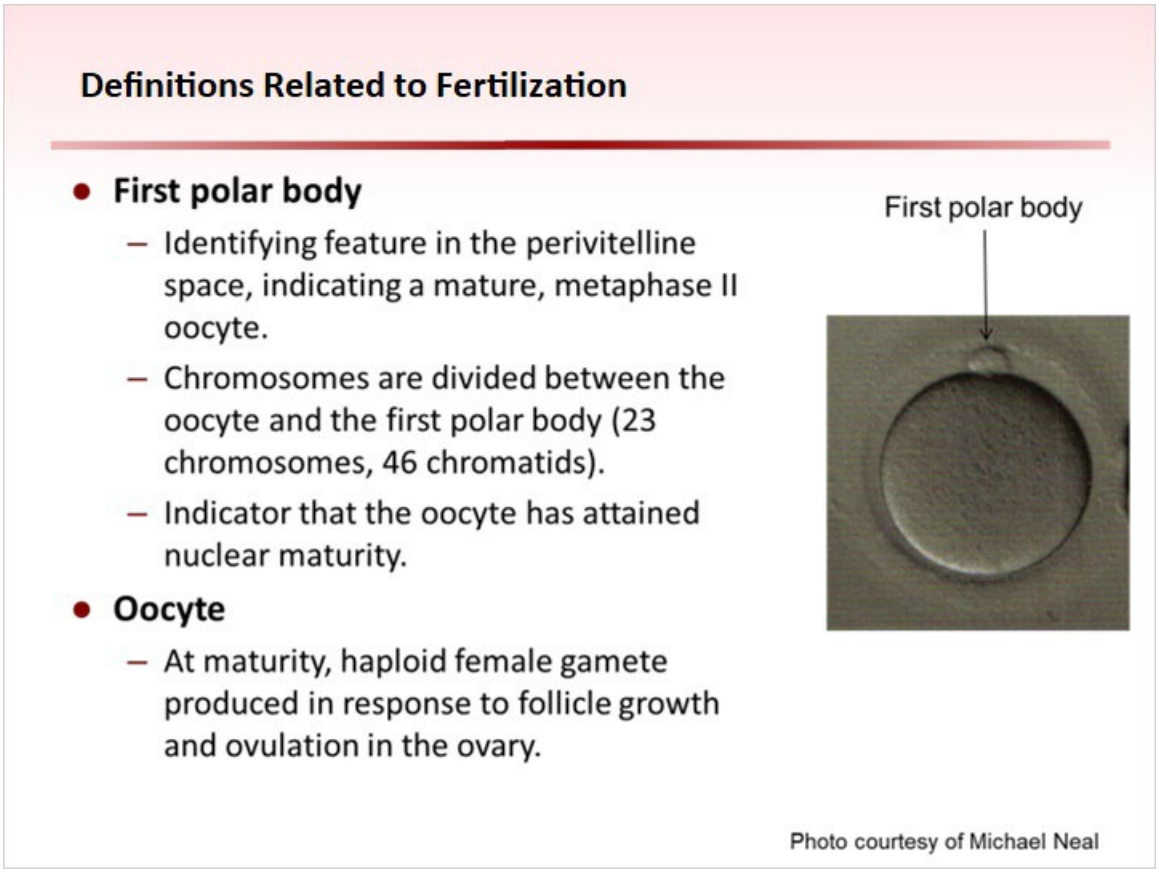


Notes:

Definitions related to fertilization will be helpful here.

Cortical-granule clearing or cortical reaction: once the sperm has penetrated the zona pellucida, the cortical reaction occurs. Cortical granules are released by exocytosis to the zona pellucida. This event causes structural modifications in the glycol-proteins in the zona pellucida rendering it impermeable to other spermatozoa.

1.11 Definitions Related to Fertilization



Notes:

First polar body is the identifying feature in the perivitelline space indicating a mature, metaphase II oocyte. Chromosomes are divided between the oocyte and the first polar body (23 chromosomes, 46 chromatids). This is an indicator that the oocyte has attained nuclear maturity.

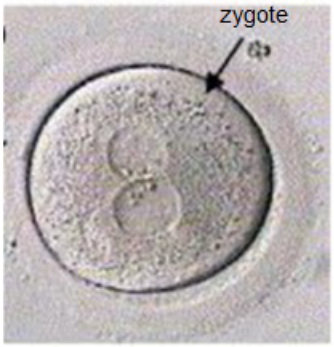
The **oocyte** is a haploid female gamete, produced in response to follicle growth and ovulation in the ovary.

1.12 Definitions Related to Fertilization

Definitions Related to Fertilization

- **Normal fertilization**
  - Identification of 2 pronuclei and presence of 2 polar bodies
  - Union of male and female gametes leads to the formation of a unique zygote (Figure below right).

- **Pronuclear formation (2PN)**
  - Process of fertilization involving mature oocyte membrane activation with sperm decondensation.
  - Fusion of male and female haploid gametes creates a diploid zygote.
  - Culmination of this process is characterized by the observation of two distinct pronuclei with two extruded polar bodies. Sometimes the polar bodies appear fragmented (polar bodies are not fragmented in the figure).



With permission from Fertil Steril. 2006 Oct;86(4):848-61.

Notes:

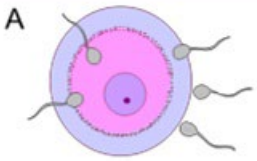
Normal fertilization is the identification of 2 pronuclei and presence of 2 polar bodies. The union of male and female gametes leads to the formation of a unique zygote (Figure displays a zygote).

Pronuclear formation (2PN) is the process of fertilization involving mature oocyte membrane activation with sperm decondensation. The fusion of male and female haploid gametes creates a diploid zygote. The culmination of this process is characterized by the observation of two distinct pronuclei with two extruded polar bodies.

1.13 Definitions Related to Fertilization

Definitions Related to Fertilization

- **Polyspermy -** the event when more than one spermatozoon penetrates the zona pellucida. Decondensation leads to multiple pronuclear formation.



