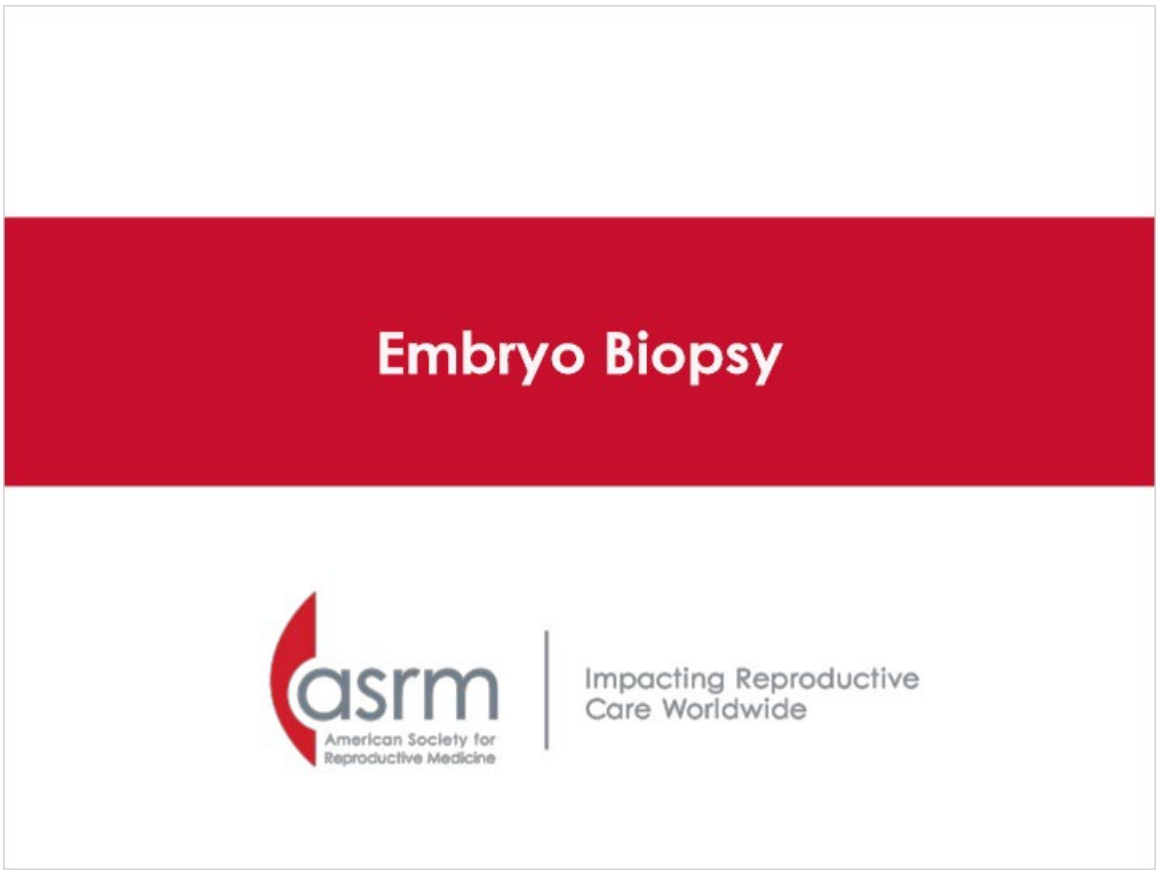
LABCC100 Lesson 7

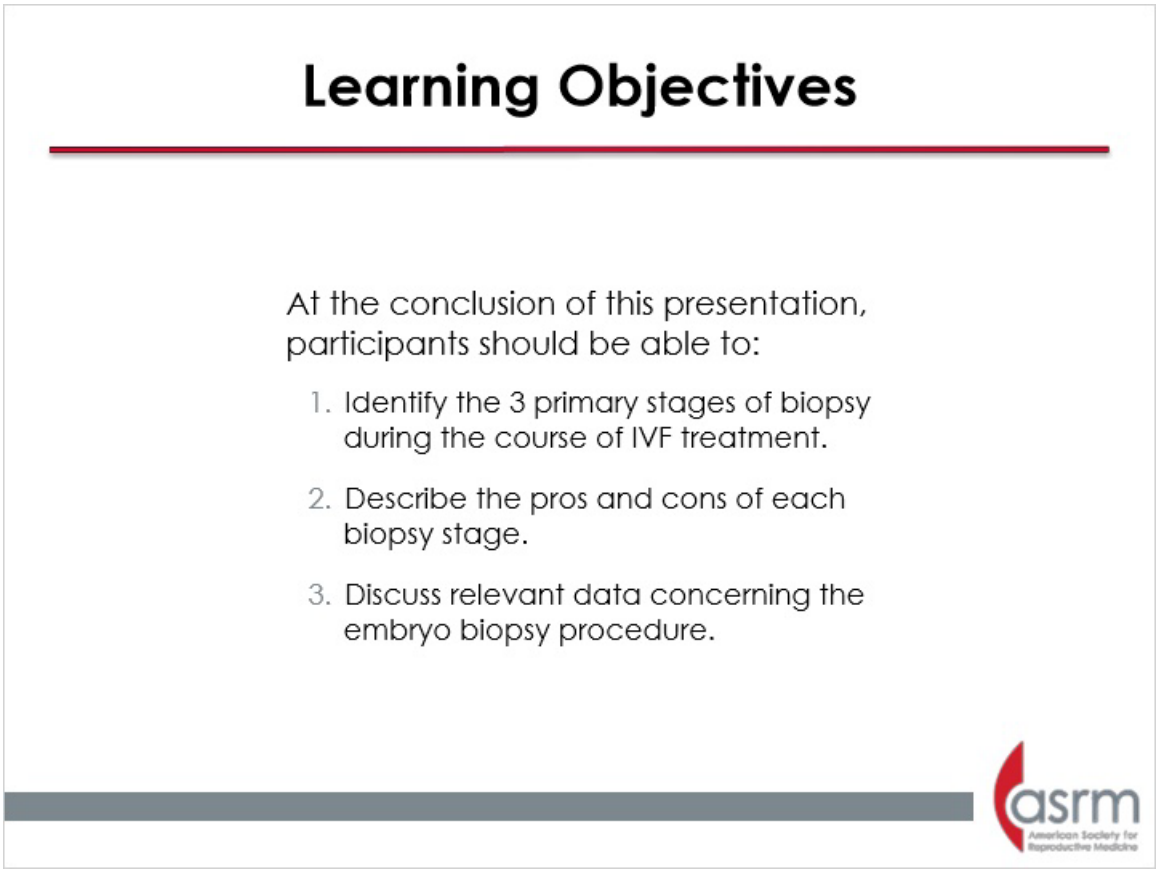
1.1 Embryo Biopsy



Notes:

Welcome to the American Society for Reproductive Medicine's eLearning modules. The subject of this presentation is Embryo Biopsy.

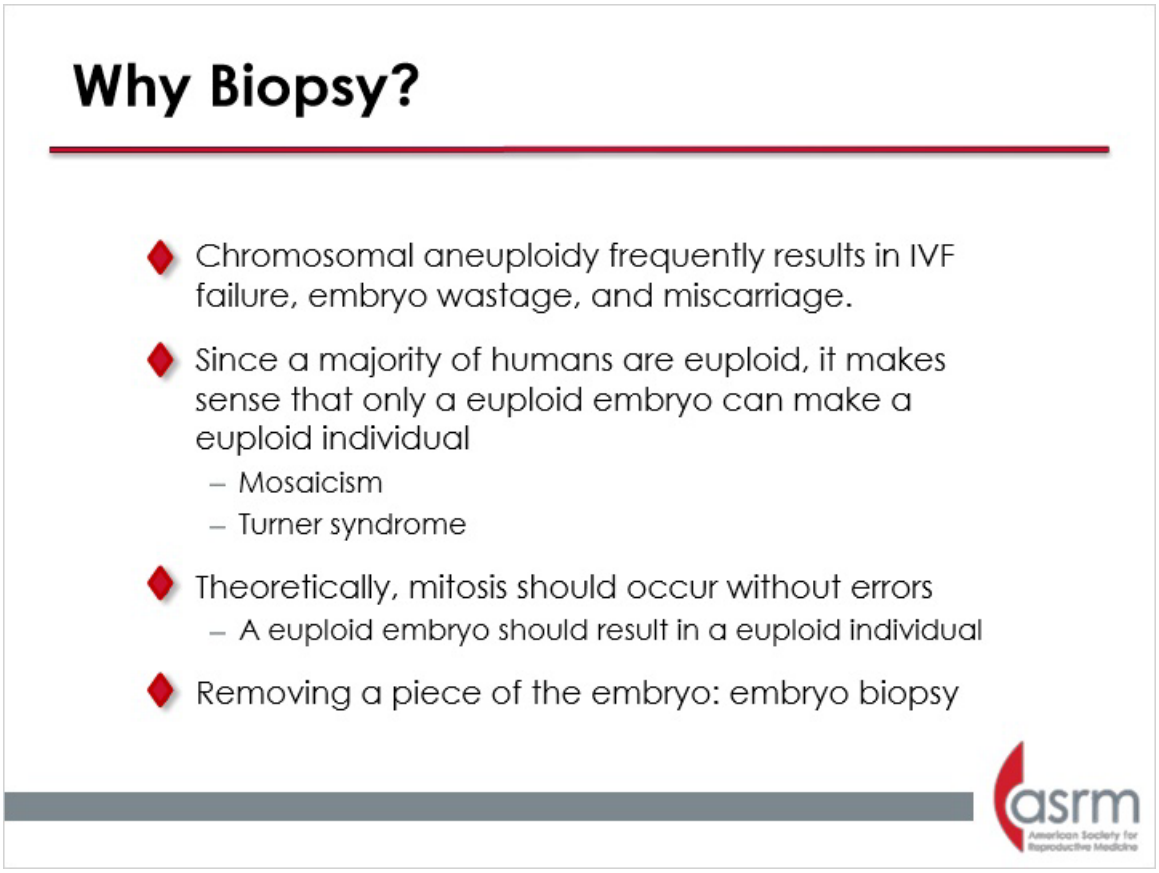
1.2 Learning Objectives



Notes:

At the conclusion of this presentation, participants should be able to:
1)Identify the 3 primary stages of biopsy during the course of IVF treatment.
2)Describe the pros and cons of each biopsy stage.
3)Discuss relevant data concerning the embryo biopsy procedure.

1.3 Why Biopsy?

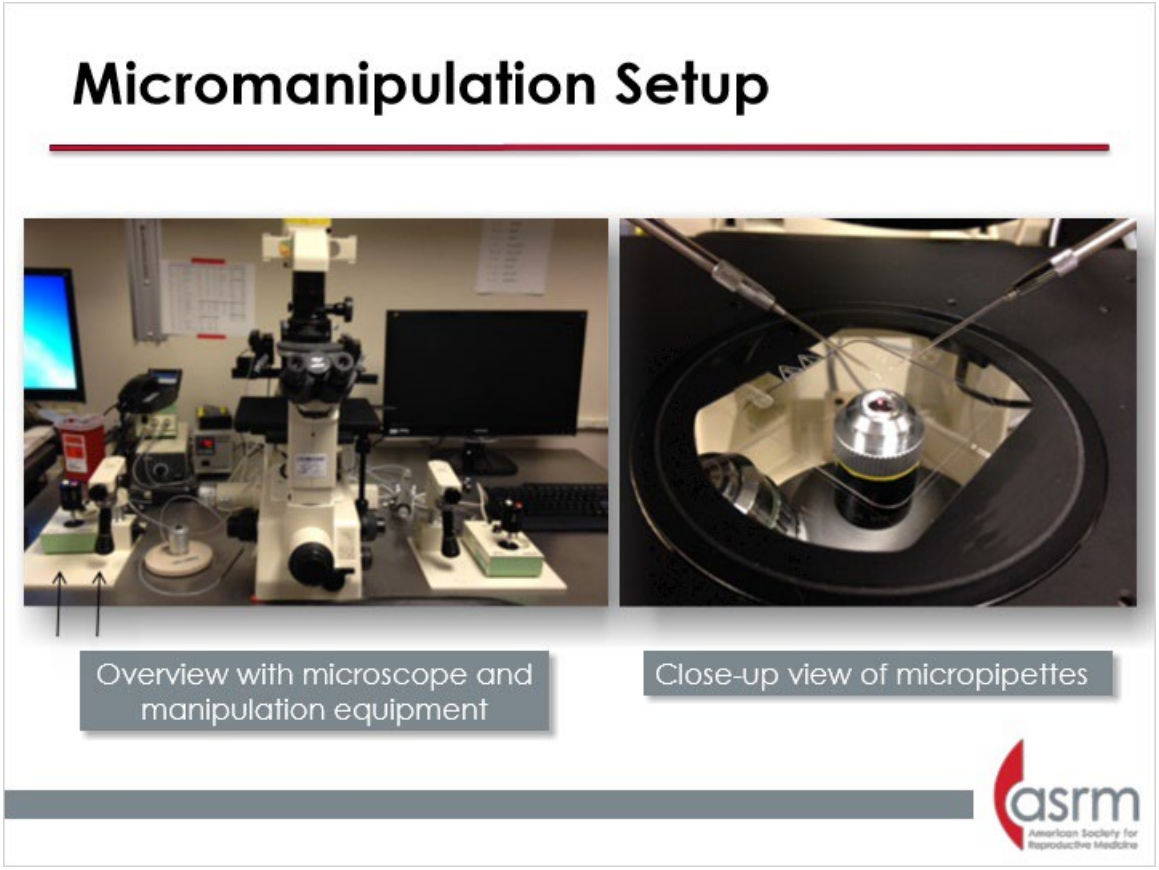


Notes:

Why is embryo biopsy performed? Given that chromosomal aneuploidy frequently results in IVF failure, embryo wastage, and miscarriages, it stands to reason that selection and transfer of embryos free of chromosomal abnormalities theoretically should result in higher pregnancy rates and lower miscarriage rates. While the majority of humans are euploid, there is a small percentage of individuals with mosaic cell lines and/or individuals who are not euploid (Turner syndrome for example). The first cellular division at the zygote stage to the last cellular division prior to birth should occur without error, and as such, a euploid preimplantation embryo should result in a euploid individual.
In order to test the genetic or chromosomal status of the embryo, a piece of it must be removed, a procedure referred to as embryo biopsy.

It should be noted that while normal chromosomal status may be the most common reason for biopsying an embryo, it is not the only reason. Identification of embryos with single gene defects or chromosomal translocations as well as gender selection may be considered valid reasons to biopsy an embryo.

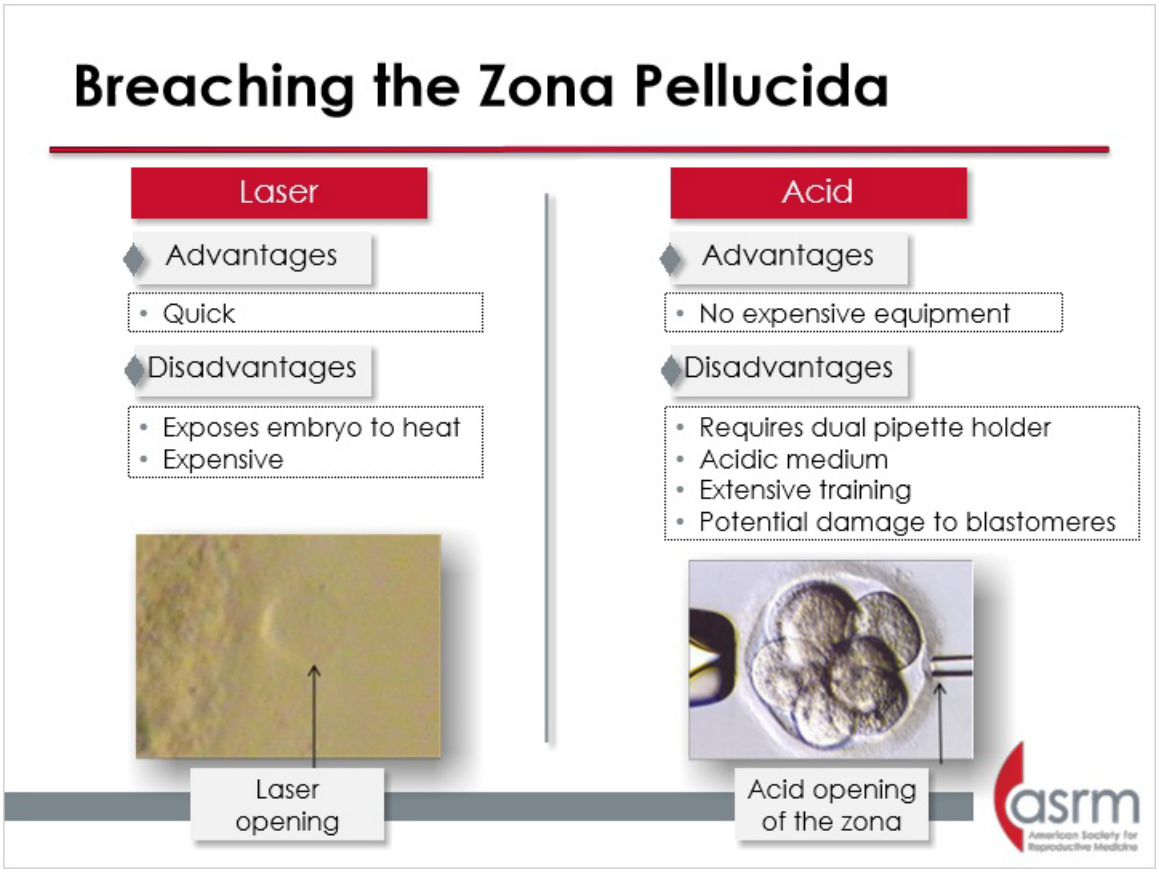
1.4 Micromanipulation Setup



Notes:

In order to perform oocyte/embryo biopsy, an inverted microscope is adapted with micromanipulators and controls as shown in the photo on the left. In this setup, the motorized controls and the hanging joysticks (indicated by the black arrows) allow for the coarse and fine movement of the micromanipulation pipettes. The photo on the right shows a closer view of the microscope stage with the micropipettes. One pipette is used to hold the oocyte or embryo in place while the other enables manipulation to remove the cell. A heated stage is used to maintain proper temperature while the dish containing the embryos is out of the incubator.