- **3 PN or Abnormal Fertilization**
  - Penetration of two sperm
  - Diploid sperm or ova
  - Failure of the extrusion of the second polar body (generally after ICSI since a single sperm was injected)

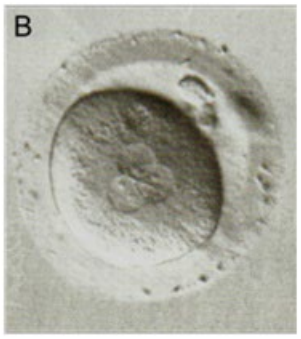


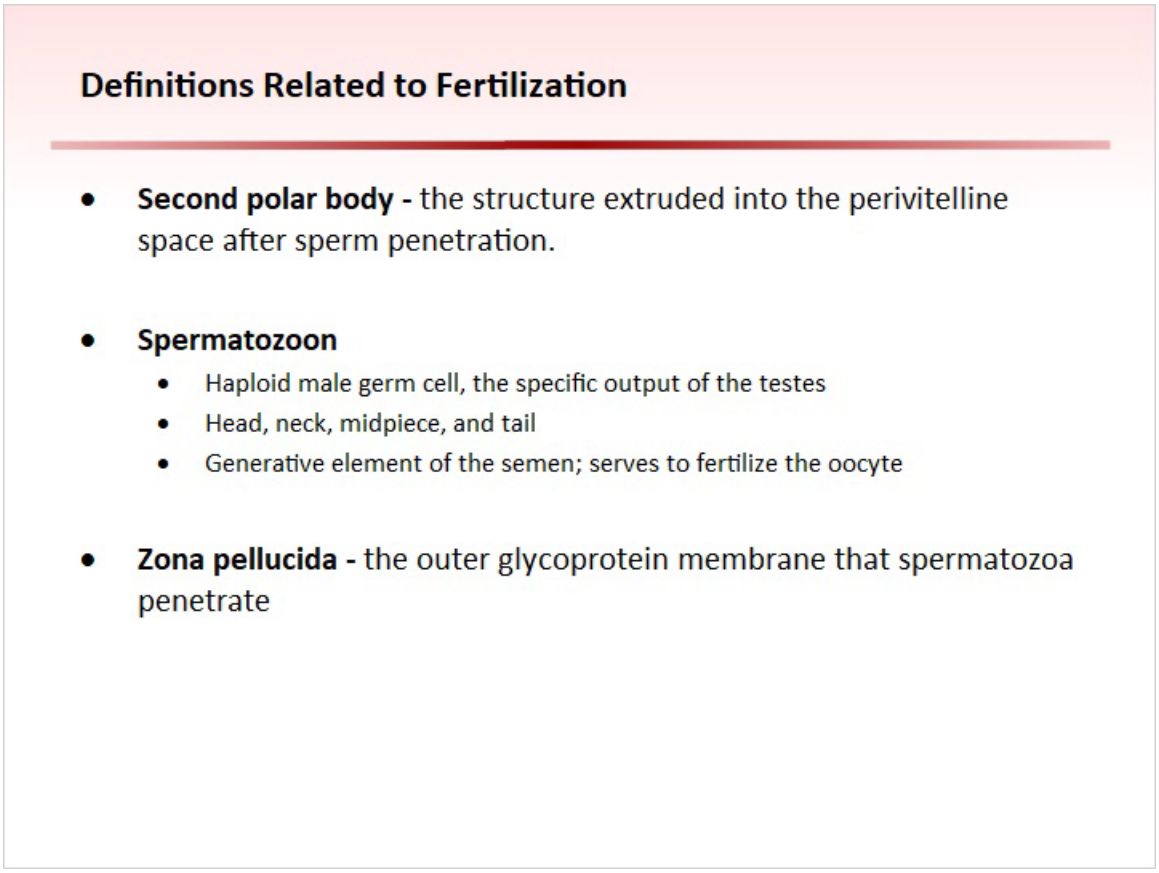
Photo courtesy of Michael Neal

Notes:

Polyspermy (A) is the event when more than one spermatozoon penetrates the zona pellucida. When decondensation occurs, this leads to multiple pronuclear formation.

3 PN or Abnormal Fertilization (B) can occur with the penetration of two sperm, result from diploid sperm or ova, or from the failure of the extrusion of the second polar body.

1.14 Definitions Related to Fertilization



Notes:

The **second polar body** is the structure extruded into the perivitelline space after sperm penetration.

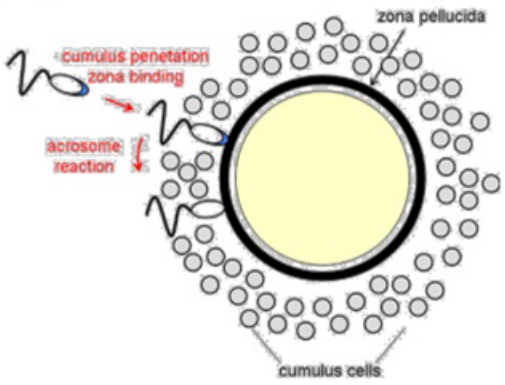
Spermatozoon is a haploid male germ cell, the specific output of the testes and consists of a head, neck, midpiece, and tail. It is the generative element of the semen and serves to fertilize the oocyte.

Zona pellucida is the outer glycoprotein membrane that spermatozoa penetrate.

1.15 Fertilization

Fertilization

- Sperm swim though the cumulus cells to get to the zona pellucida (ZP)
- A spermatozoon binds to the ZP
- Acrosome reaction occurs
- The spermatozoon penetrates through the ZP and enters the oocyte
- Cortical granule reaction

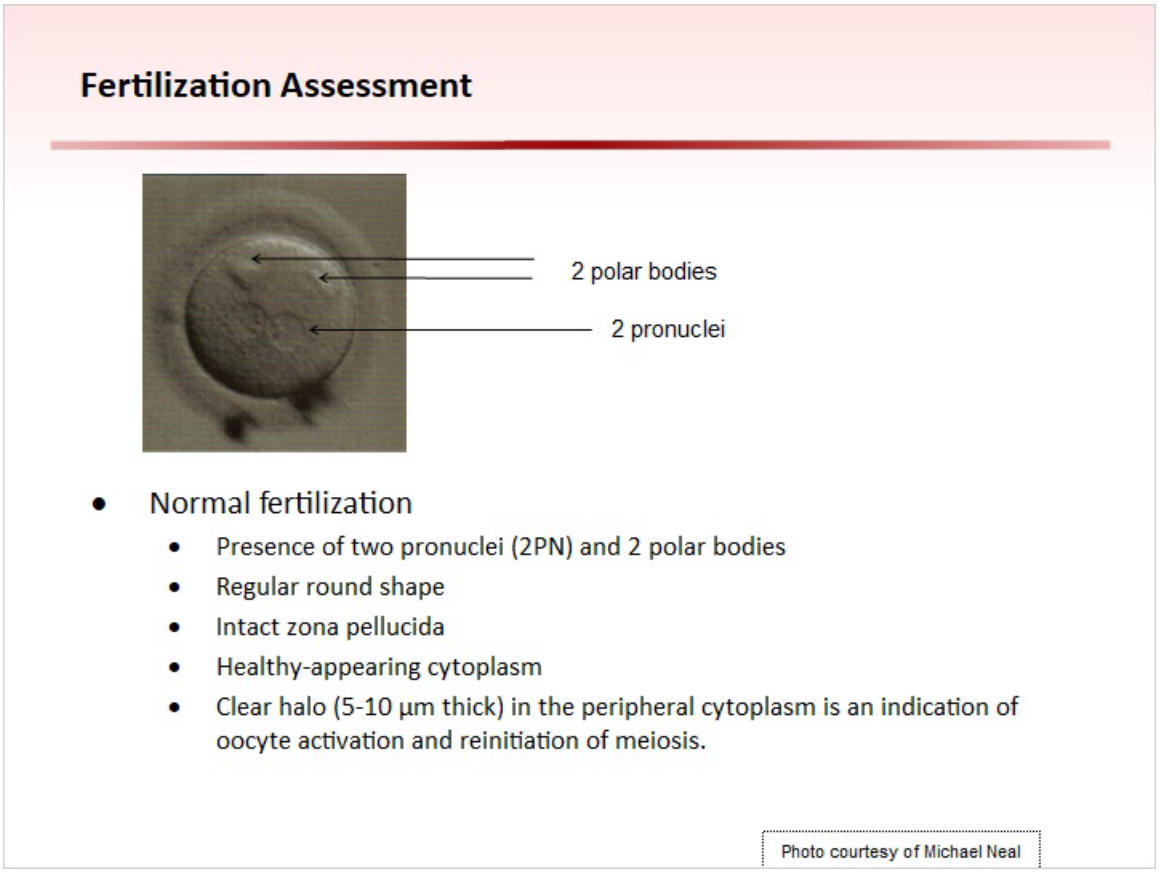


With permission from Florman H M et al. PNAS 2007;104:11123-11124. National Academy of Sciences

Notes:

In the case of conventional in vitro fertilization, sperm are introduced into a dish containing one or more cumulus-oocyte complexes. The sperm must then swim and break their way through the mass of cumulus cells surrounding the egg until a single spermatozoon reaches and successfully binds to the zona pellucida. The ability to breach the zona pellucida is a critical step in fertilization. Binding of the spermatozoon to the egg induces the acrosome reaction. During this process enzymes are released that dissolve the zona pellucida and create a pathway for the sperm to enter the oocyte. Cortical granule exocytosis from the ooplasmic periphery cause a chemical alteration of the zona pellucida, which generally renders it impermeable to other sperm. The best studied enzymes involved in this process include hyaluronidase and acrosin. Intracytoplasmic sperm injection (ICSI) overcomes the hurdle of a spermatozoon getting to and past the zona pellucida.

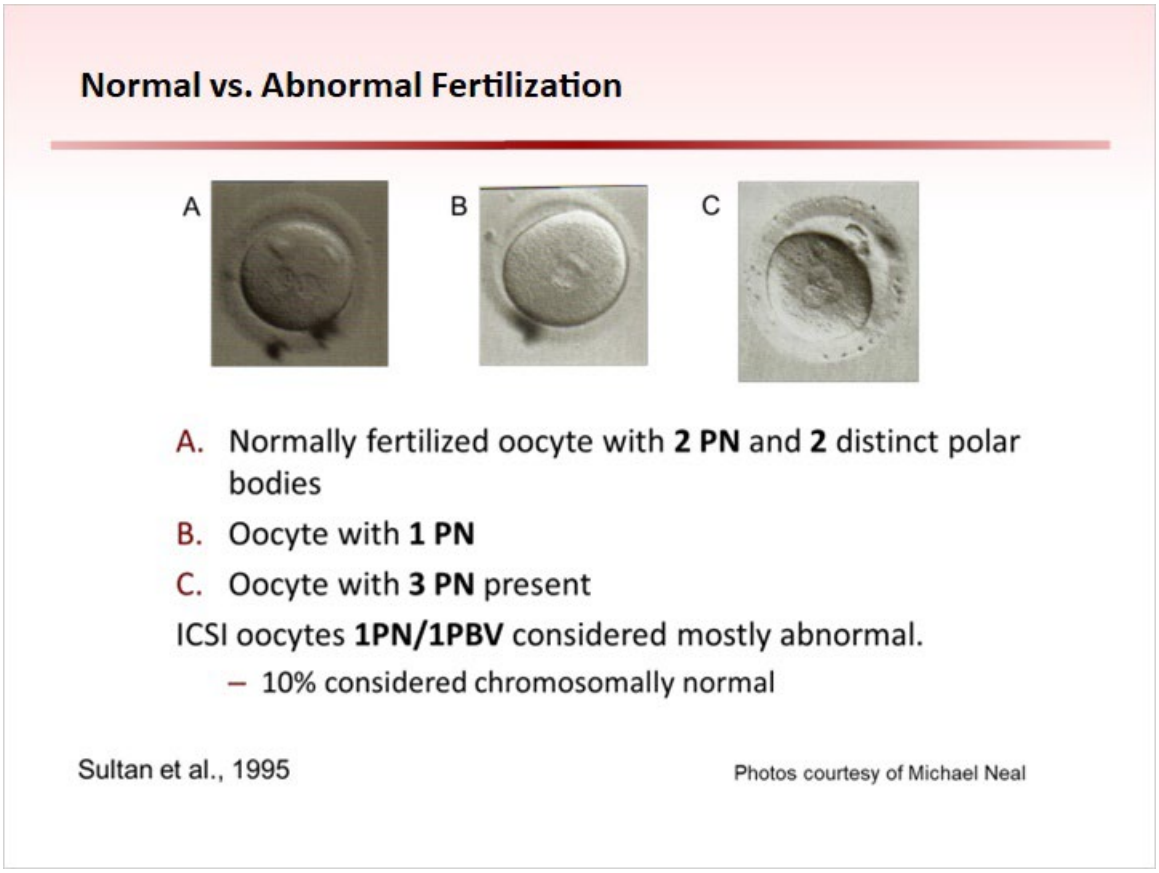
1.16 Fertilization Assessment



Notes:

Pronuclei can be observed under the dissecting microscope. However, it is recommended that fertilization assessments be performed using an inverted microscope. This will allow the embryologist to assess greater detail and distinguish normal pronuclei from vacuoles or other cytoplasmic irregularities. Furthermore, it allows for pronuclei assessment. The presence of 2 pronuclei (2PN) and 2 polar bodies indicates a normally fertilized oocyte or zygote. Other features of a normally fertilized egg include: regular round shape, an intact zona pellucida, and a healthy appearing cytoplasm. A clear halo (5-10 µm thick) in the peripheral cytoplasm is an indication of oocyte activation and reinitiation of meiosis.
Observation of acceptable development documents the presence of the pronuclei (PN) and polar bodies visible (PBV).