1.5 Breaching the Zona Pellucida



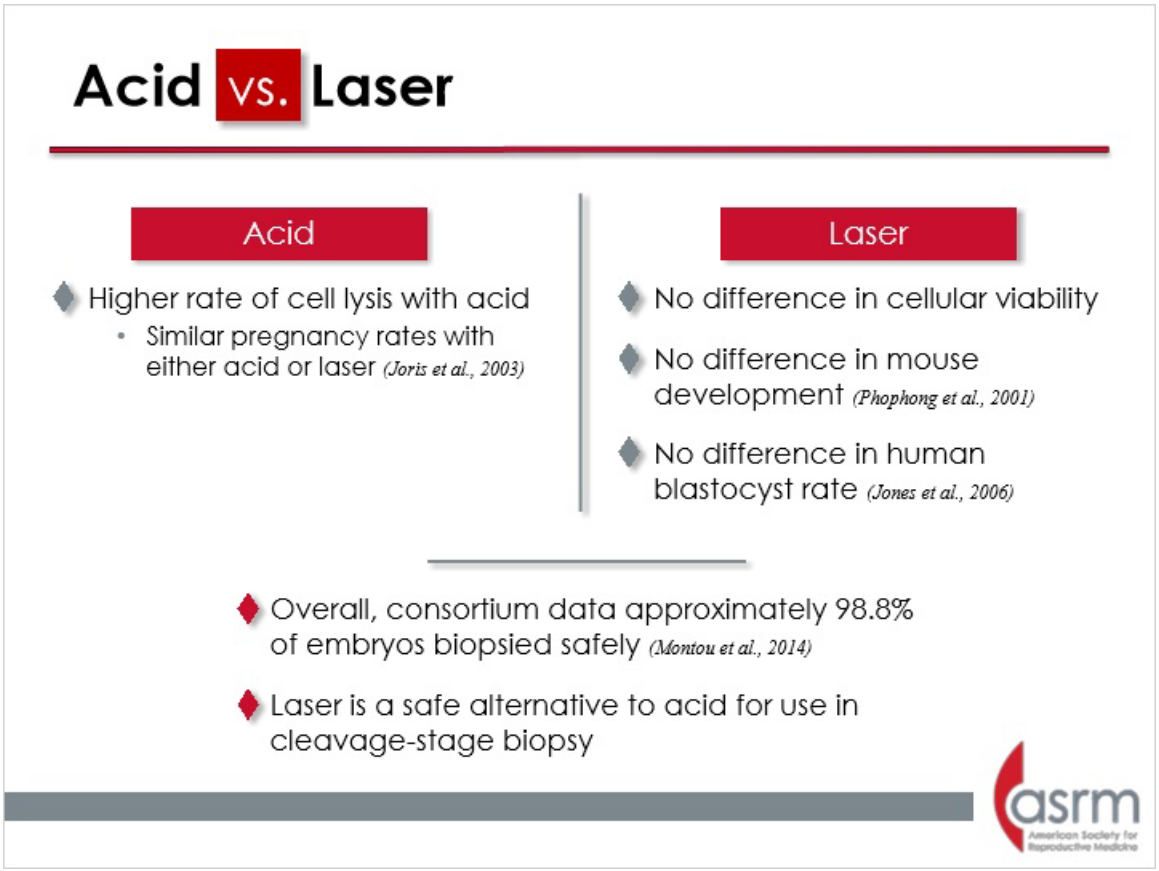
Notes:

The zona pellucida must be breached in order to remove the cell or cells from the oocyte or embryo. Two techniques are commonly used to breach the zona: laser or the use of an acidified medium. Partial zona dissection is also used by some technicians, especially when performing polar body biopsy. Each has advantages and disadvantages. The laser is quick and easy to operate once properly trained. However, the laser pulse heats the culture medium and the zona pellucida, which may potentially damage the embryo. The laser is an expensive piece of equipment for laboratories to purchase and usually is linked to a Windows-based computer system. For laboratories working with a biopsy specialist who comes in just to perform biopsy cases, the specialist may bring a laser, but it may or may not adapt to the inverted microscope in the laboratory.

An alternative to breaching the zona with a laser is the use of an acidified medium such as acidified Tyrode's. This method was used before the advent of the laser method; however, the majority of biopsy cases today are performed using a laser. The acid technique does not require expensive equipment, only an additional pipette holder for the micromanipulation setup and the acid medium. This technique requires extensive training. It is possible that the acidified medium will be expelled into the embryo,

exposing the blastomeres to the acid and potentially damaging the embryo.

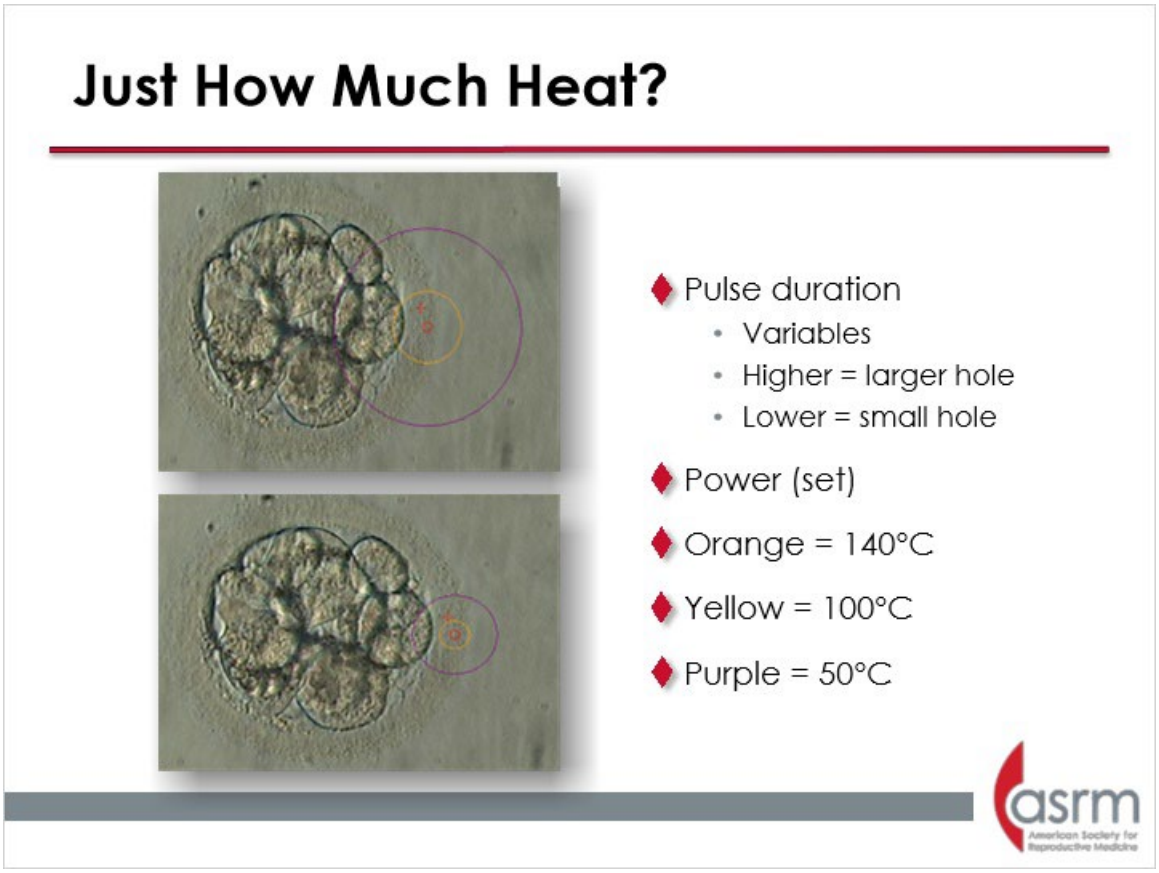
1.6 Acid vs. Laser



Notes:

The majority of research has shown no difference in embryo development between use of laser and acid to breach the zona pellucida. Some researchers demonstrated a higher rate of cell lysis with the acid than with the laser, which may be attributed to technician training and experience. Regardless, research has shown that the laser is a safe alternative to acid for use in cleavage-stage biopsy. Overall, the largest consortium to collect specific preimplantation genetic diagnosis (PGD) data from over 60 centers worldwide reports that 98.8% of embryos were successfully biopsied.

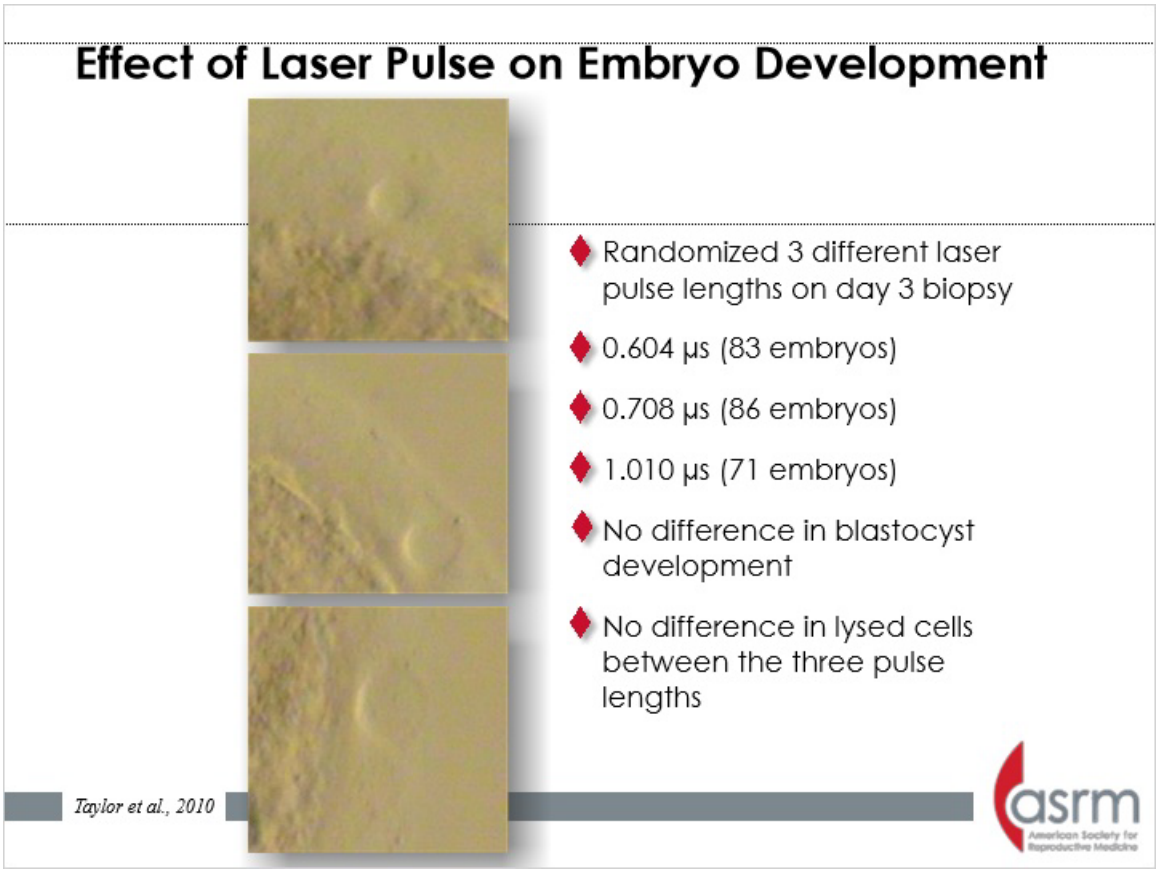
1.7 Just How Much Heat?



Notes:

The amount of heat generated by a laser pulse depends upon a variety of factors, including pulse duration (time of the light pulse activation) and the power of the laser (in mW). The power is usually set by the manufacturer; the only way to increase the size of the hole created is to increase the length of time the laser fires (or the pulse length). The longer the pulse length, the larger the hole. These images each show the heat in concentric circles generated by the laser pulse. These are both from the same laser. The upper image represents a laser pulse length of 3000 µs (microseconds) while the lower panel shows a pulse length of 510 µs. Note the large concentric circles extending out from a single laser shot. The heat generated from the laser shot extends halfway into the embryo. Contrast that with a much shorter pulse duration of 510 µs. The heat generated is much lower and does not extend very far into the embryo. For reference, the colors of the concentric circles are the different temperature gradients. The purple circle (furthest from the laser pulse) is 50° C while the small orange circle is 140° C with a single pulse.

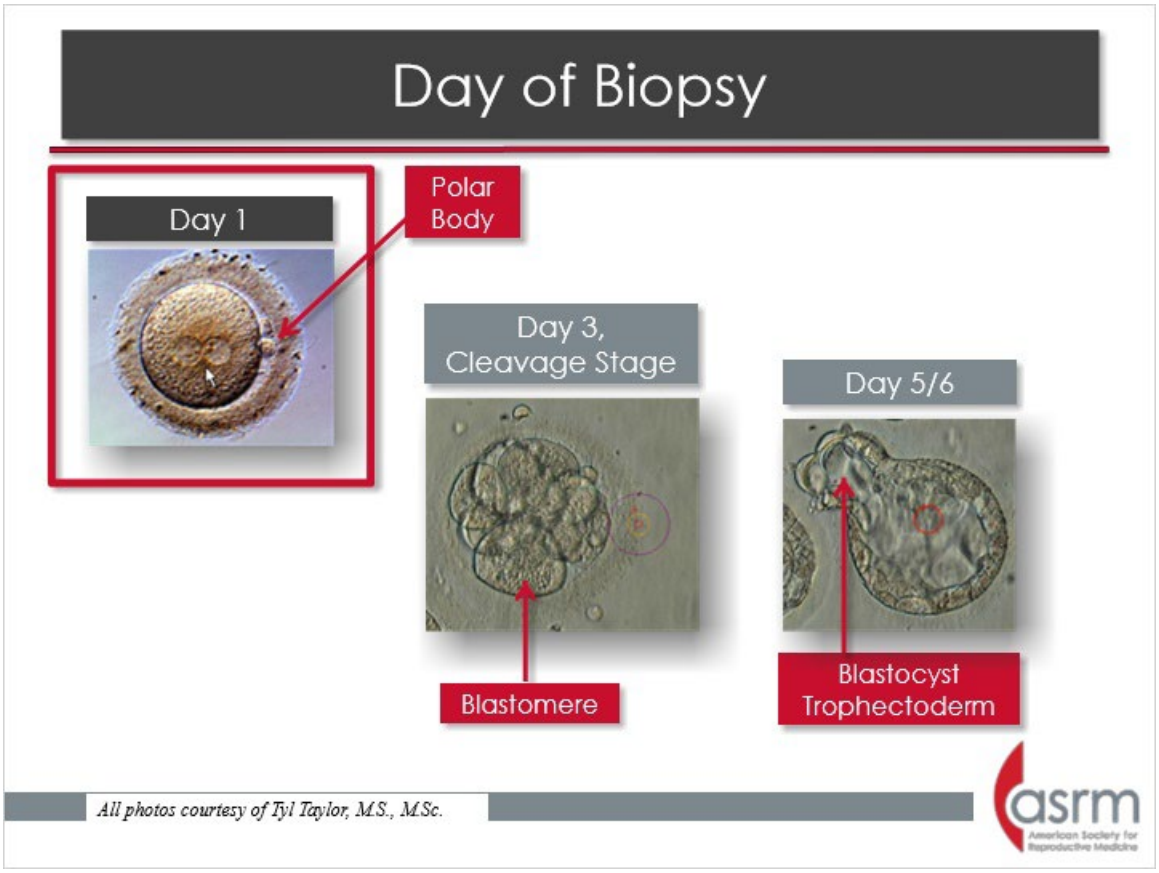
1.8 Effect of Laser Pulse on Embryo Development



Notes:

To examine the effect of different laser pulse lengths on subsequent embryo development, Taylor and colleagues randomized their day 3 biopsy procedure utilizing 3 different laser pulse lengths. They observed no difference in the number of lysed blastomeres or blastocyst development following the biopsy procedure. Unfortunately, this study did not examine pregnancy or live-birth rates between the 3 different pulse lengths.

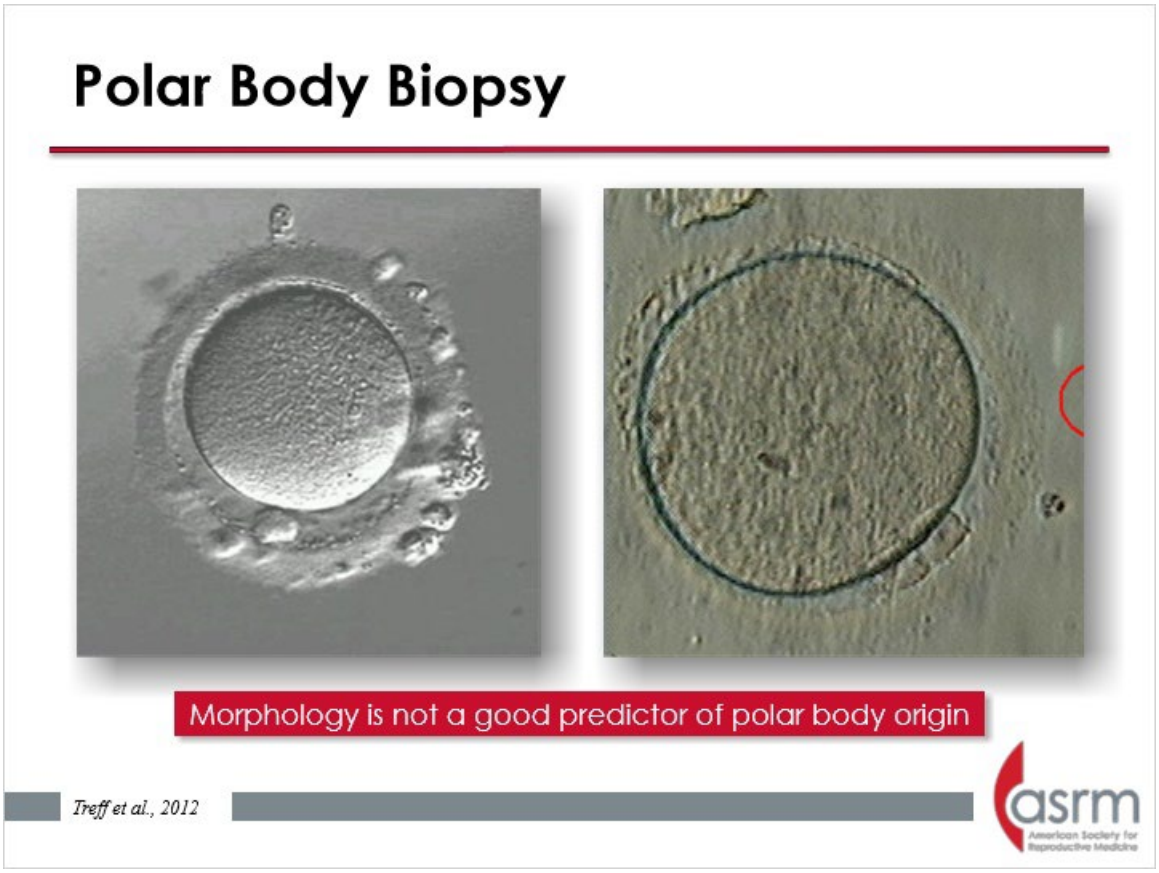
1.9 Day of Biopsy



Notes:

On what day should biopsy be performed? Each day has its pros and cons. During the course of an IVF cycle, biopsy may be conducted at one of 3 stages, polar body (day 0 and day 1), cleavage (day 3), or blastocyst (day 5 and day 6).