1.17 Normal vs. Abnormal Fertilization



Notes:

Zygotes with more than 2PN present have abnormal fertilization. **1PN/2PBV** is sometimes seen with IVF since the actual time that the sperm reaches and fuses with the egg is not known, and the zygote may have progressed to syngamy of the pronuclei. This is considered normally fertilized; however this embryo is not usually selected for transfer due to the unknown status in the presence of other embryos with known normal fertilization. It may be cultured and cryopreserved instead of being selected for transfer unless no other embryo is available for transfer. This is not considered normal development with ICSI oocytes.

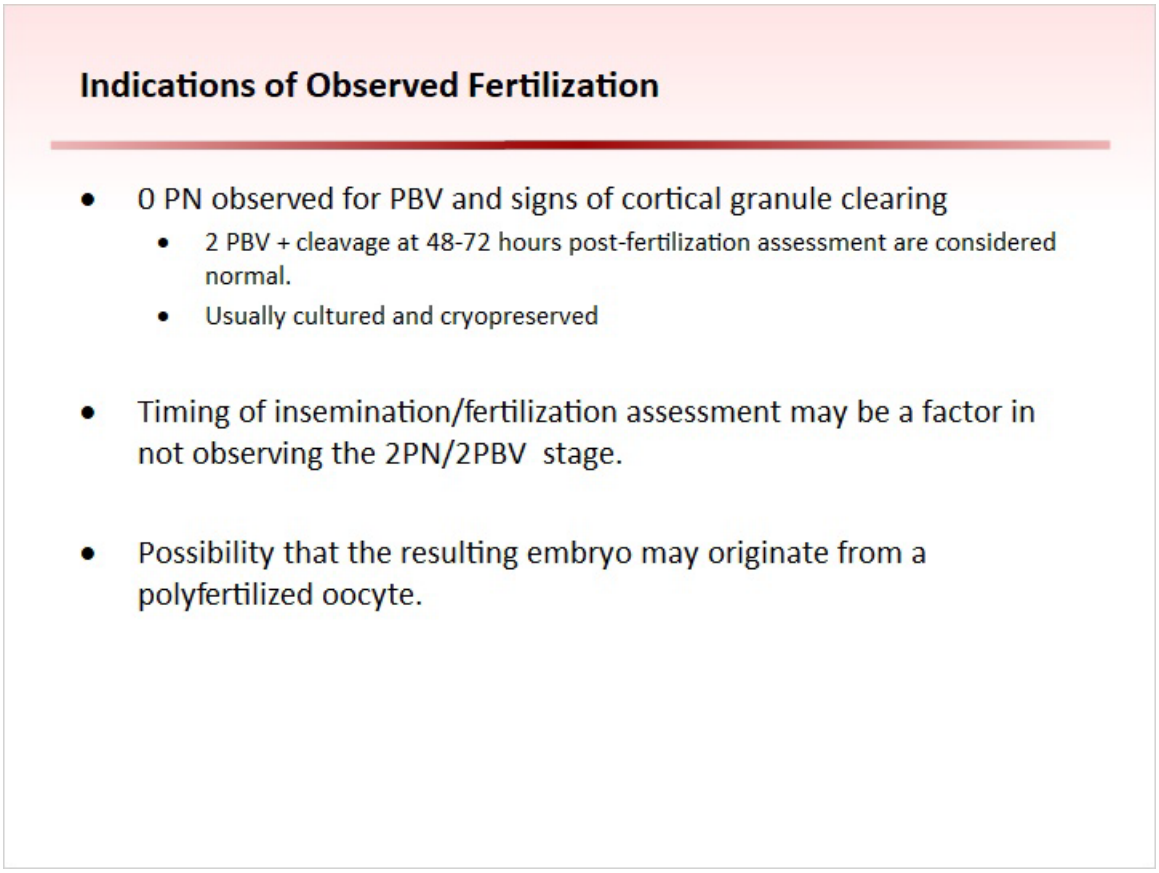
3PN/2PBV in IVF/ICSI may be caused by a diploid oocyte or diploid spermatozoa or by polyfertilization in IVF only.

3PN/1PBV denotes a digenic oocyte. In ICSI, since these are monospermic zygotes, it is assumed that the oocyte failed to extrude its second polar body or that the centriole has divided in a triploid manner.

ICSI oocytes that exhibit **1PN/1PBV** are considered mostly abnormal. Statistically, 10% are

considered chromosomally normal. There is a high haploid and diploid mosaic development in these oocytes/embryos. With ICSI, these usually indicate haploid development, suggesting that sperm-oocyte interaction may have failed (Sultan et al., 1995). These are not considered for transfer; unless no other embryos are available.

1.18 Indications of Observed Fertilization



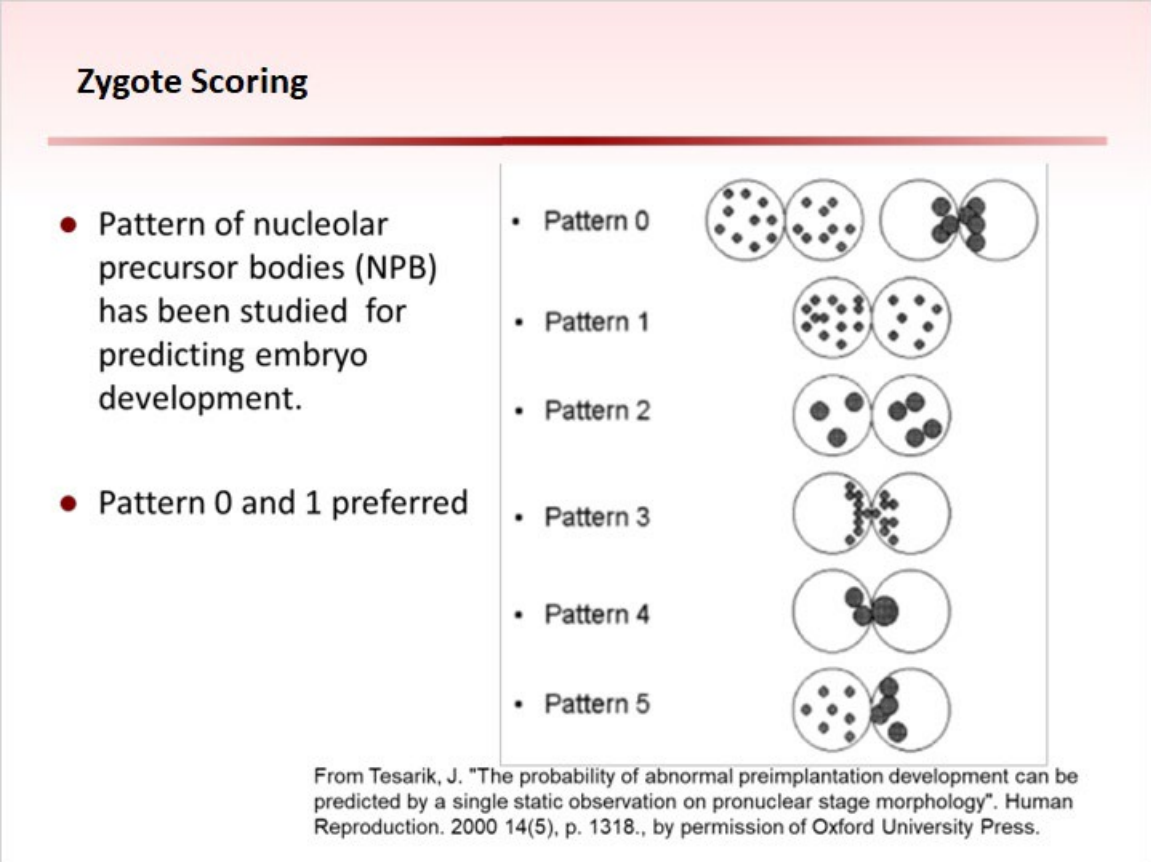
Notes:

Oocytes that exhibit **0**PN at the time of fertilization assessment are observed for polar body number and signs of cortical granule clearing. IVF oocytes that exhibit two polar bodies and cortical granule clearing and cleave at the time of the 48-72-hour embryo assessment procedures may be considered to be normally developing. These zygotes will typically be cultured and cryopreserved instead of being selected for embryo

transfer due to the unknown status in the presence of other embryos with known normal fertilization.

Timing of insemination/fertilization assessment may be a factor in not observing the 2PN/2PBV stage. However, because the pronuclear stage was not appropriately assessed, there is a possibility that the resulting embryo may originate from a polyfertilized oocyte.

1.19 Zygote Scoring

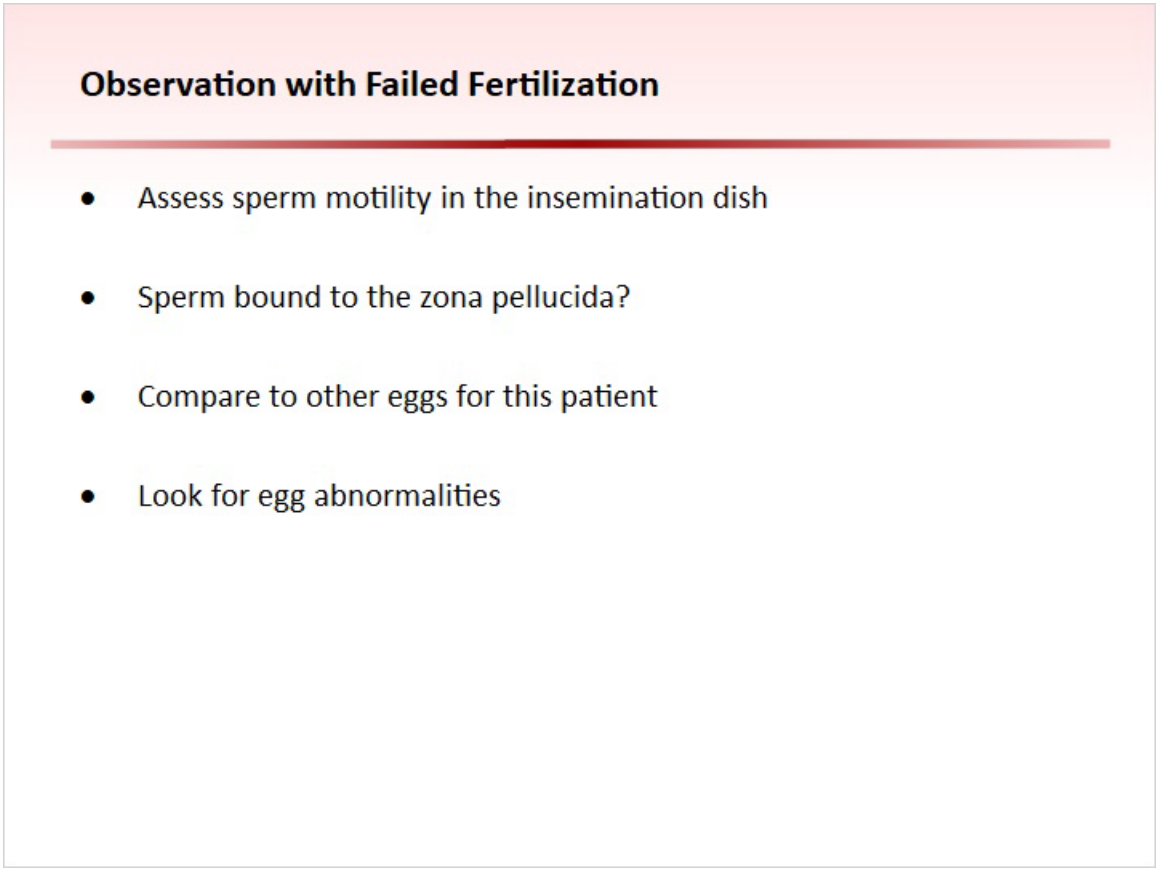


Notes:

The timing and pattern of pronuclei formation have been studied in relation to the ability to predict which embryos in a cohort have the best implantation potential. While several papers have shown a positive prognostic effect (Scott et al., 2003; Scott 2000; Tesarik & Greco, 1999; Tesarik et al., 2000; Balaban et al., 2001; Nagy et al., 2003; Gianoroli et al., 2003; Edirisinghe et al., 2005), others have shown no beneficial predictive value (Salmuts et al., 2001; James et al., 2006; Weitzman, 2010). Typically, pronuclei are similar in size and centrally located in the fertilized oocyte.

Pronuclear scoring is based on the symmetry and alignment of the pronuclei. It involves rolling the zygote to ensure proper evaluation of the number and relative position of the nucleolar precursor bodies (NPB) which are established in the pronuclei. Ideally, each pronucleus should contain 5-7 NPB with each of the male and female pronuclei having a similar number of NPB. Inequity between the male and female pronuclei is considered abnormal and is graded at a lower score. Animal studies have correlated the importance of NPB for normal embryo development and competence (Svarcova et al., 2009).

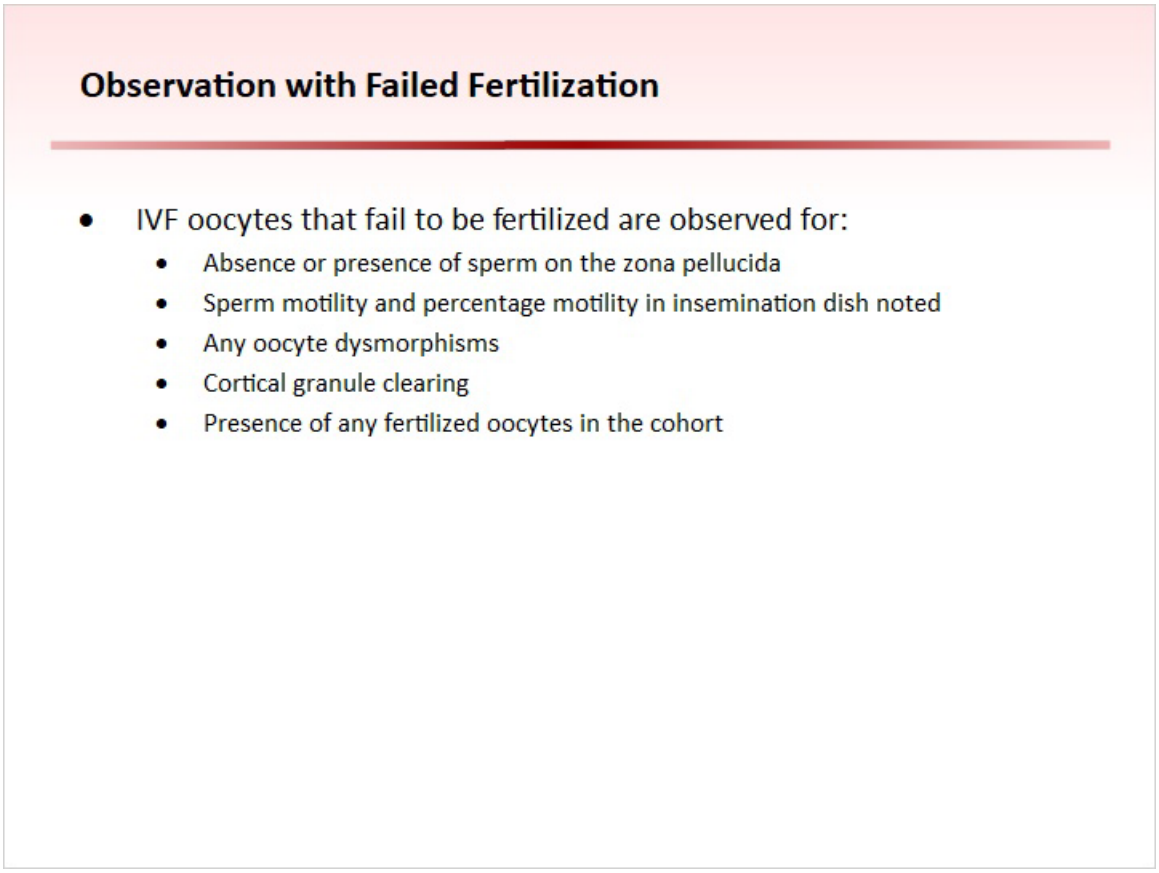
1.20 Observation with Failed Fertilization



Notes:

The following observations should be made when fertilization fails:
Assess sperm motility in the insemination dish, determine whether sperm are bound to the zona pellucida, compare with other eggs for this patient, and look for egg abnormalities.

1.21 Observation with Failed Fertilization

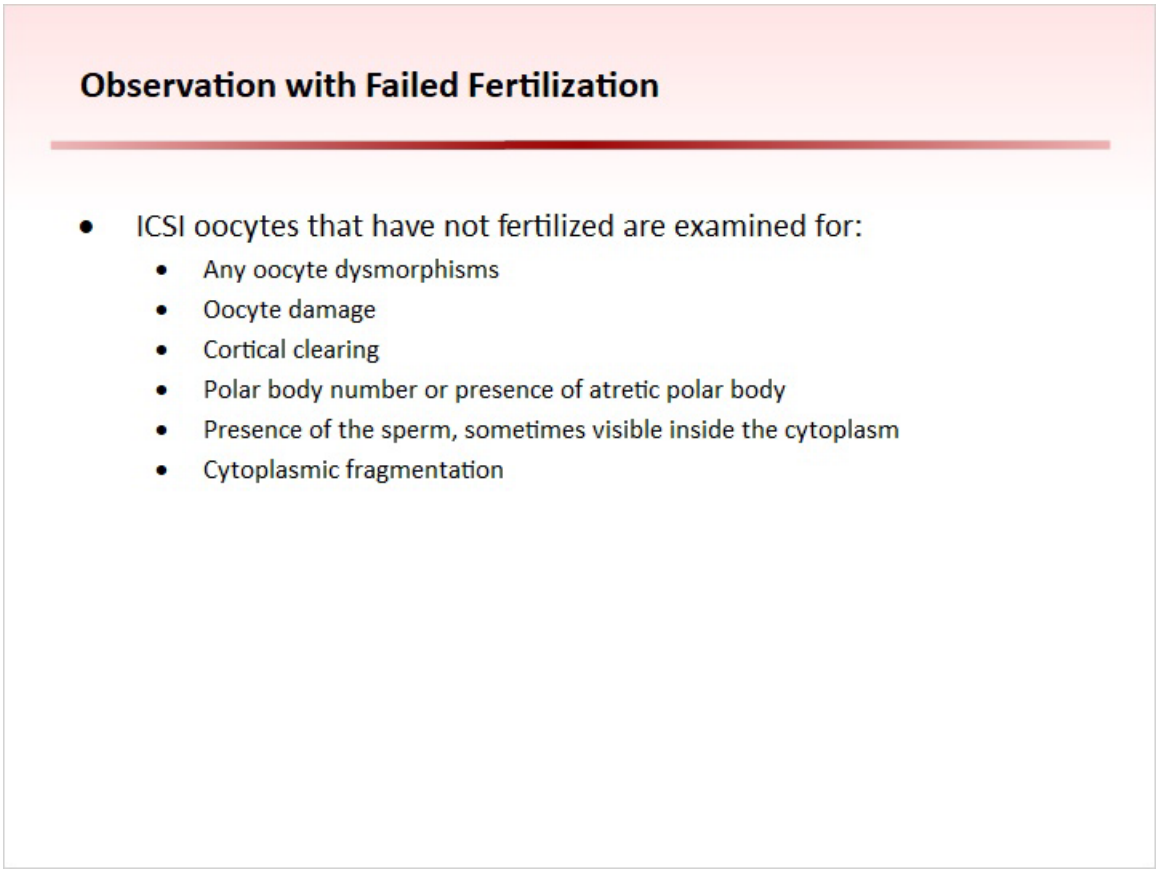


Notes:

IVF oocytes that fail to be fertilized are observed for the following:

- Absence or presence of sperm on the zona pellucida
- Sperm motility and percentage motility in insemination dish noted
- Any oocyte dysmorphisms
- Cortical granule clearing
- Presence of any fertilized oocytes in the cohort

1.22 Observation with Failed Fertilization

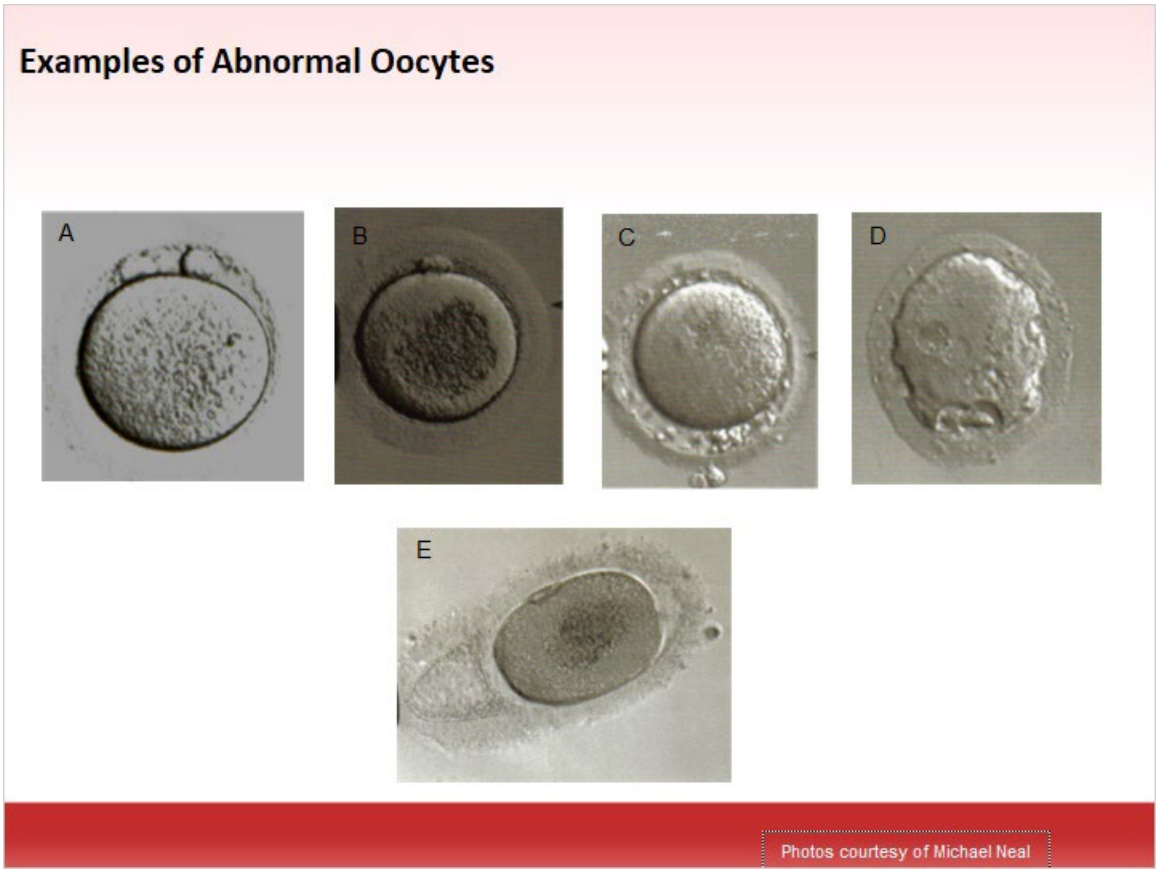


Notes:

ICSI oocytes that have not fertilized are examined for the following:

- Any oocyte dysmorphisms
- Oocyte damage
- Cortical clearing
- Polar body number or presence of atretic polar body
- Presence of the sperm, sometimes visible inside the cytoplasm
- Cytoplasmic fragmentation