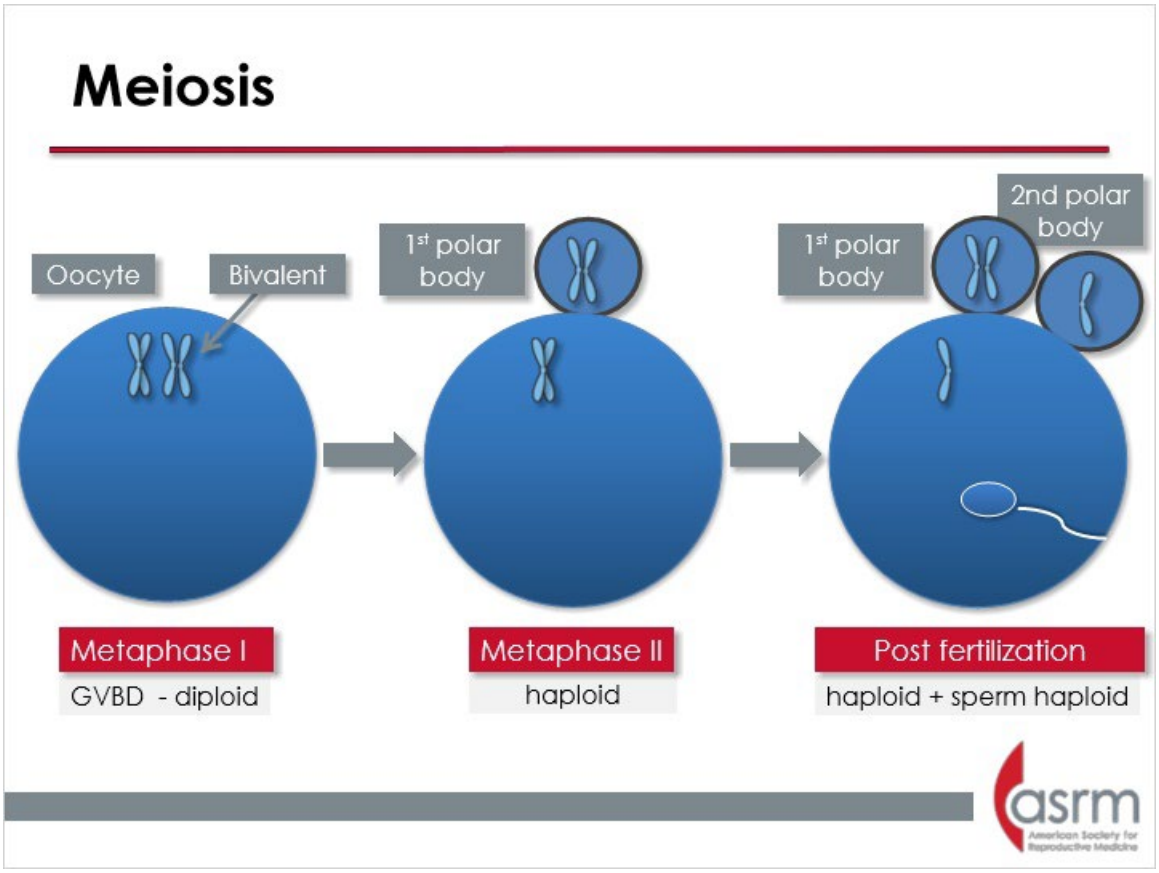
1.10 Polar Body Biopsy



Notes:

Polar body biopsy will be discussed first. The image on the left is a mature egg with an extruded polar body at the 6 o'clock position. The image on the right is a zygote. Two polar bodies can be seen at the 5 o'clock position. Some laboratories biopsy only the first polar body on day 0, plus or minus the second polar body on day 1, while others biopsy both polar bodies on day 1 and then use morphology to predict which polar body is the first and which is the second. Research has shown that morphology is not a good predictor of polar body origin so it may be difficult to ascertain which is the first versus the second polar body. This is important in accurately determining meiosis I vs. meiosis II chromosomal status.

1.11 Meiosis

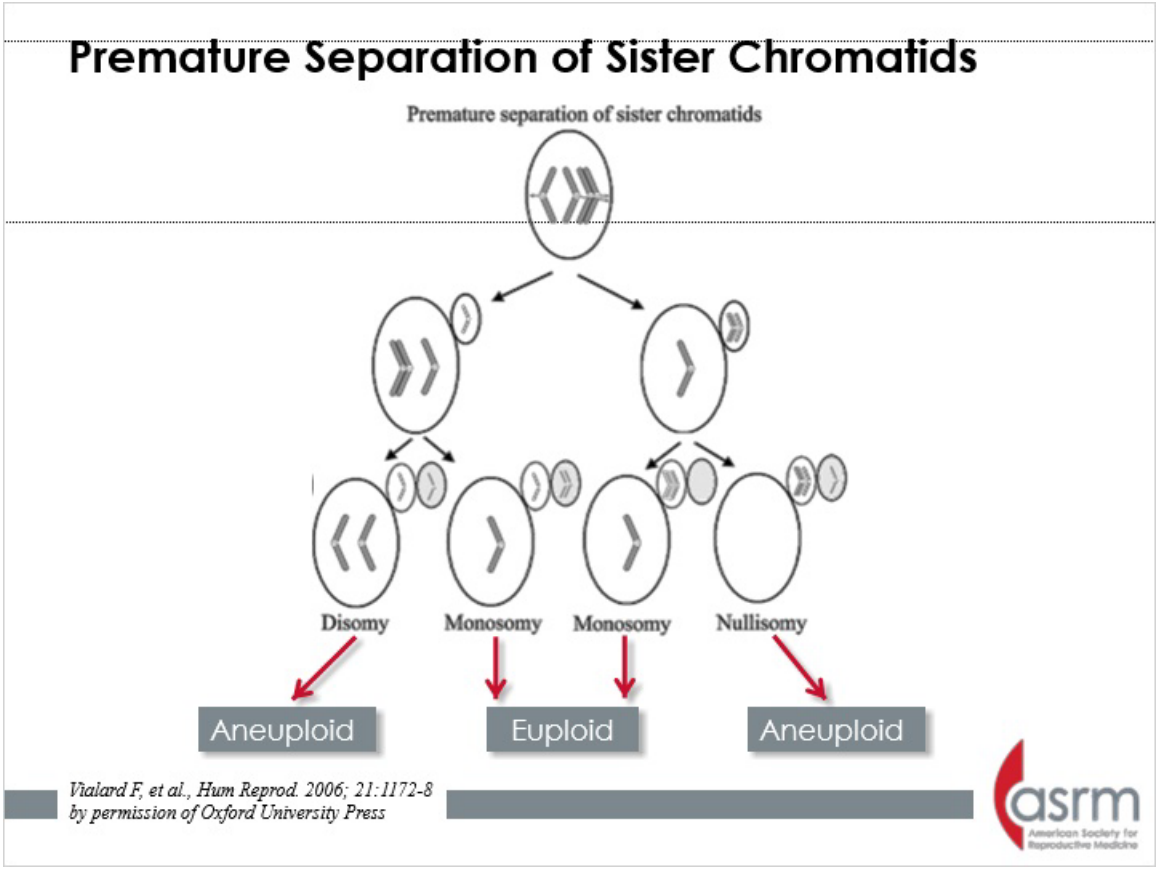


Notes:

A brief review of oocyte maturation will help with understanding polar body biopsy. The immature oocyte is generated while the female fetus is in the womb. From this point until follicular recruitment, the oocyte is in the germinal vesicle or GV stage. At this stage, the oocyte contains 2 sets of chromosomes with 4 chromatids in a bivalent configuration.

The germinal vesicle breaks down, and the oocyte is now in the metaphase I stage (as shown in the drawing on the left). Next, the chromosomes migrate to the periphery of the oocyte. From there, the first meiotic spindle forms, extruding a bivalent (2 chromatids) into the first polar body. The oocyte is now a mature metaphase II (MII) oocyte as shown in the center drawing. The first polar body contains 2 chromatids from each pair, while the oocyte also contains 2 chromatids from each pair. The chromosomes within the oocyte now align on the second meiotic spindle and await the introduction of the sperm. The sperm contains a single set of chromosomes; therefore, when the sperm is introduced, the oocyte must reduce its chromosome content from 2 chromatids to 1. This is done by extruding a single chromatid into the second polar body. Now, the sperm and the oocyte's chromosomes can merge together, forming a zygote, and begin mitotic divisions.

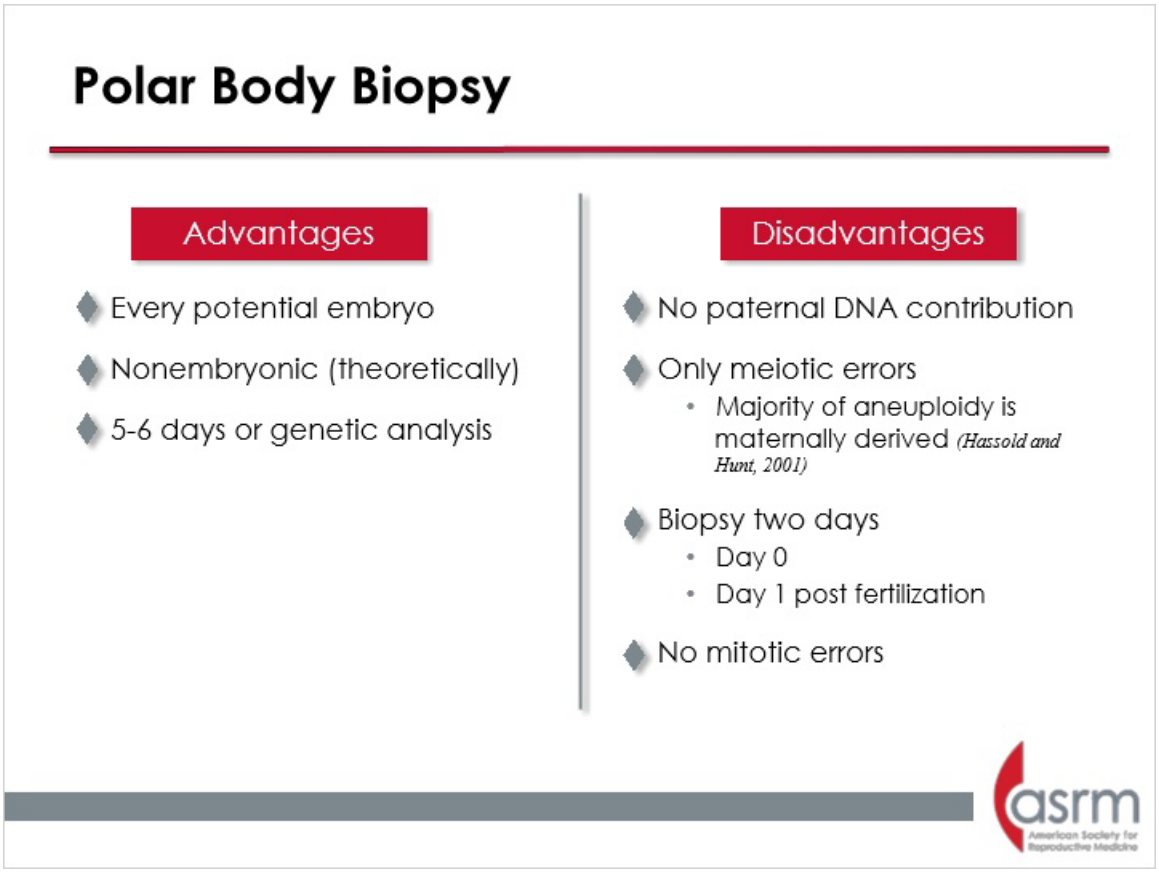
1.12 Premature Separation of Sister Chromatids



Notes:

One of the most common errors during meiosis is premature separation of sister chromatids. There are multiple scenarios here, but this diagram makes it easy to see the significance of results from the first versus second polar body biopsy. What is important to notice is that even if the first polar body is aneuploid, the resultant oocyte may still be euploid, depending on the results of the second polar body diagnosis. This poses a problem if only the first polar body is biopsied. Potentially viable embryos may be discarded even though there is a possibility that the oocyte could be euploid after the second polar body extrusion.

1.13 Polar Body Biopsy



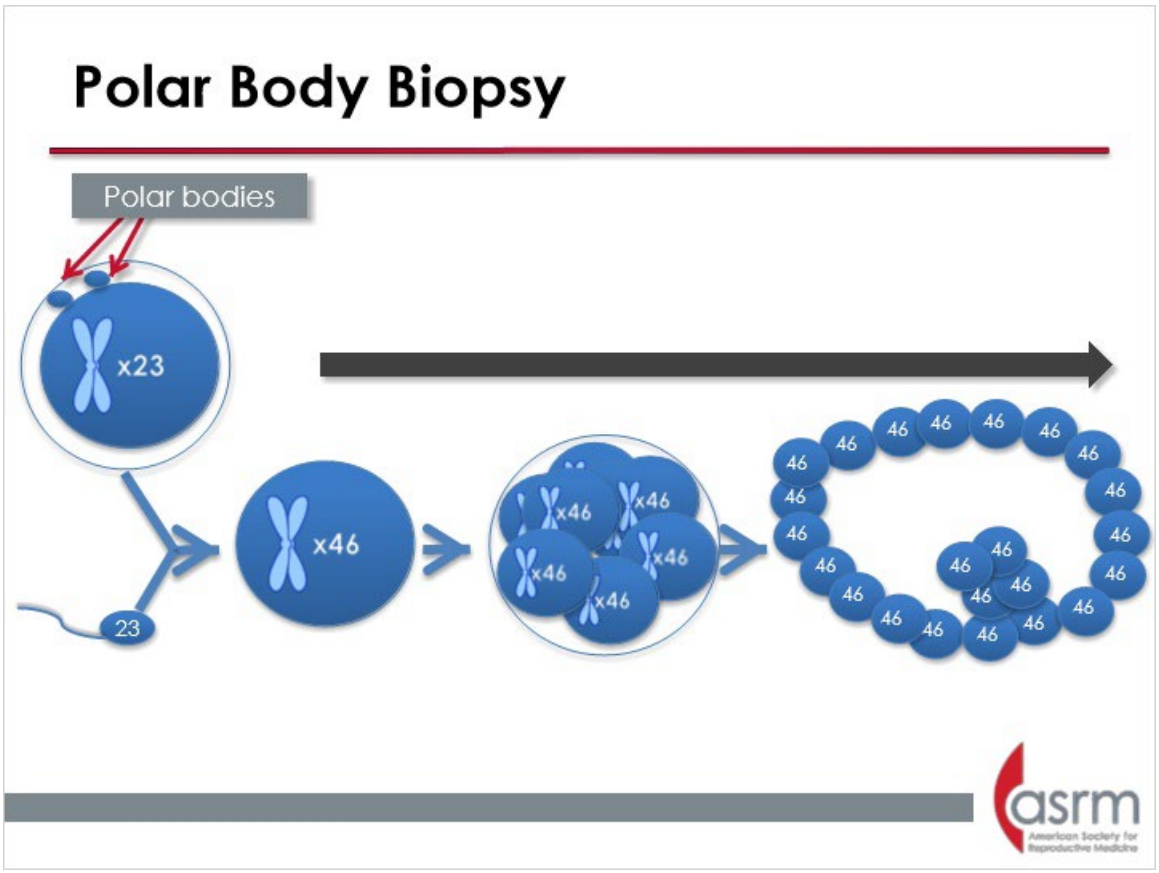
Notes:

There are multiple advantages and disadvantages for polar body biopsy. One of the primary advantages is that every mature oocyte can be biopsied (as opposed to blastocyst-stage biopsy where the embryos must meet a certain stage requirement to perform the biopsy). Theoretically, since polar bodies are byproducts of meiosis, the removal of this nonembryonic material will not affect subsequent embryo development. Moreover, results of polar body biopsy are generally available in less than 5-6 days, which would still allow for transfer of fresh embryos.

In terms of disadvantages, the polar bodies can only be related back to the oocyte, not the embryo, so the paternal DNA contribution to the embryo is unknown. However, it is believed that a majority of aneuploidy is maternally derived; therefore, is information on the oocyte (maternal) only prudent for a majority of cases? This concurs with the next point that only meiotic errors can be detected, not mitotic errors. Lastly, typically both polar bodies are removed for analysis. This can be done on day 0 (when the first polar body is present) and then again on day 1 (when the second polar body is present). Alternatively, the biopsy can be done on day 1 (when both polar bodies are present). This would also contradict one of the original advantages of polar body biopsy in that the first polar body biopsy was performed on the unfertilized oocyte, circumventing any

ethical consideration of the fertilized embryo. Most laboratories that perform polar body biopsy will also remove the second polar body that is present after fertilization.

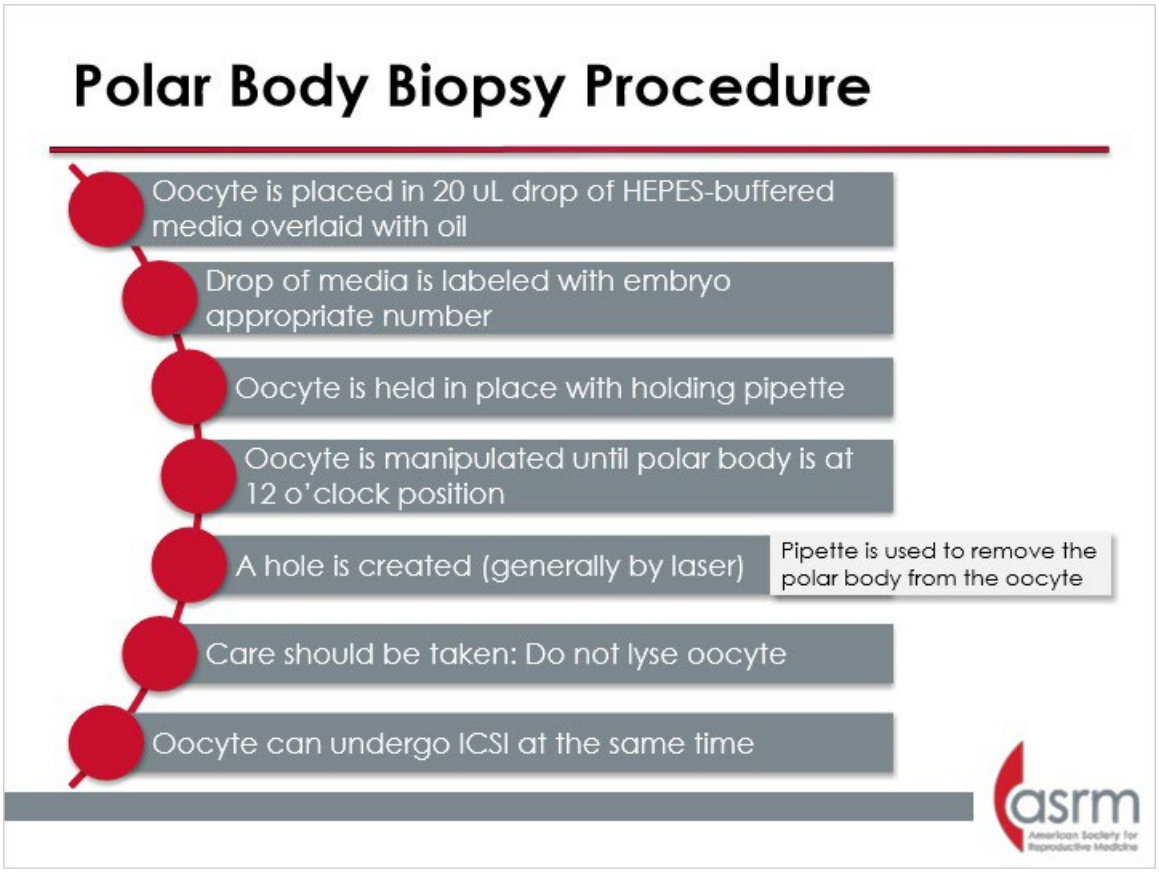
1.14 Polar Body Biopsy



Notes:

Theoretically, if the polar bodies are euploid, then so is the oocyte, and so should be the embryo.

1.15 Polar Body Biopsy Procedure



Notes:

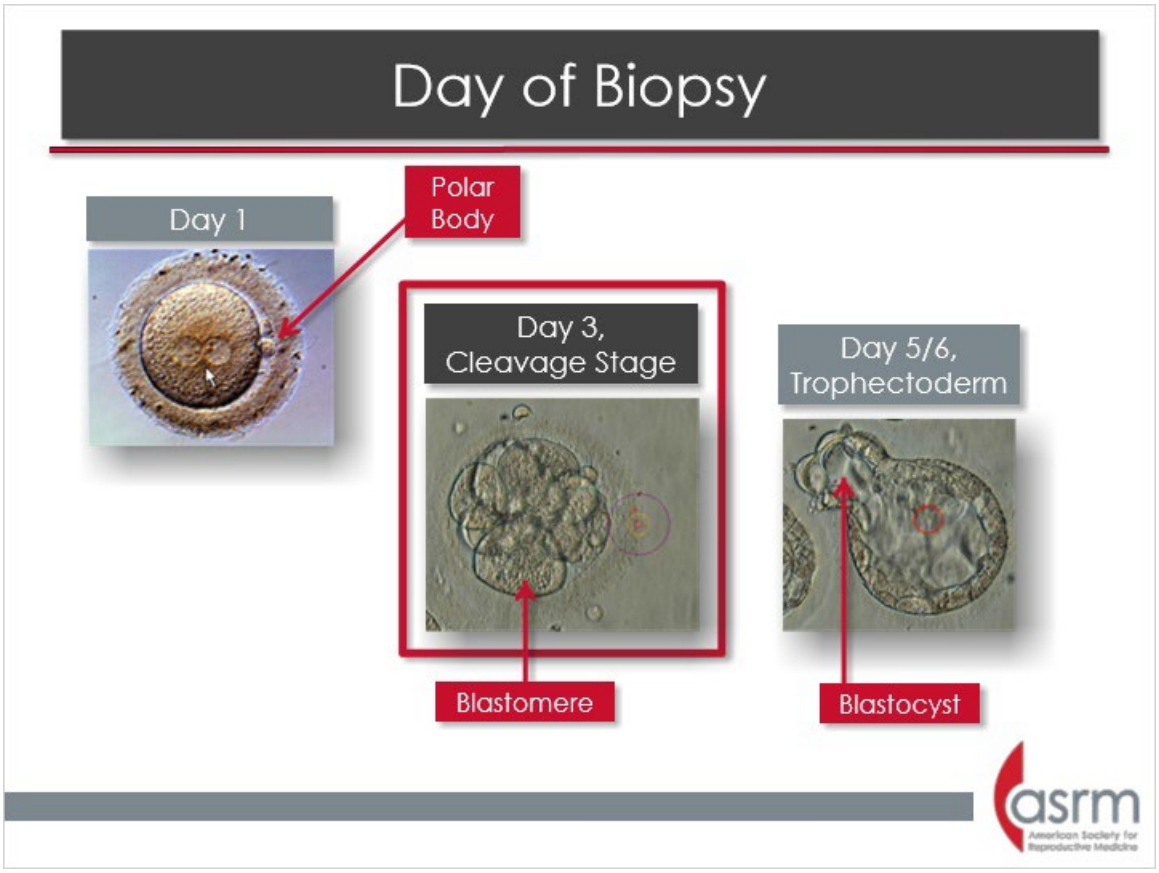
Polar body biopsy can be performed on day 0 prior to ICSI, and/or on day 1, at fertilization check. First, the oocyte is placed in small drops of HEPES-buffered media, overlaid with oil. The drop containing the oocyte is labeled with the appropriate number. A holding pipette is used to hold the oocyte in the correct position, and the oocyte is rotated until the polar body(s) are visible. The zona is breached, and the polar body is suctioned out of the oocyte. If desired on day 0, ICSI can be performed. The oocyte is placed back into the incubator. The removed polar body is moved through a series of wash drops and then placed in a labeled microcentrifuge tube for molecular analysis, which will be discussed later. This tube containing the polar body is then sent to the genetics laboratory for processing.

1.16 Video

Notes:

This video shows a polar body biopsy. The micropipette is inserted through the laser-created opening at the 1-2 o'clock position and the polar body is removed.

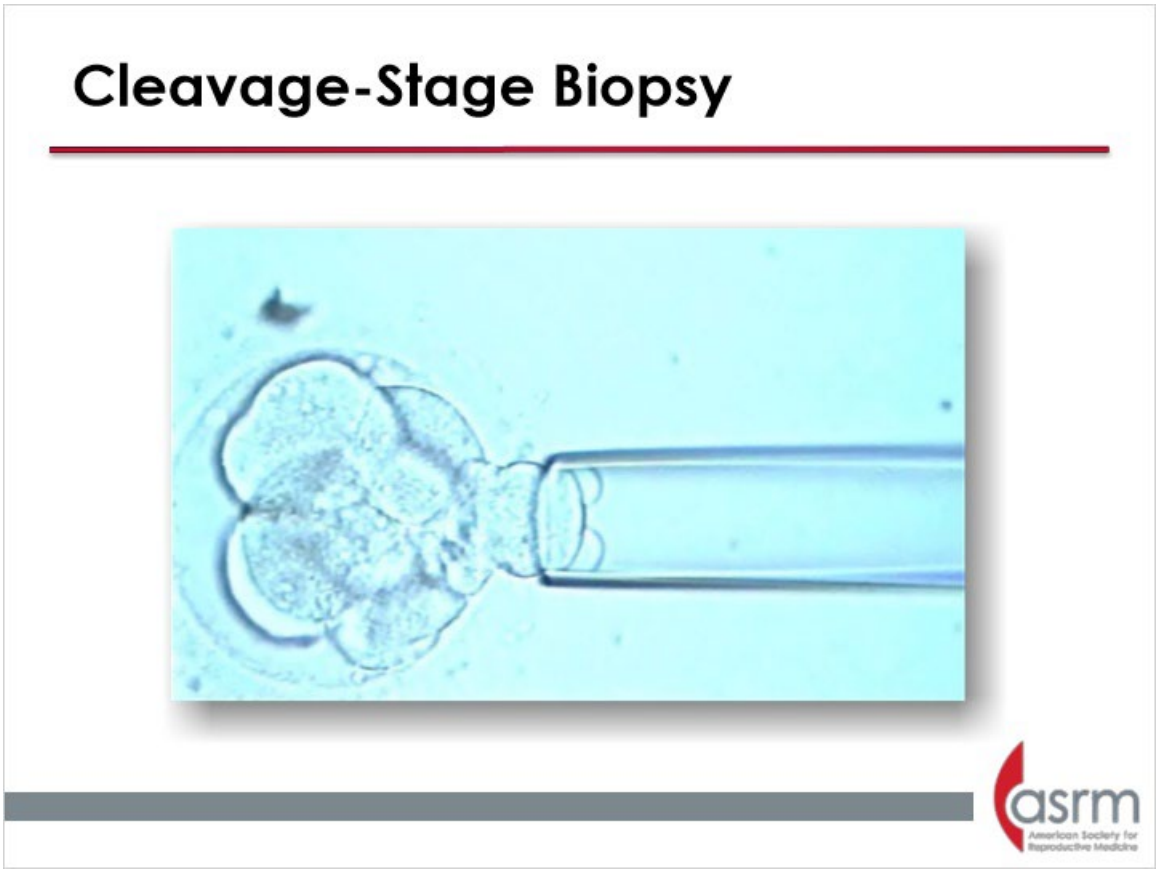
1.17 Day of Biopsy



Notes:

Day 3 or cleavage-stage biopsy will be reviewed in this section.

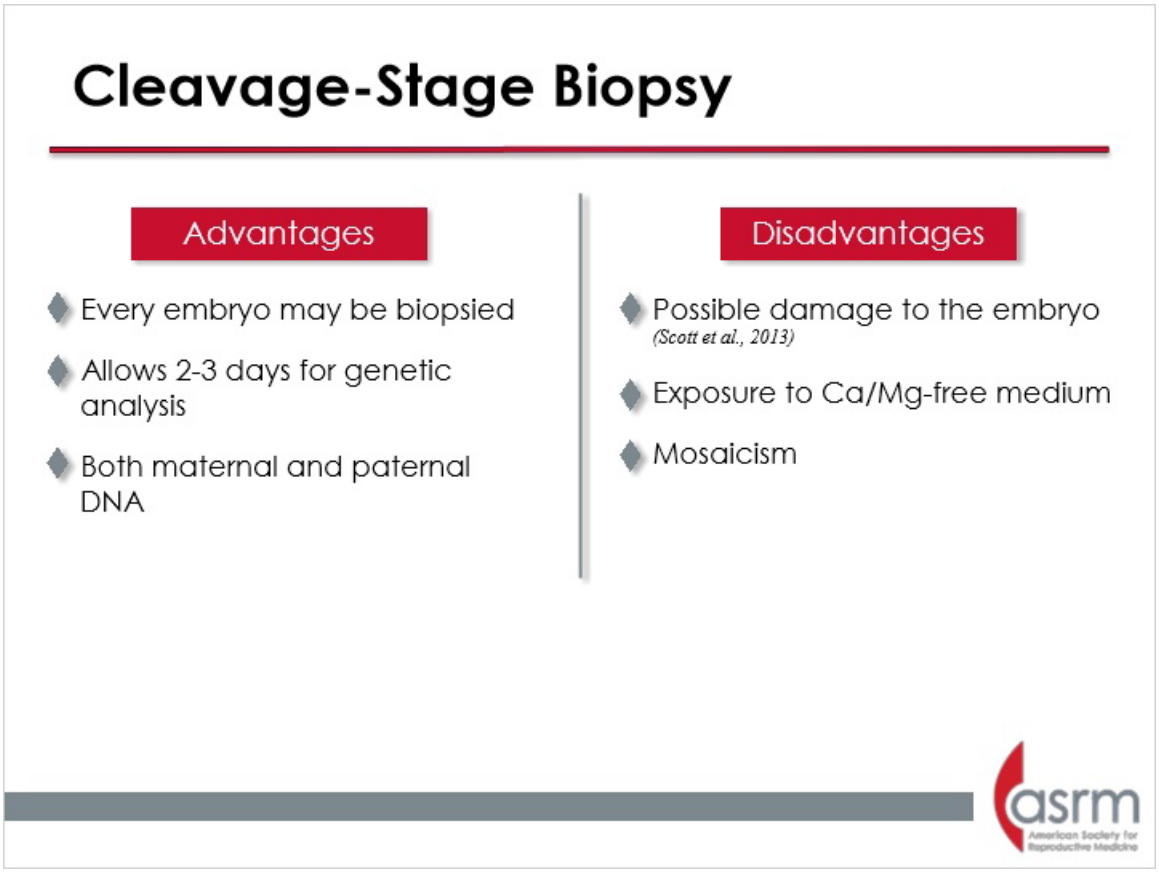
1.18 Cleavage-Stage Biopsy



Notes:

A single blastomere is typically removed at the cleavage stage, although there are instances when some researchers have biopsied two blastomeres.

1.19 Cleavage-Stage Biopsy

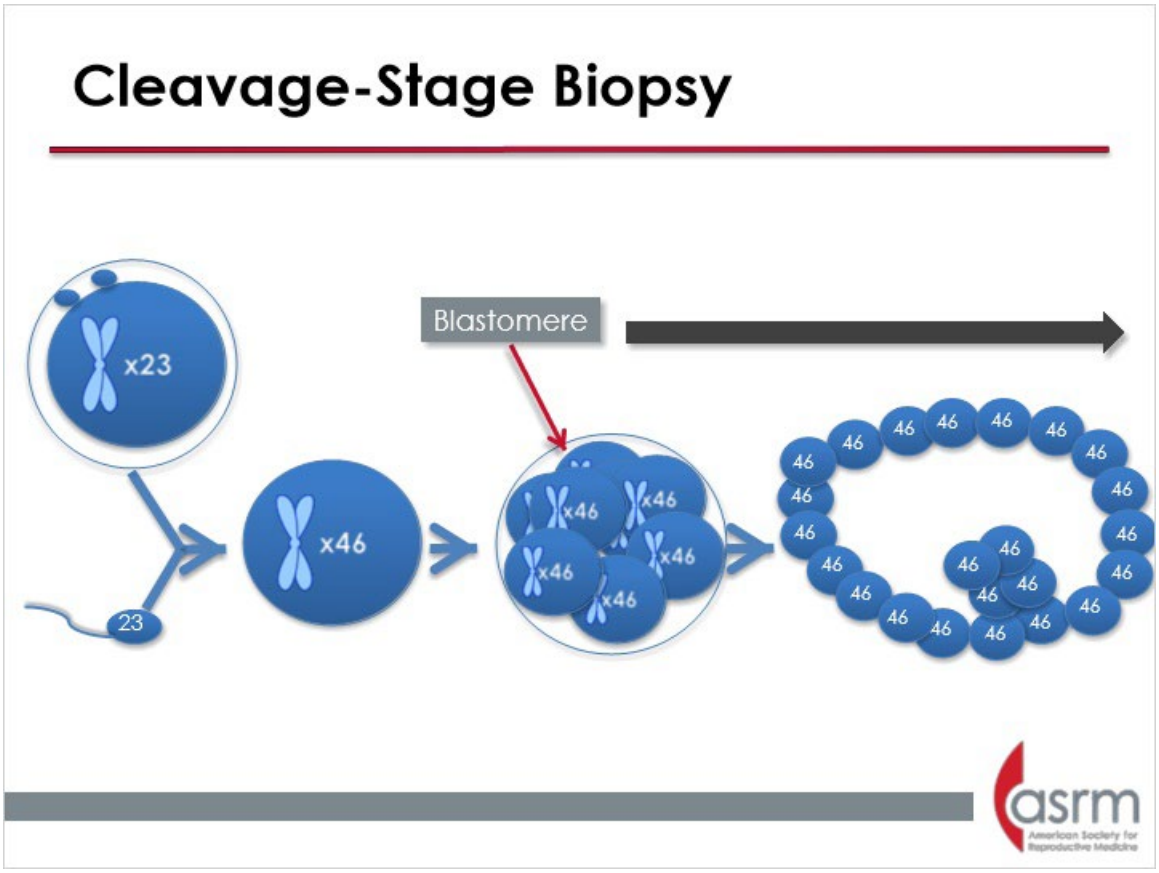


Notes:

As noted, cleavage-stage or day-3 biopsy may enable the sampling of every embryo. Moreover, results of biopsy at the cleavage stage are available within 2 to 3 days, allowing for a fresh transfer at the blastocyst stage. Unlike polar body biopsy that samples only maternal DNA, cleavage-stage biopsy samples both maternal and paternal DNA in the embryo.

Research has shown that cleavage-stage biopsy may significantly impair implantation and live-birth rates. It is recommended that cleavage-stage biopsy be performed while the embryo is exposed to a calcium and magnesium-free medium. Cell-to-cell bonds are mediated by calcium, so removal of calcium from the medium allows separation of the blastomeres, which lends itself to an easier biopsy. Lastly, mosaicism seems to be pronounced within the preimplantation embryo, so the chromosomal status of a single cell compared with the rest of the embryo is still in question.