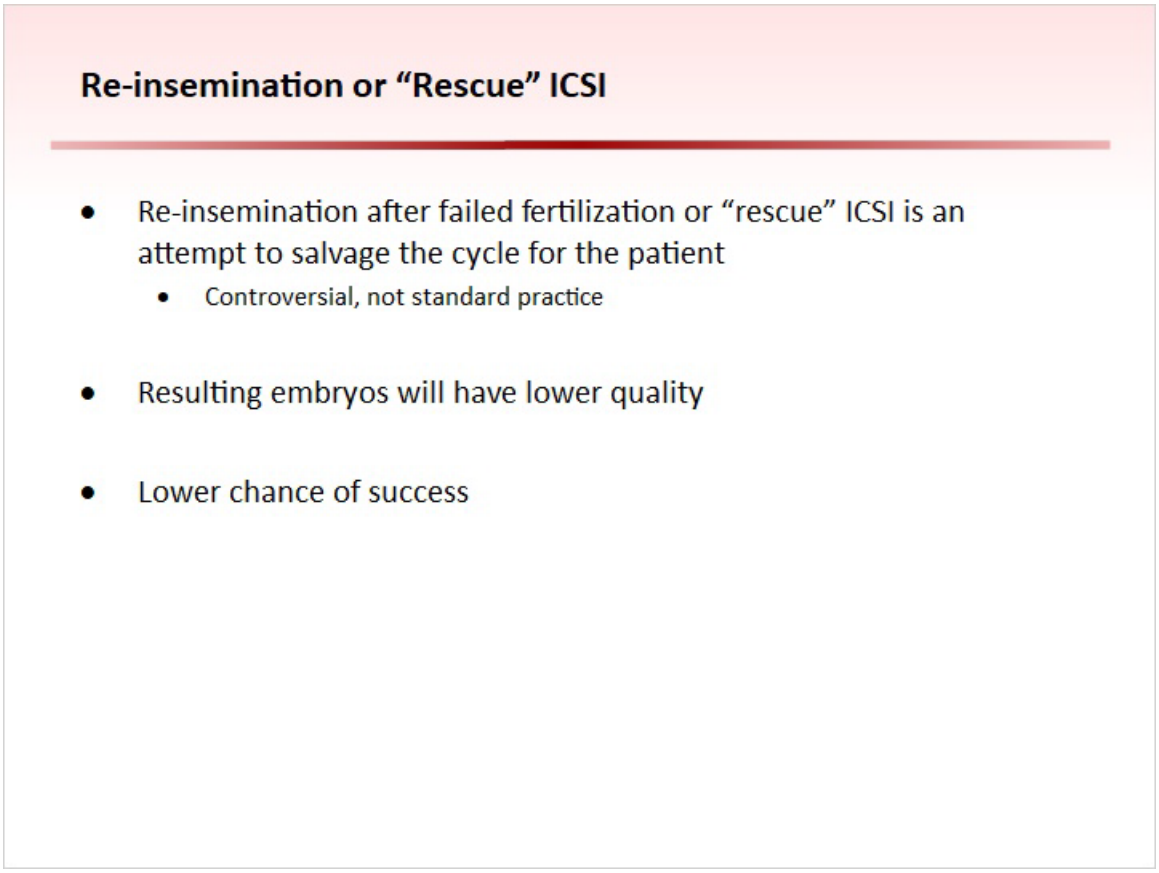
1.23 Examples of Abnormal Oocytes



Notes:

Examples of abnormal oocytes:
A: oocyte with 2 distinct polar bodies
B: oocyte with central granularity
C: oocyte with a high degree of perivitelline debris
D: fertilized oocyte with irregular cytoplasm
E: oocyte with a thick irregular zona pellucida, oval, flat polar body and central granularity

1.24 Re-insemination or "Rescue" ICSI

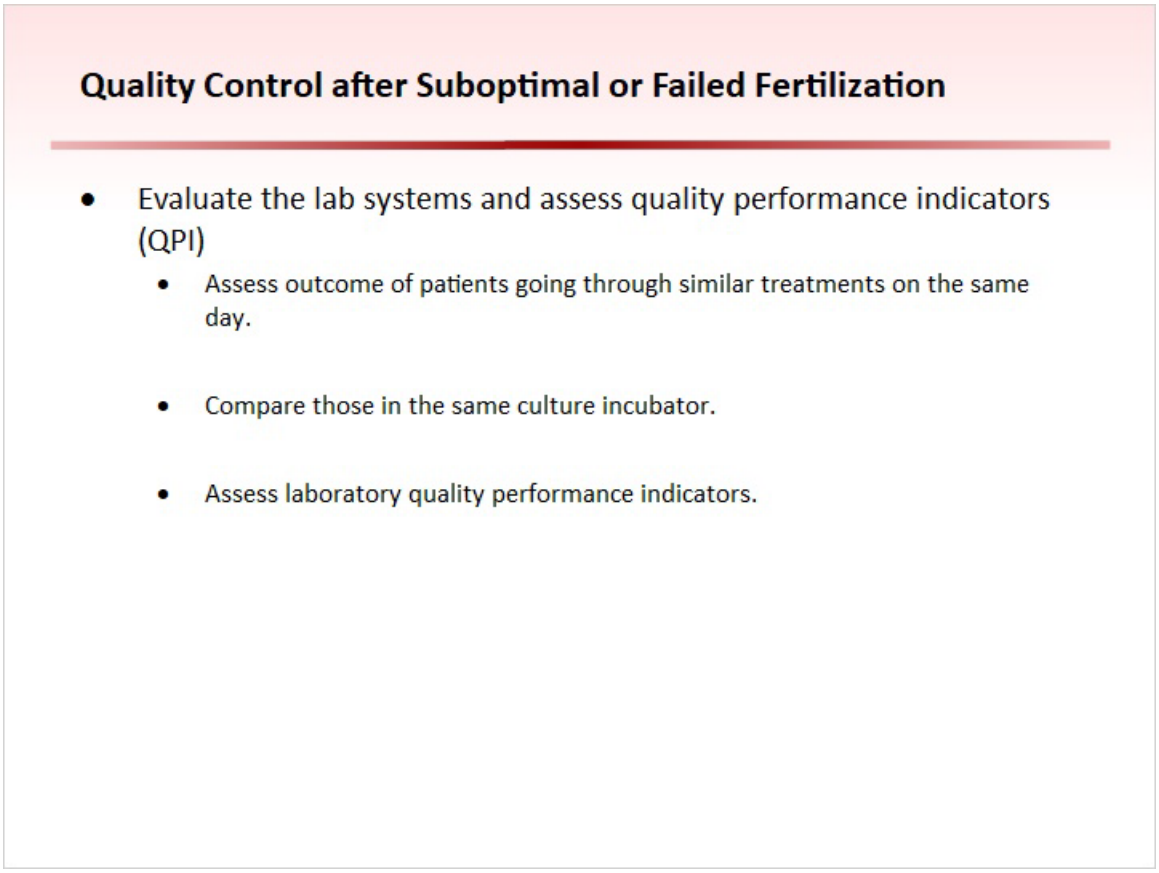


Notes:

If oocytes do not exhibit clear signs of fertilization at the time of assessment, they can be re-inseminated or a "rescue" ICSI procedure used. However, this practice has been the topic of much scientific debate, and it is not a standard practice. If re-insemination occurs, the time of observation for fertilization and cleavage needs to be adjusted according to the time of re-insemination. It is preferred to perform re-insemination as soon as possible with a fresh sperm sample. If this is not possible, a portion of the partner's leftover sperm sample from the initial insemination can be used.

Embryos from "rescue" ICSI or re-insemination typically are of lesser quality and have a poor prognosis for implantation and pregnancy. However, the news of failed fertilization is devastating for the couple. Many patients appreciate the continued attempt to create embryos for transfer despite the low chance of success.

1.25 Quality Control after Suboptimal or Failed Fertilization



Notes:

After a case of suboptimal or failed fertilization it is important to evaluate the lab systems and assess the lab's quality performance indicators (QPI). Fertilization success should be compared with other patients who underwent retrieval on the same day to ensure that the lack of fertilization is not a universal problem. It is ideal if embryos of other patients undergoing the same procedures (i.e., IVF) cultured in the same incubator can be used for comparison. If the comparison reveals that the failed fertilization event is isolated to one patient then this review should be documented. If the comparison shows an effect on more than one patient, then a broader investigation of QPI should be undertaken and documented to ensure that the culture conditions and laboratory environment are working properly.

1.26 Thank you!

Notes:

Thank you for participating in this educational activity.