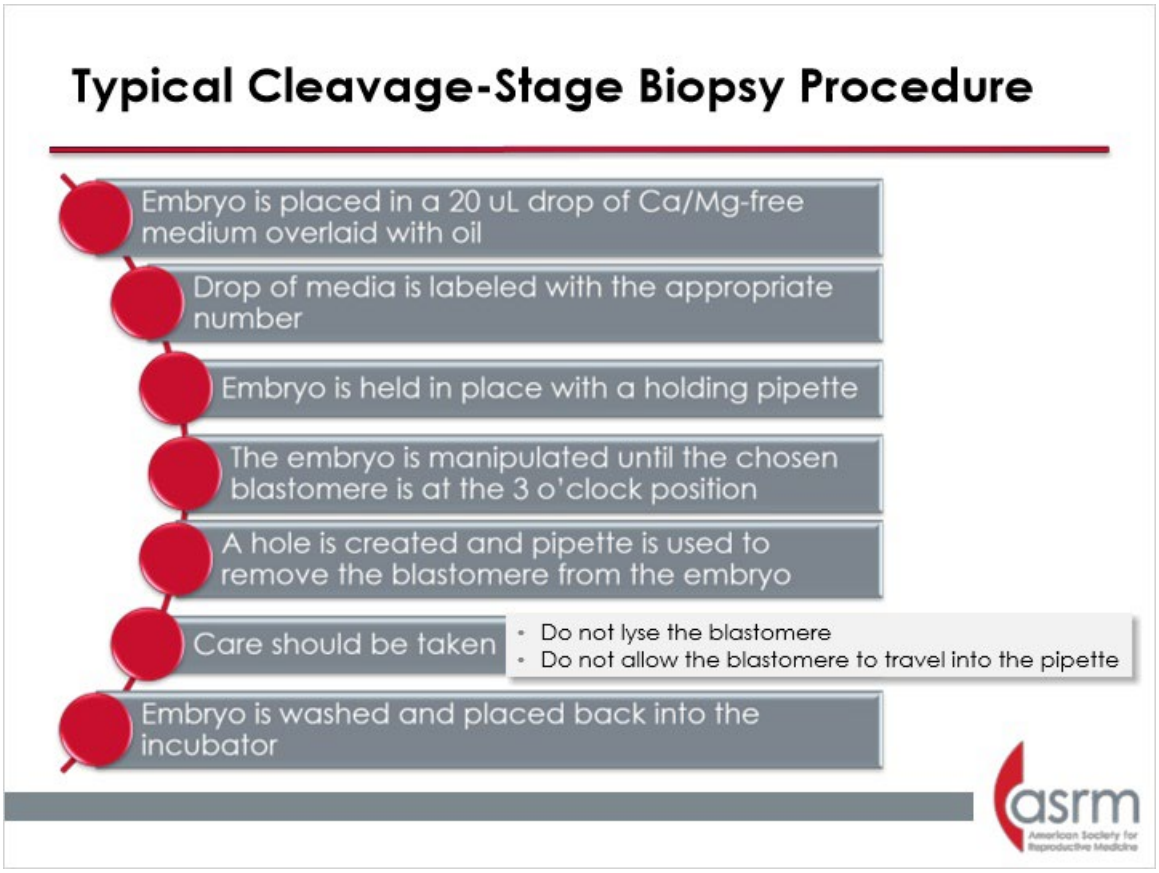
1.20 Cleavage-Stage Biopsy



Notes:

The biopsy of a blastomere from a cleavage-stage embryo should relate to the chromosomal status throughout the rest of the embryo.

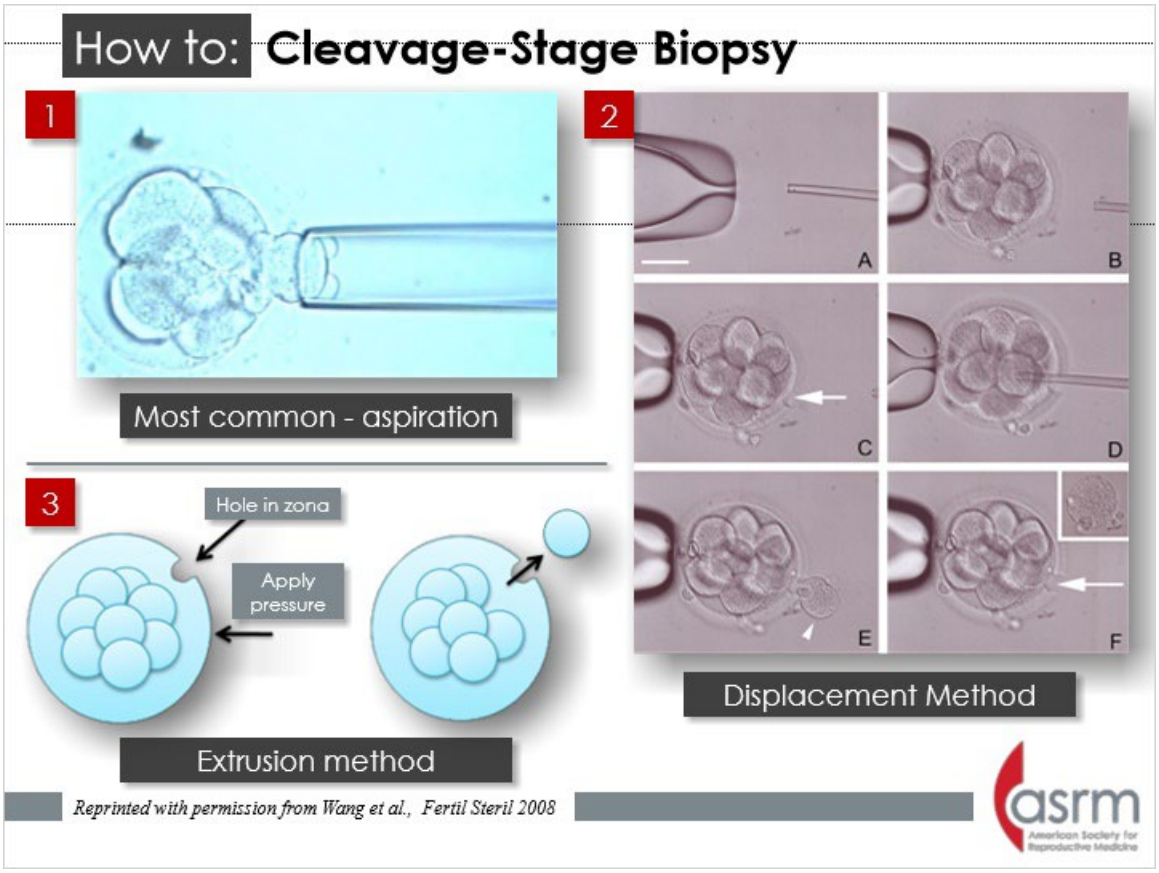
1.21 Typical Cleavage-Stage Biopsy Procedure



Notes:

Cleavage-stage biopsy consists of multiple steps. First, the embryo is placed in small drops of calcium and magnesium-free medium, overlaid with oil. This medium assists in the breakdown of gap junctions between blastomeres and facilitates easier removal of the blastomeres from the embryo. The drop containing the embryo is labeled with the appropriate number. A holding pipette is used to hold the embryo in the correct position. The embryo is rotated until the desired blastomere is chosen (usually with a visible nucleus). The zona is breached, and the blastomere is gently suctioned out of the embryo. The embryo is then washed in culture media and returned to the incubator. The removed cell is moved through a series of washes and then placed within a PCR tube. This PCR tube is packed and shipped to the genetics company for processing.

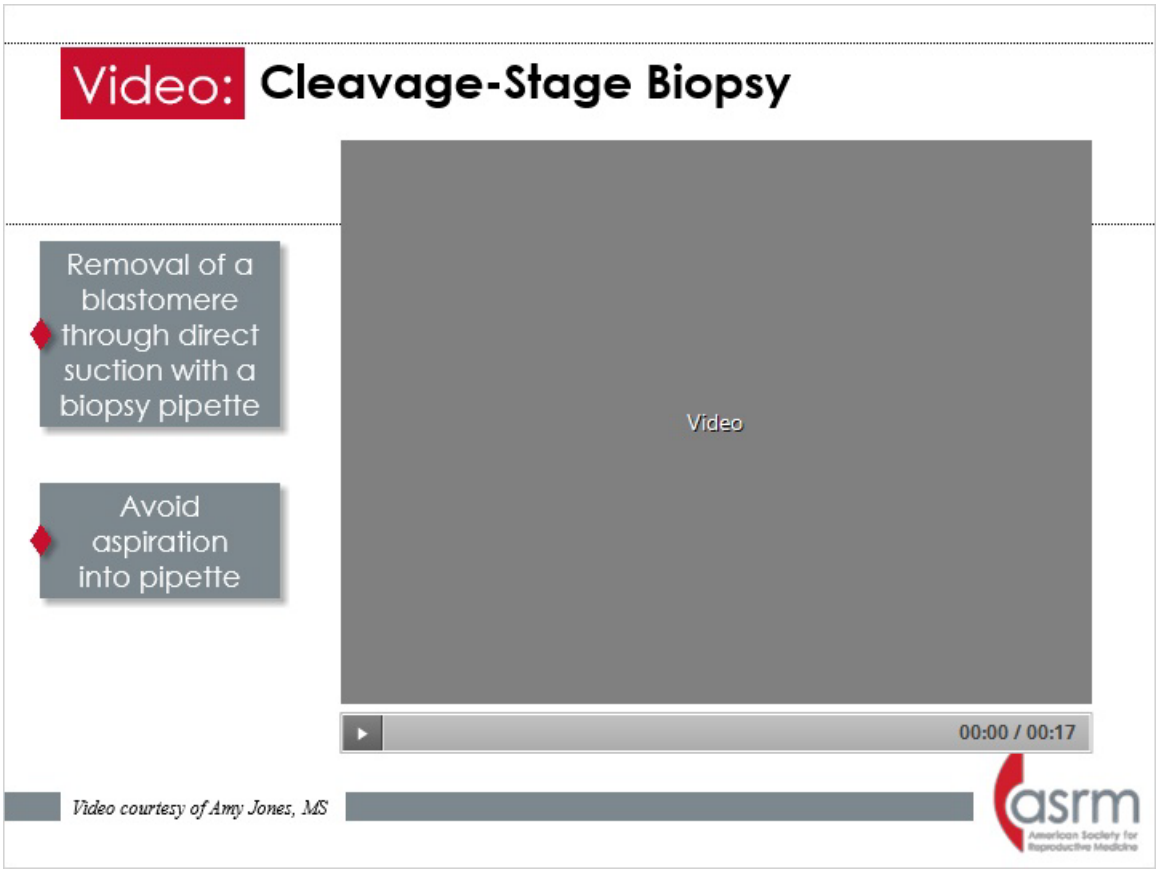
1.22 How to Cleavage-Stage Biopsy



Notes:

There are actually several ways to biopsy. At least 3 completely different techniques may be used that all appear equally effective.
In panel 1, a blastomere is removed using direct suction via the biopsy pipette. This technique is sometimes referred to as the aspiration technique.
In panel 2, the displacement method is shown. Panel 2A shows the setup of both holding and assisted-hatching micropipettes. In panel 2B, a day-3 embryo is held in place by a holding micropipette. In panel C, a laser is used to make a hole in the zona pellucida at approximately the 3 o'clock position as indicated by the white arrow. In panel 2D an assisted hatching micropipette is inserted into the hole, and medium is gently expelled inside the zona. Panel E shows that the fluid pushes the blastomere out of the hole in the zona by positive pressure. In panel F one can see that the blastomere is successfully isolated from the embryo. The arrowhead indicates the isolated blastomere in Panel E.
In panel 3, a blastomere is removed by first breaching the zona. The micropipette is then used to apply gentle pressure on the zona, as opposed to the suction of the blastomere. This method causes the blastomere to essentially "pop" out of the zona.

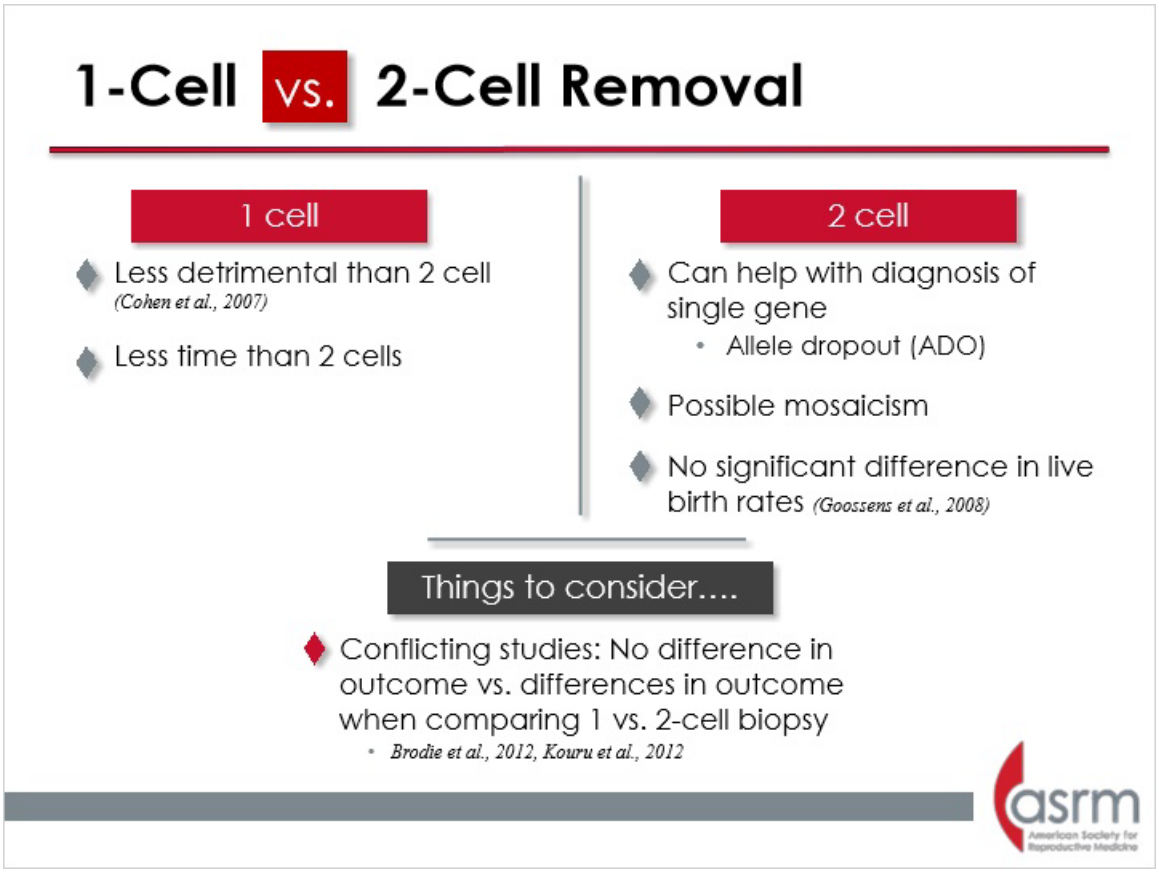
1.23 Video Cleavage State Biopsy



Notes:

This video depicts removal of a single blastomere using the aspiration technique. As the embryo is held in place with the holding pipette, an opening is made in the zona pellucida, exposing the selected blastomere. A suction pipette is used to gently aspirate the blastomere from the embryo. Note that it is not necessary to aspirate the blastomere into the pipette. In fact, this should be avoided if possible, as it may increase the likelihood of blastomere lysis. The biopsied blastomere is then released into the embryo drop.

1.24 1-Cell vs. 2-Cell Removal



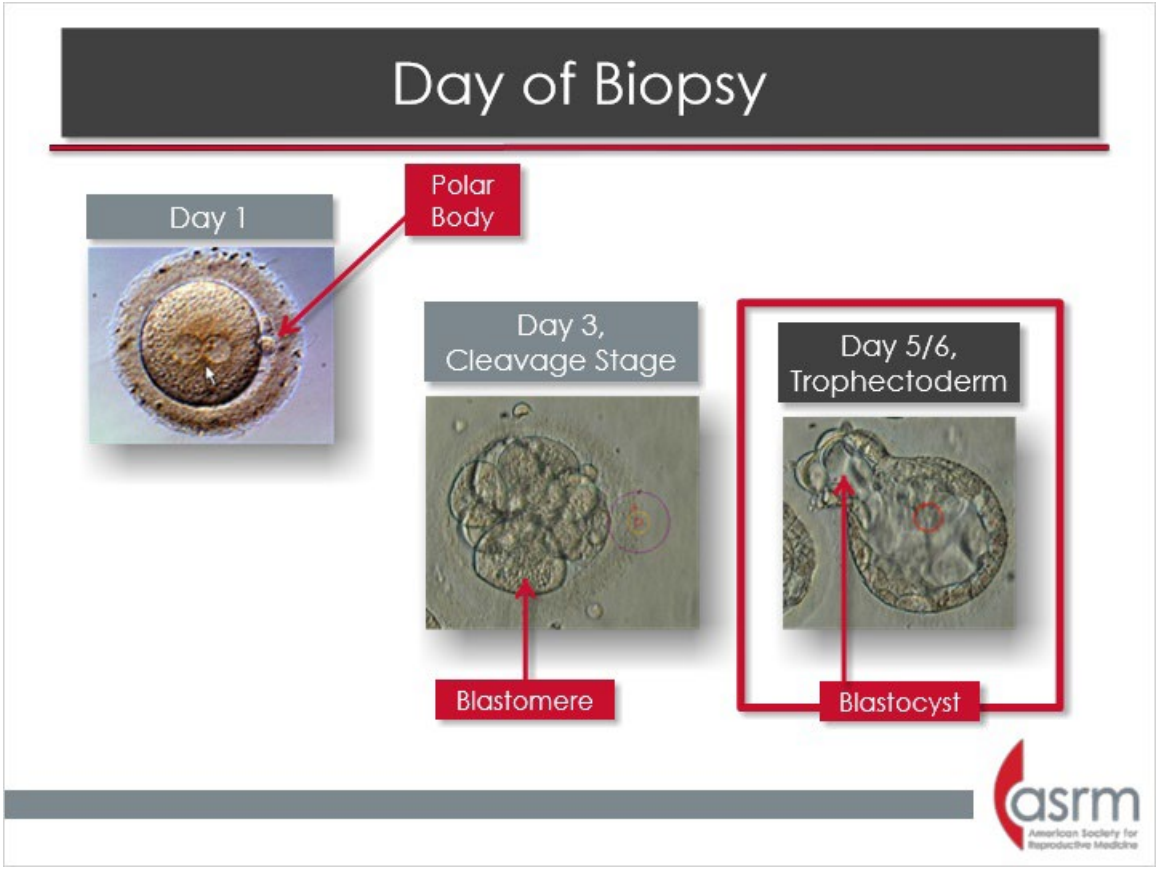
Notes:

As previously mentioned, there are reports examining the effects of the removal of 2 cells during cleavage-stage biopsy. The removal of 1 cell appears to be less detrimental than the removal of 2 cells. However, a randomized controlled trial demonstrated that although blastocyst formation was significantly higher when only one cell was removed, there was no significant difference in live-birth rates whether one or two cells were removed.

The biopsy procedure itself does not take as long if the technician is removing only a single cell. This means that the embryo spends less time within the calcium and magnesium-free medium, which may be detrimental to growth. However, the removal of 2 cells may increase the rate of obtaining a diagnosis with single gene defects, particularly to combat allele dropout. Biopsy of 2 cells may also help detect mosaicism, which seems to be relatively common during preimplantation development.

It is difficult to determine the exact effects of the removal of 2 cells. These typically are removed from more progressed embryos (6-8 cells), whereas the biopsy of 1 cell is performed on less-progressed embryos. There are multiple observational studies that show conflicting results: some found no significant difference between cell number and embryo progression, while others did show a difference.

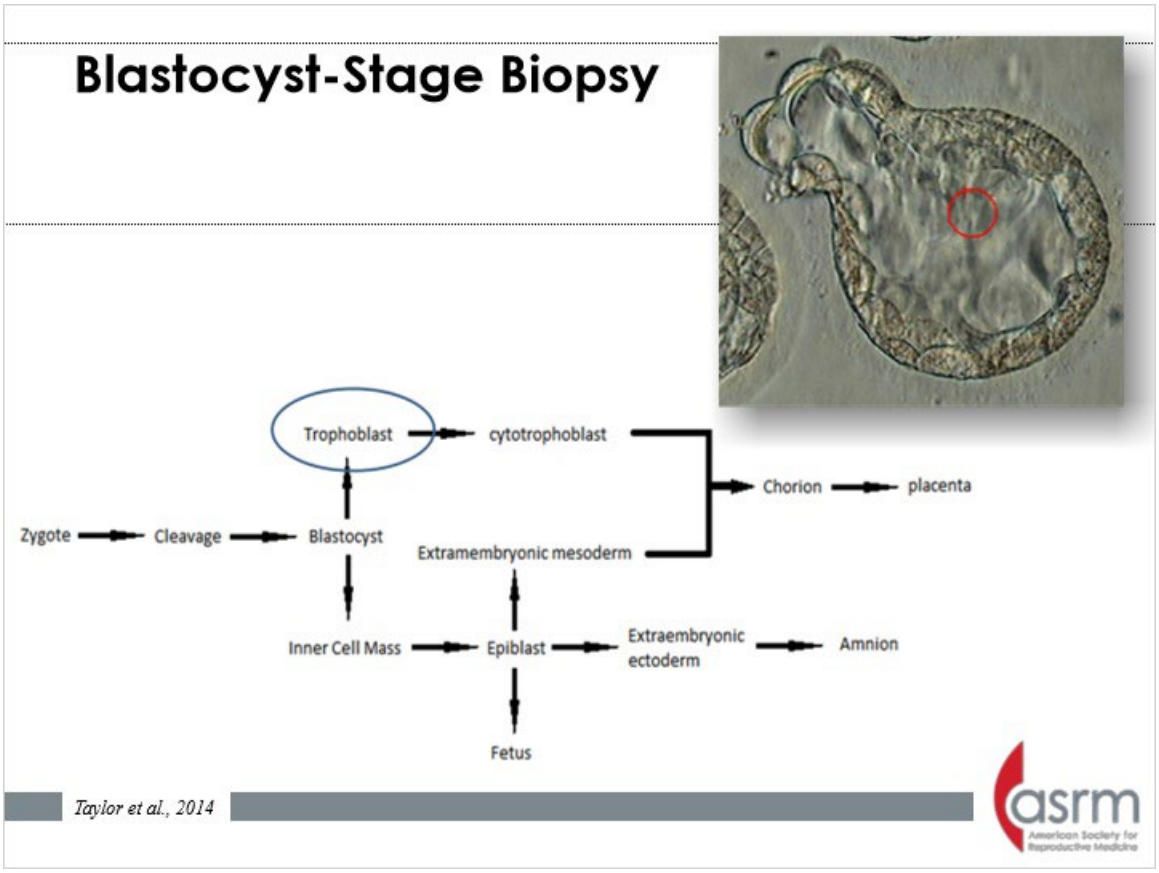
1.25 Day of Biopsy



Notes:

Embryos can also be biopsied at the blastocyst stage.

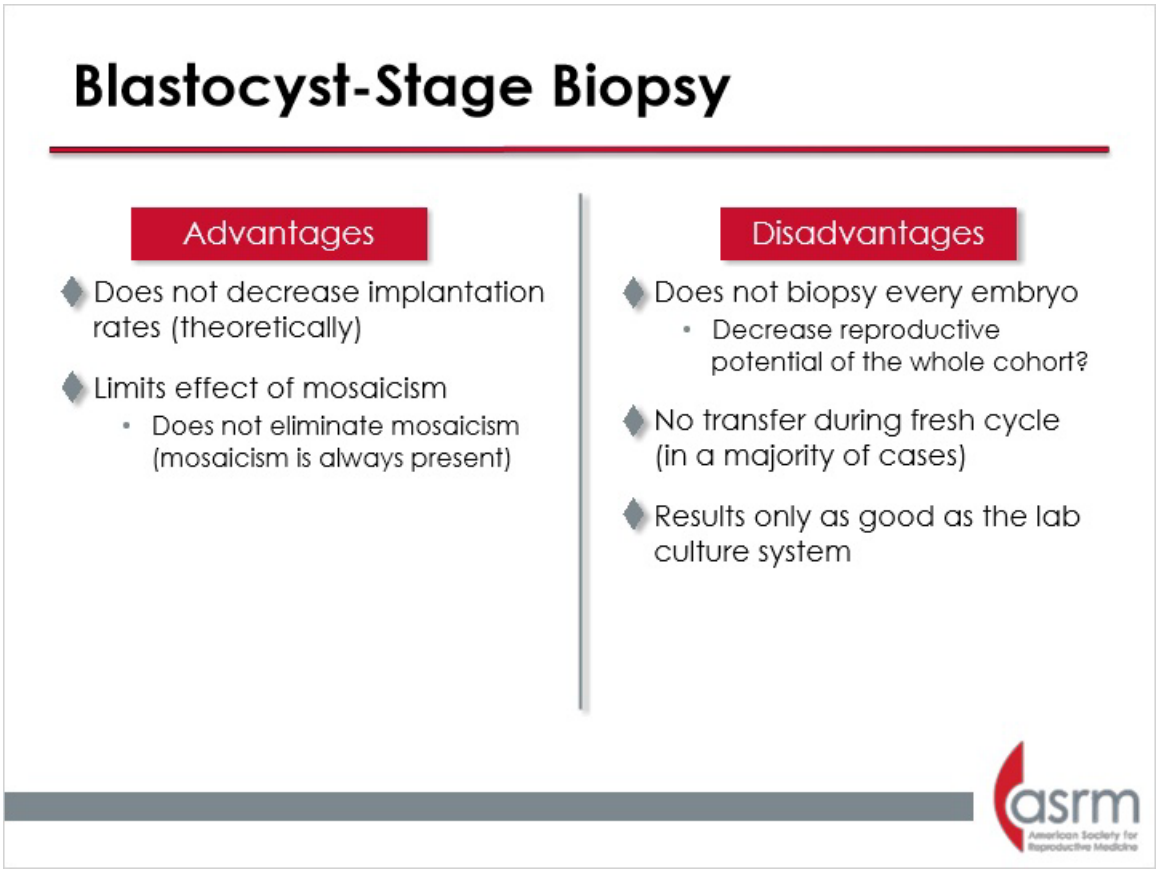
1.26 Blastocyst-Stage Biopsy



Notes:

Blastocyst biopsy involves removal of a portion of the trophectoderm, avoiding the inner cell mass. During the developmental process, the trophoblast does not form the fetus, but rather forms the placenta. Therefore, trophectoderm biopsy does not remove a piece of the embryo that will become the child.

1.27 Blastocyst-Stage Biopsy



Notes:

As previously discussed, trophectoderm biopsy does not significantly reduce implantation. Also, comprehensive chromosome screening techniques return a single result for any number of cells. Therefore, if one biopsies 5-10 cells (as with trophectoderm biopsy), then only a majority of those have to be diagnosed as euploid to return a euploid diagnosis. This approach limits the effects of mosaicism by not determining an aneuploid result unless a majority of cells are aneuploid.

Some of the disadvantages of blastocyst-stage biopsy are as follows:

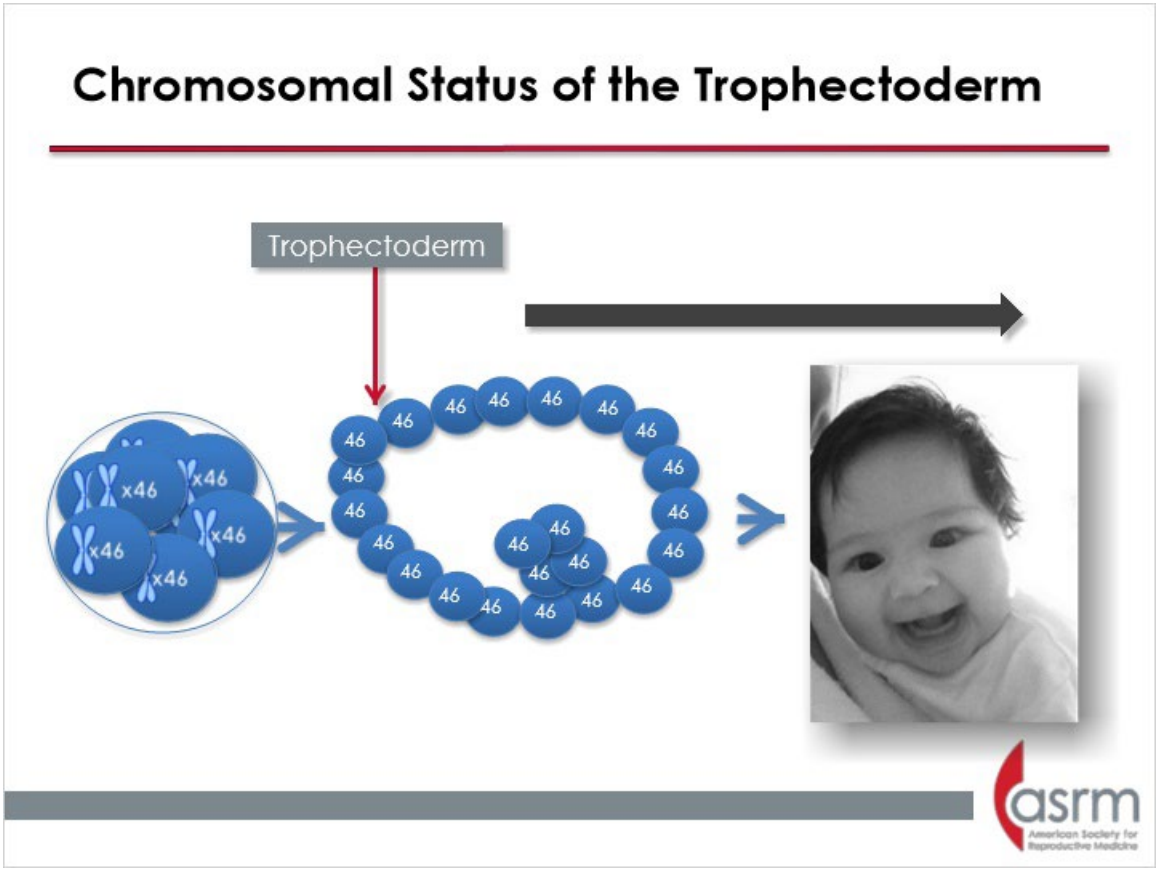
1)A majority of labs do not biopsy every embryo, but rather biopsy only those that develop to a viable blastocyst (presenting with good quality inner cell mass and trophectoderm). With this approach, it is possible to discard potentially viable embryos that may have slower progression.

2)If the biopsy occurs on day 6, for a majority of IVF labs it is not possible to transfer embryos the same day, as it typically takes 24-48 hours to get results. This means that embryos must be cryopreserved, and the patient will subsequently undergo a cycle using frozen blastocysts. This may increase the cost of a cycle to the patient. Moreover, although freezing and thawing techniques have improved tremendously in the last 5 years (vitrification warming rates may approach 98%), it is possible that a

euploid blastocyst will not survive the thaw, leading to a difficult consult for both the patient and physician. This possibility should be covered in freeze/thaw consent forms as well.

3) Lastly, because only blastocysts are biopsied, the results of this approach are only as good as the laboratory's culture system. Low oxygen, type of culture media, and type of incubator may all affect blastocyst development. If a lab has a low blastocyst formation rate, blastocyst biopsy may not be a beneficial option.

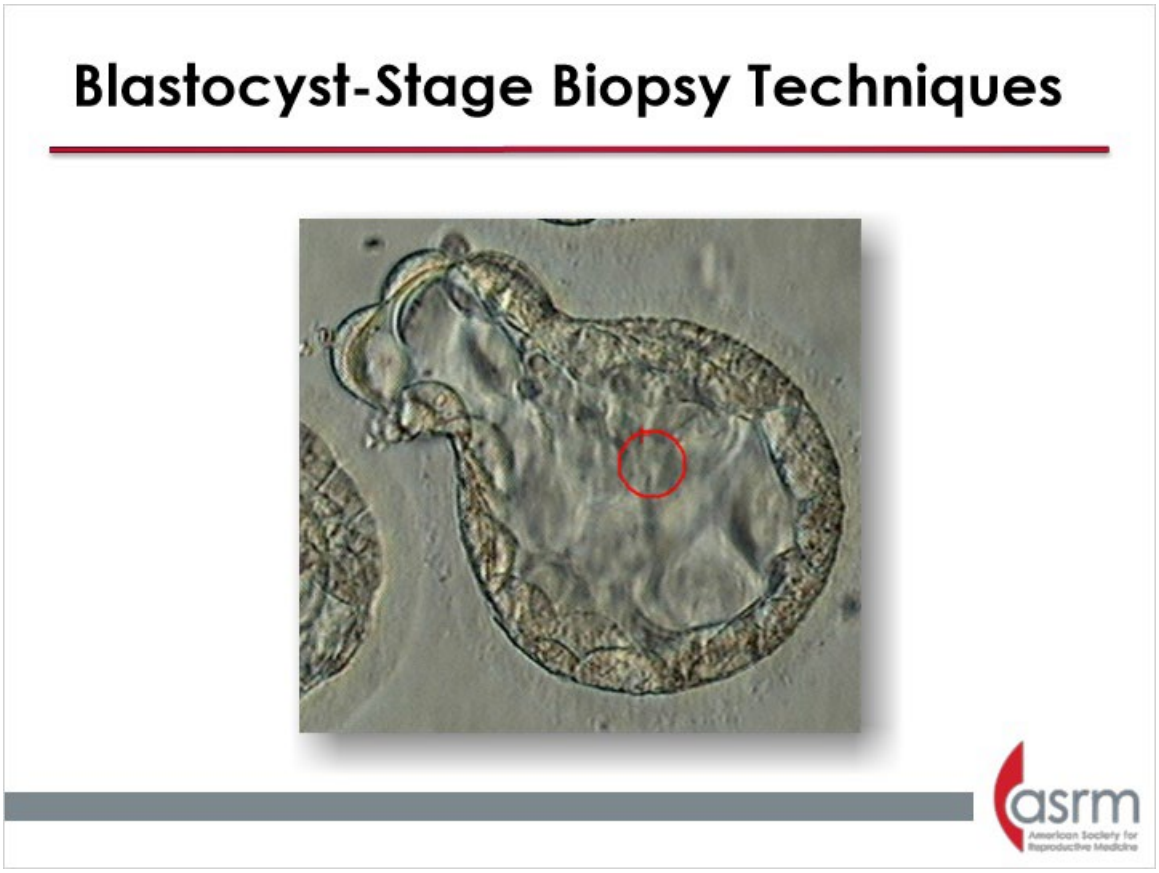
1.28 Chromosomal Status of the Trophectoderm



Notes:

Theoretically, the chromosomal status of the trophectoderm relates to the chromosomal status of the child.

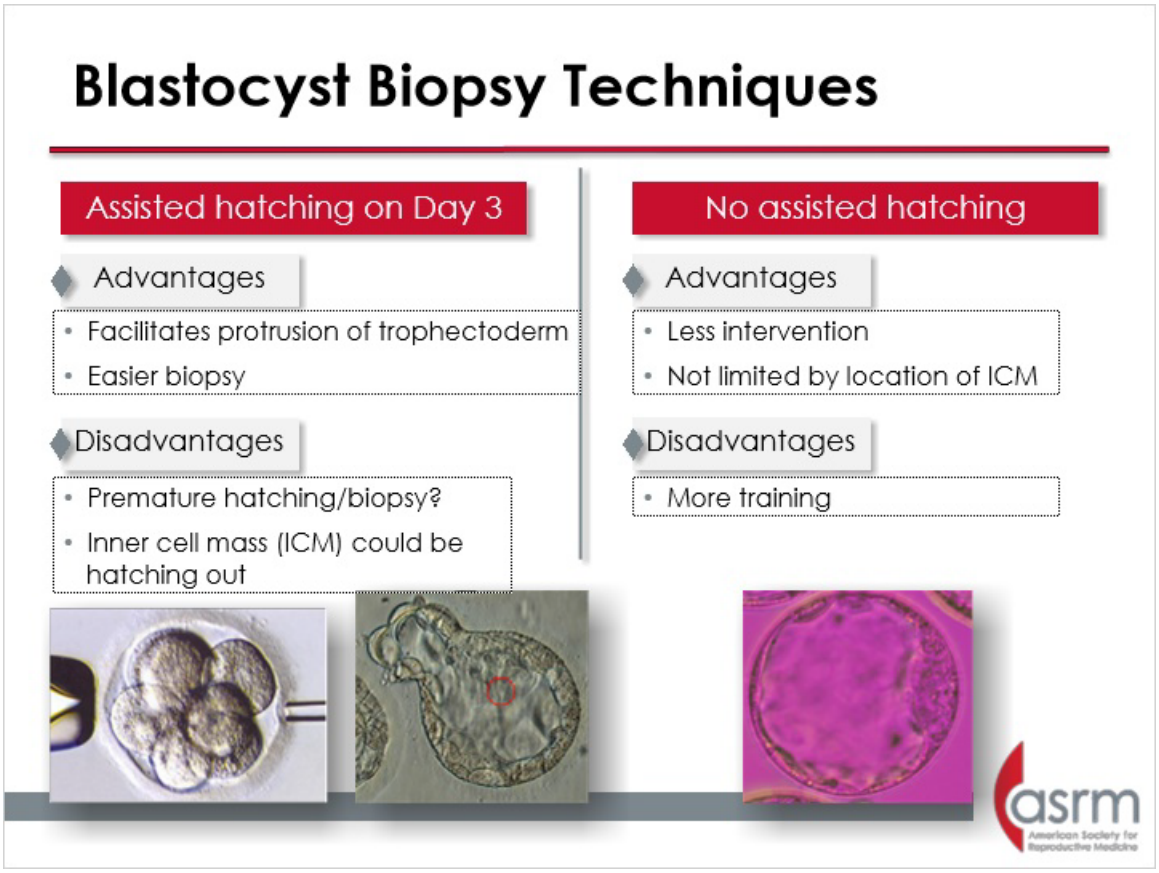
1.29 Blastocyst-Stage Biopsy Techniques



Notes:

Different techniques can be utilized in conjunction with blastocyst biopsy.

1.30 Blastocyst Biopsy Techniques



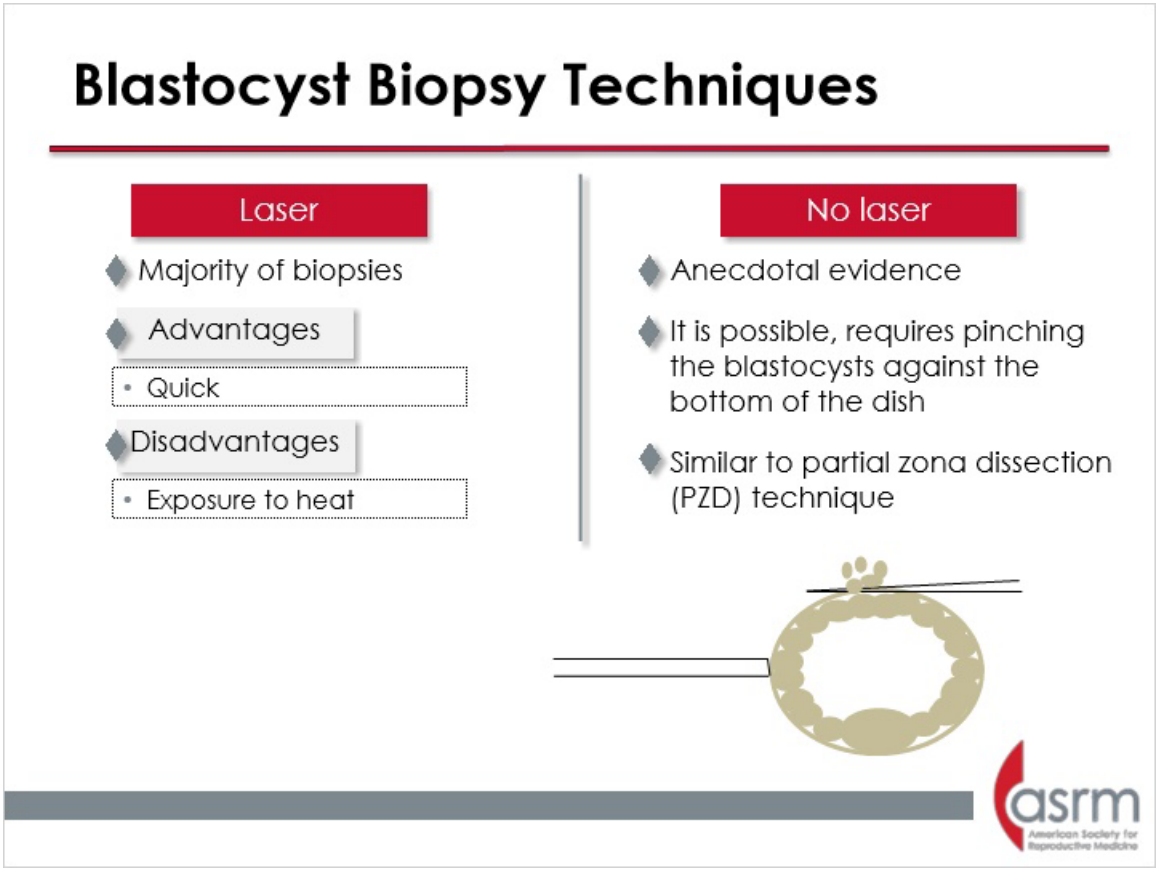
Notes:

The actual biopsy technique at the blastocyst stage is fairly universal. What does differ is what takes place on day 3. To facilitate the protrusion of the trophectoderm from the zona pellucida, assisted hatching may be performed on day 3 as shown in the photo on the left. Assisted hatching is the purposeful breaching of the zona pellucida, either by mechanical or acidic means, creating a hole within the zona pellucida that allows for an easier biopsy (as the trophectoderm to be removed typically will be herniating from the zona). However, assisted hatching on day 3 may cause premature hatching, which may affect embryo development, and possibly lead to biopsy of non-expanded embryos. Lastly, it is possible that the inner cell mass will hatch out of the zona. This would require a separate hole to be created, away from the inner cell mass, so a piece of the trophectoderm can be removed.

Alternatively, Capalbo and colleagues (2013) described blastocyst biopsy without the aid of day-3 assisted hatching by simply culturing the embryos to day 5. At the blastocyst stage, the embryo is expanded, and the zona is intact as shown in the photo on the right. This allows the technician to remove a piece of the trophectoderm directly across from the inner cell mass. This also allows the laser pulses to be concentrated furthest from the inner cell mass, theoretically causing less damage. While this technique certainly

requires less intervention prior to biopsy, more technician experience is needed to minimize the number of laser shots needed to breach the zona and “cut” the trophectoderm.

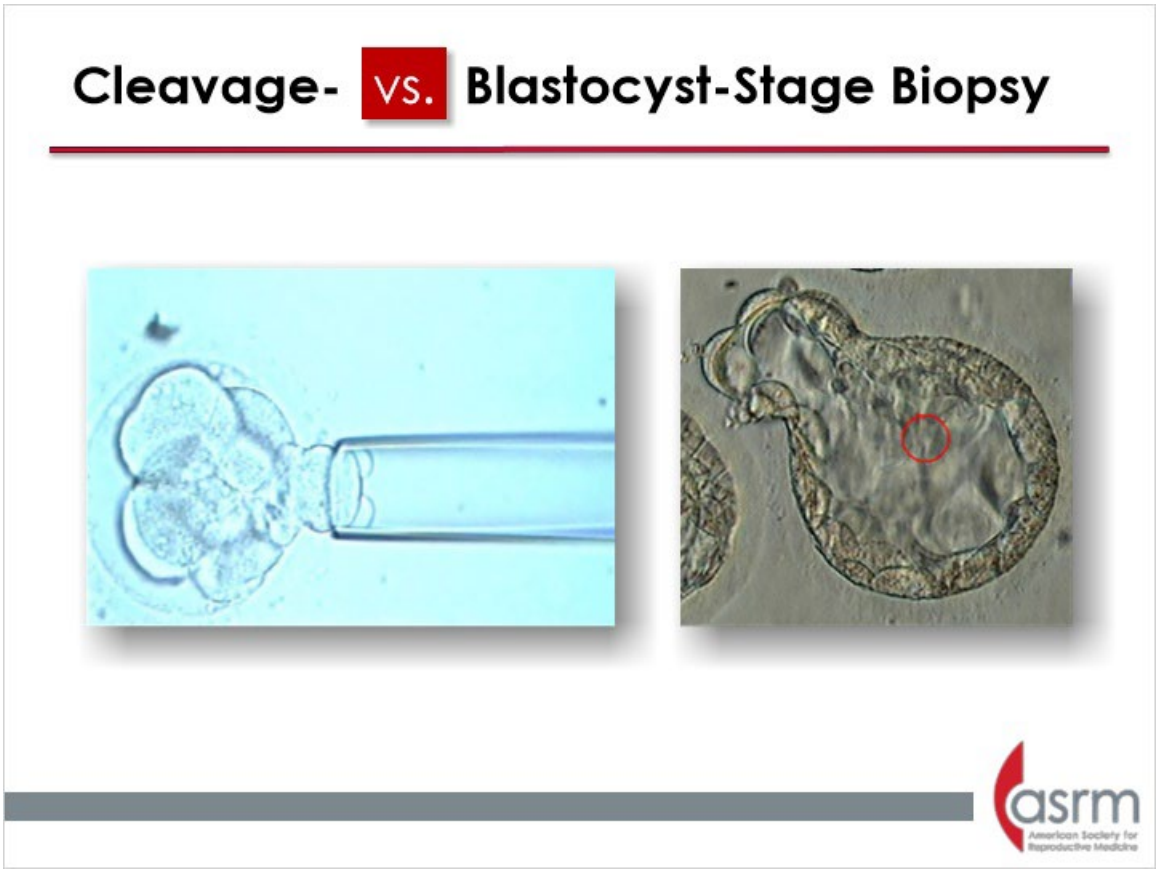
1.31 Blastocyst Biopsy Techniques



Notes:

One may think that a laser is needed to “cut” a piece of the trophectoderm, but before the advent of laser breaching, other methods were used. Cutting the trophectoderm may require pinching the blastocyst against the bottom of the dish**,** or it can be held in place with a micropipette while a microneedle is inserted under the zona, as shown in the drawing. The needle can be used to rub an opening in the zona using the partial zona dissection technique known as PZD. This allows several trophectoderm cells to be extruded and collected. It is unknown if this procedure is more or less damaging to the embryo than the laser. Regardless, there is more than one way to remove a piece of the trophectoderm although the use of a laser appears to be the most common at this time.

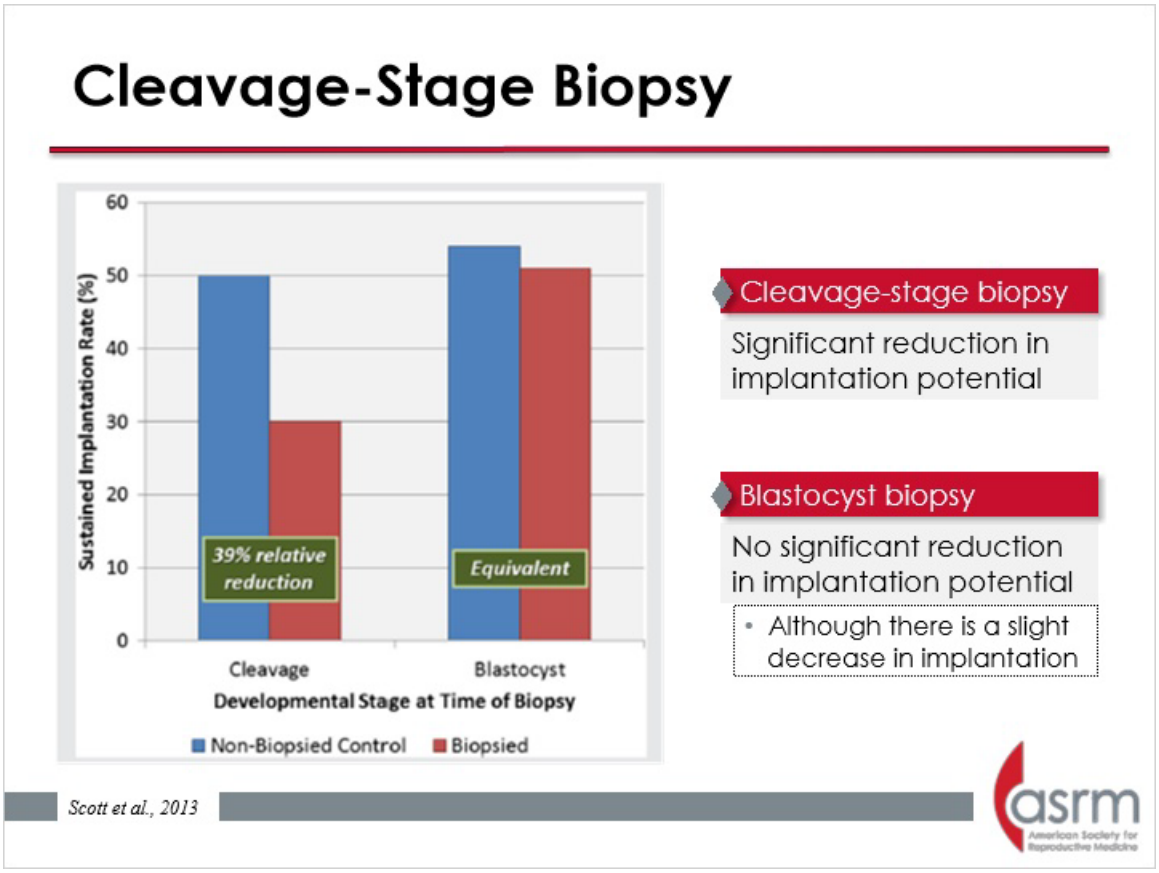
1.32 Cleavage- vs. Blastocyst-Stage Biopsy



Notes:

To follow is a discussion of the differences between cleavage- and blastocyst-stage biopsy.

1.33 Cleavage-Stage Biopsy

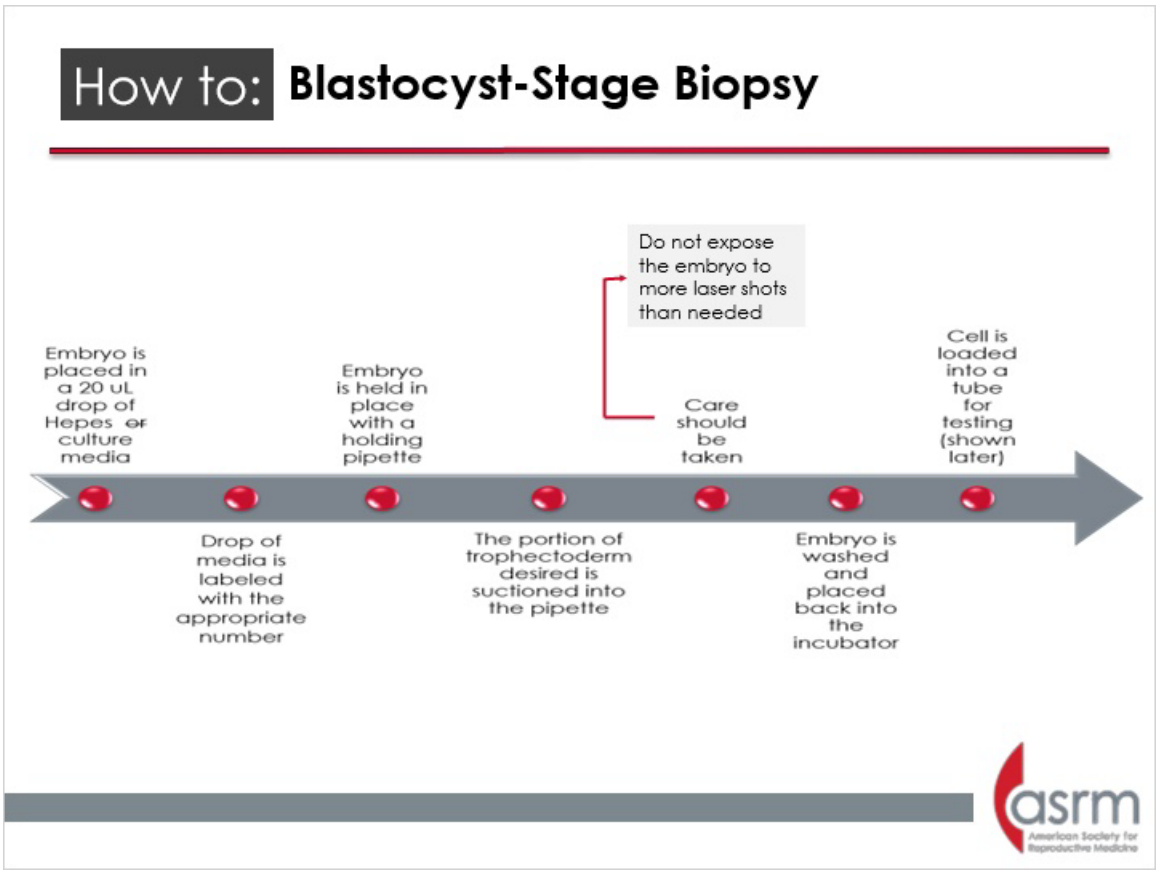


Notes:

In a randomized controlled trial by Scott and colleagues (2013), one group had embryos transferred at the cleavage stage, and the other at the blastocyst stage. One embryo for each patient was biopsied, while the other was not biopsied. Embryos were tracked by DNA if a live birth resulted to determine which embryo implanted. The biopsied cleavage-stage embryos had significantly reduced implantation rates; however, those that were biopsied at the blastocyst stage presented with similar ability to implant. There was no significant decrease in implantation with blastocyst biopsy. It is interesting to note that the implantation rates were approximately the same for a day-3 cleavage-stage embryo and a day-5 blastocyst.

While the study suggests that cleavage-stage biopsy can harm embryos and blastocyst biopsy does not, one must be careful to remember that with cleavage-stage biopsy, every embryo could potentially be biopsied. With blastocyst biopsy, typically only those embryos that have shown sufficient progression are biopsied and all others are discarded. Therefore, only the “best of the best” are biopsied. It is unknown how many viable embryos are discarded because they do not reach the blastocyst stage by day 6. Live births from day 7 blastocysts have occurred, although at a lower rate than their day-5 and day-6 counterparts.

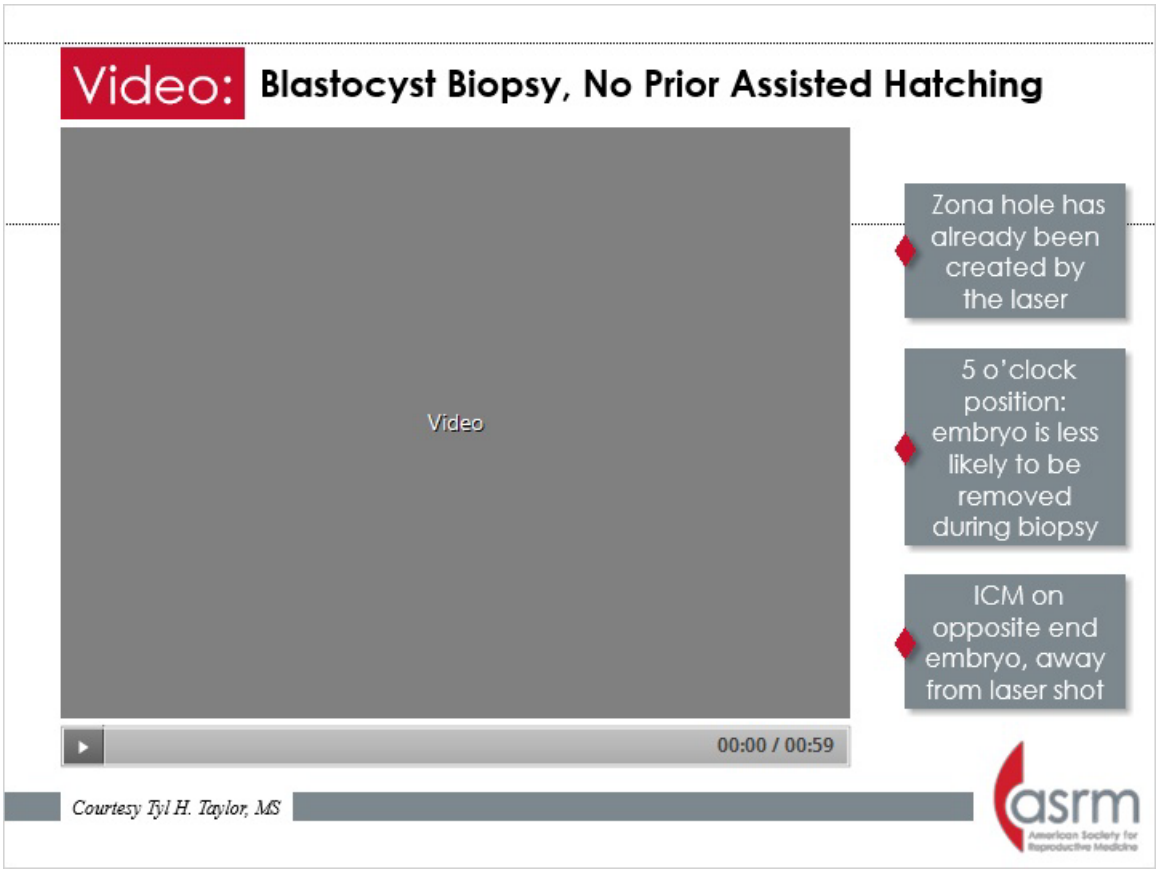
1.34 How to Blastocyst-Stage Biopsy



Notes:

Blastocyst-stage biopsy consists of multiple steps. First, the embryo is placed in small drops of HEPES culture media, overlaid with oil. Calcium and magnesium-free medium is not needed for this procedure. The drop containing the embryo is labeled with the appropriate number. A holding pipette is used to hold the embryo in the correct position. A piece of the trophectoderm is suctioned into the biopsy pipette and essentially "cut" using a laser. The piece of trophectoderm is then loaded into a tube for testing.

1.35 Video Blastocyst-Biopsy



Notes:

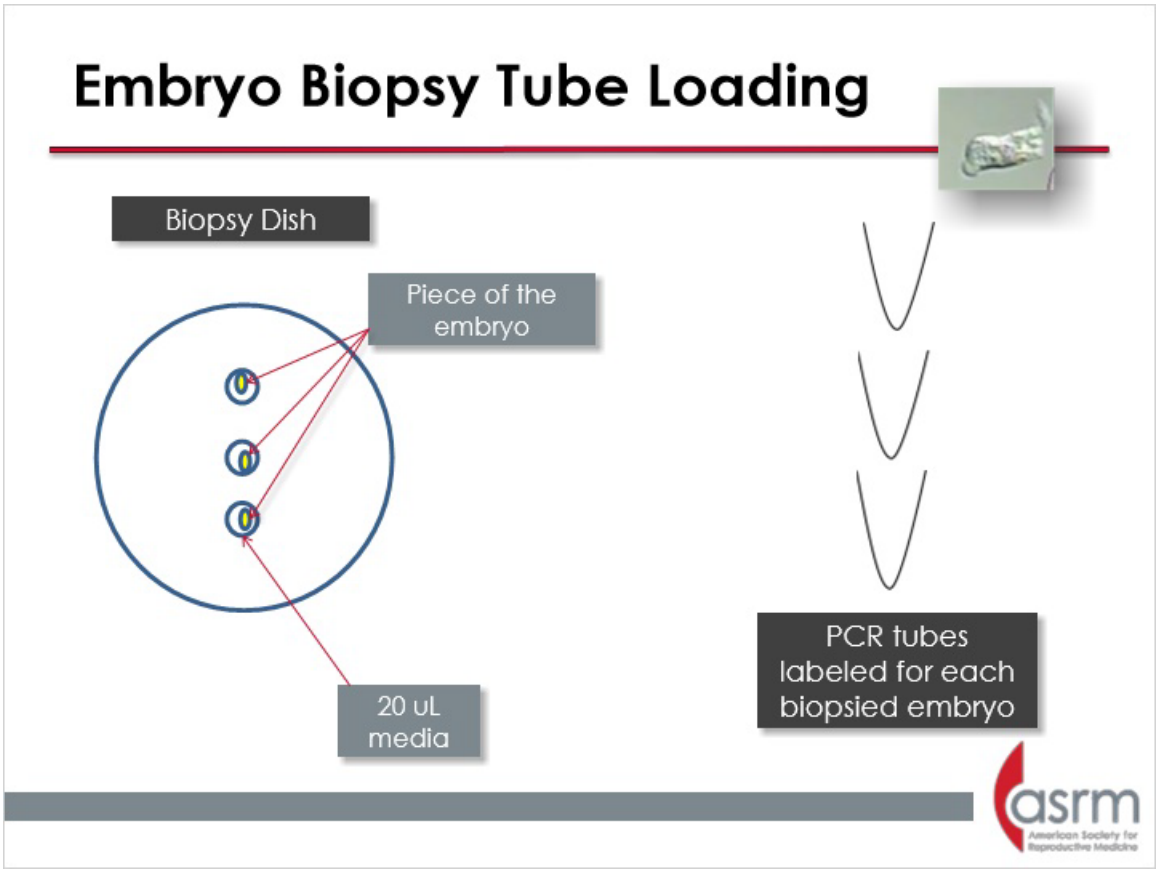
This video depicts biopsy of trophectoderm cells from an expanded blastocyst that was not previously hatched on day 3. The laser is used to make a small opening at the 5 o'clock position with the inner cell mass positioned opposite the opening and hence the laser pulses. The biopsy pipette is used to aspirate several trophectoderm cells through the zona opening. The laser is used to separate the cells from the trophectoderm. The biopsied cells are then released back into the drop containing the embryo.

1.36 Video Blastocyst-Biopsy Day 3 Hatching

Notes:

This video depicts biopsy of trophectoderm cells from an expanded blastocyst embryo that had been previously hatched on day 3. Note the trophectoderm cells protruding from the zona pellucida, facilitating the biopsy procedure.

1.37 Embryo Biopsy Tube Loading

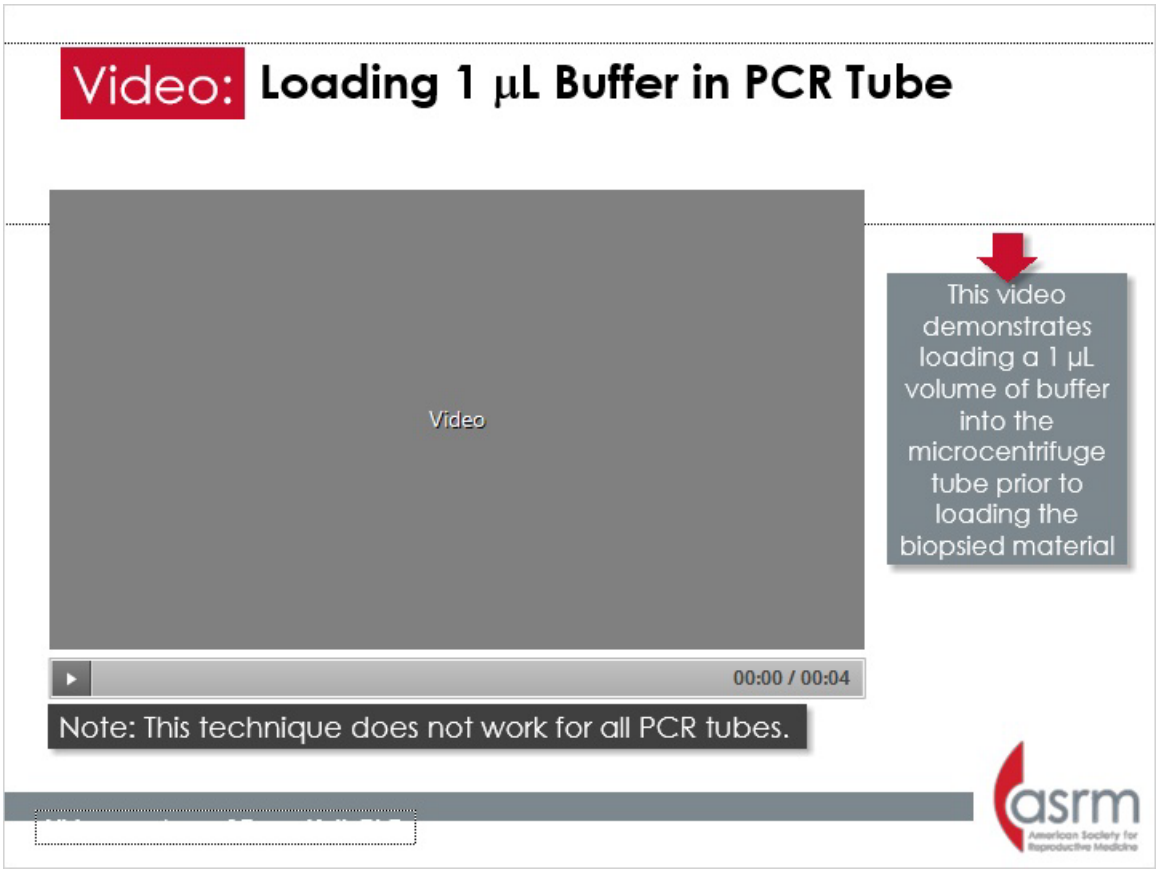


Notes:

A photo of the biopsied trophectoderm is shown at the top. While there are a variety of techniques for loading biopsied cells into the PCR tube, it is critical to minimize the volume of medium that is moved with the piece of the embryo (or individual cell with day-3 biopsy). Alterations in the volume may affect the concentration of the solutions for the molecular testing. The first technique involves simply placing the biopsy material into the tube, referred to as dry loading. Attach a 140 µL stripper tip to a pipetting device and turn down the volume to read 1 µL. This assures minimal volume will be expelled into the tube. Carefully pick up the biopsied material and pipette it through the multiple washes as specified by the reference lab. These wash steps are important to remove any extraneous cells that might contaminate the molecular test. After the final wash, the piece of trophectoderm is moved and the cells expelled into the microcentrifuge tube while visualizing underneath the dissecting microscope. Visualization of the embryo biopsy material can be difficult at times; however, it becomes easier with practice. Dry loading makes visualizing the embryo piece more difficult; however it minimizes media volume, and helps assure a proper read during the testing procedure.
Another technique that allows for easier visualization of the biopsied material when loading requires a preloading of media into the PCR tube. The embryo biopsy material is easier to see expelling from the pipette when the tip is in media, as opposed to dry loading. Although easier to visualize, there is less room for error in terms of media volume. Since the microcentrifuge

tube is already loaded with media, extreme care should be taken to minimize the amount of media carried over during the loading procedure.
One of the problems with loading is that it can be difficult to see the biopsied material being expelled into the tube under the microscope. In order to circumvent this, try placing the tube so that it leans against a 60mm dish. This will allow the tube to be at the proper angle to allow for easy visualization when loaded.

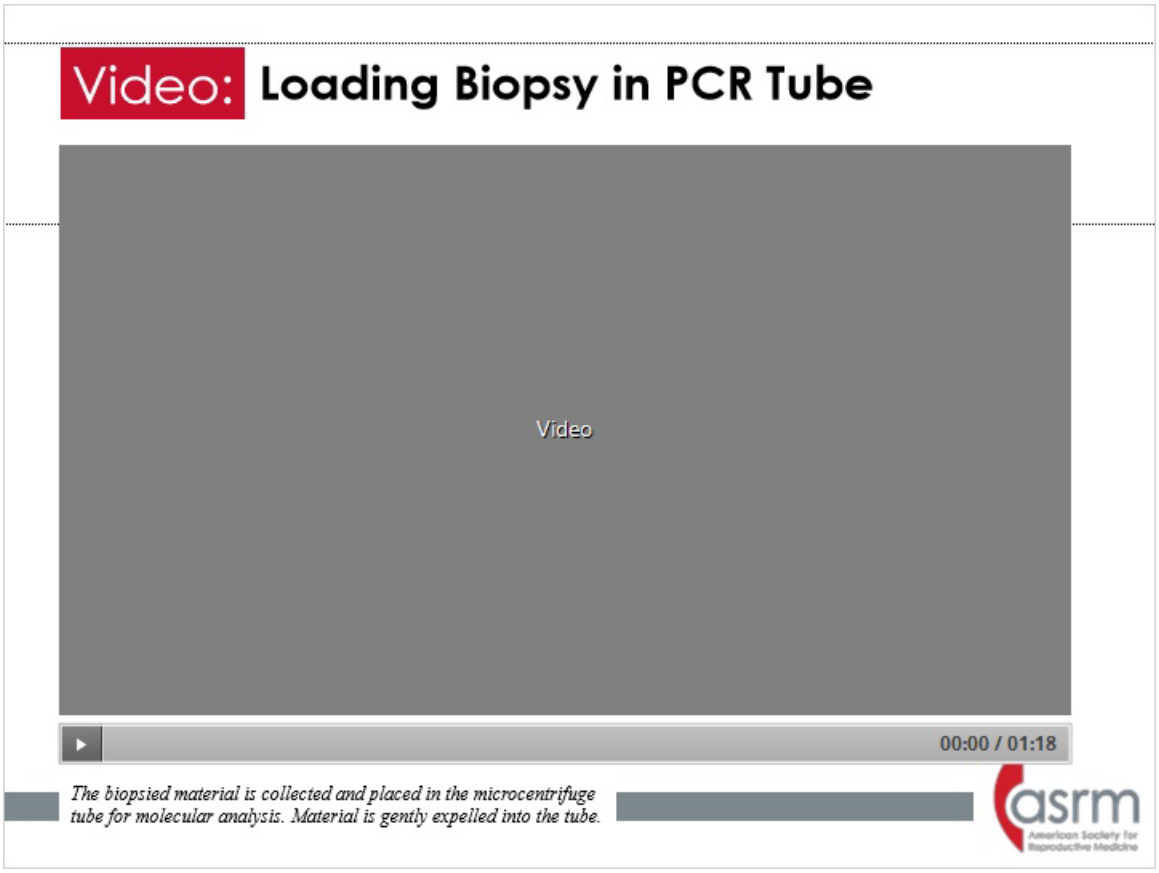
1.38 Loading 1 mL Buffer in PCR Tube



Notes:

This video demonstrates loading a 1 µL volume of buffer into the microcentrifuge tube prior to loading the biopsied material.

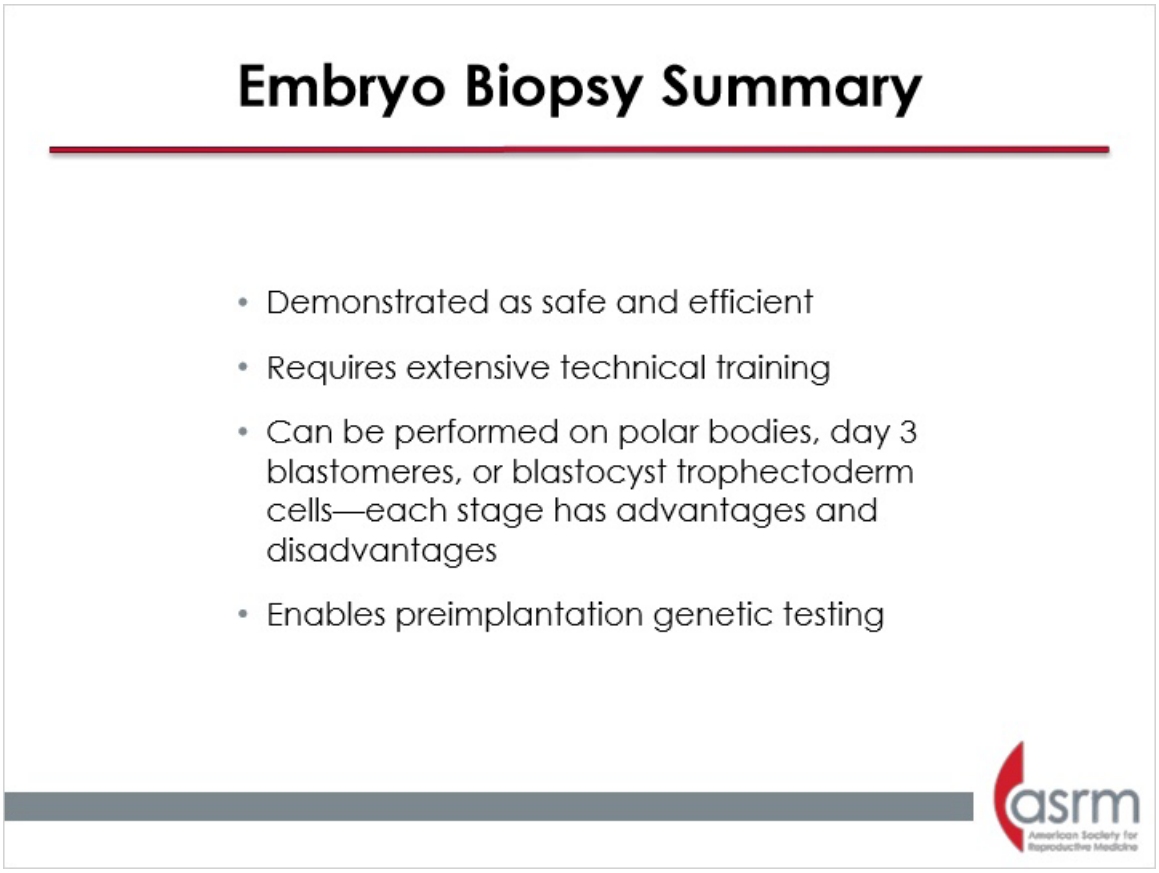
1.39 Loading Biopsy in PCR Tube



Notes:

The biopsied material is collected and placed in the microcentrifuge tube for molecular analysis. Material is gently expelled into the tube.

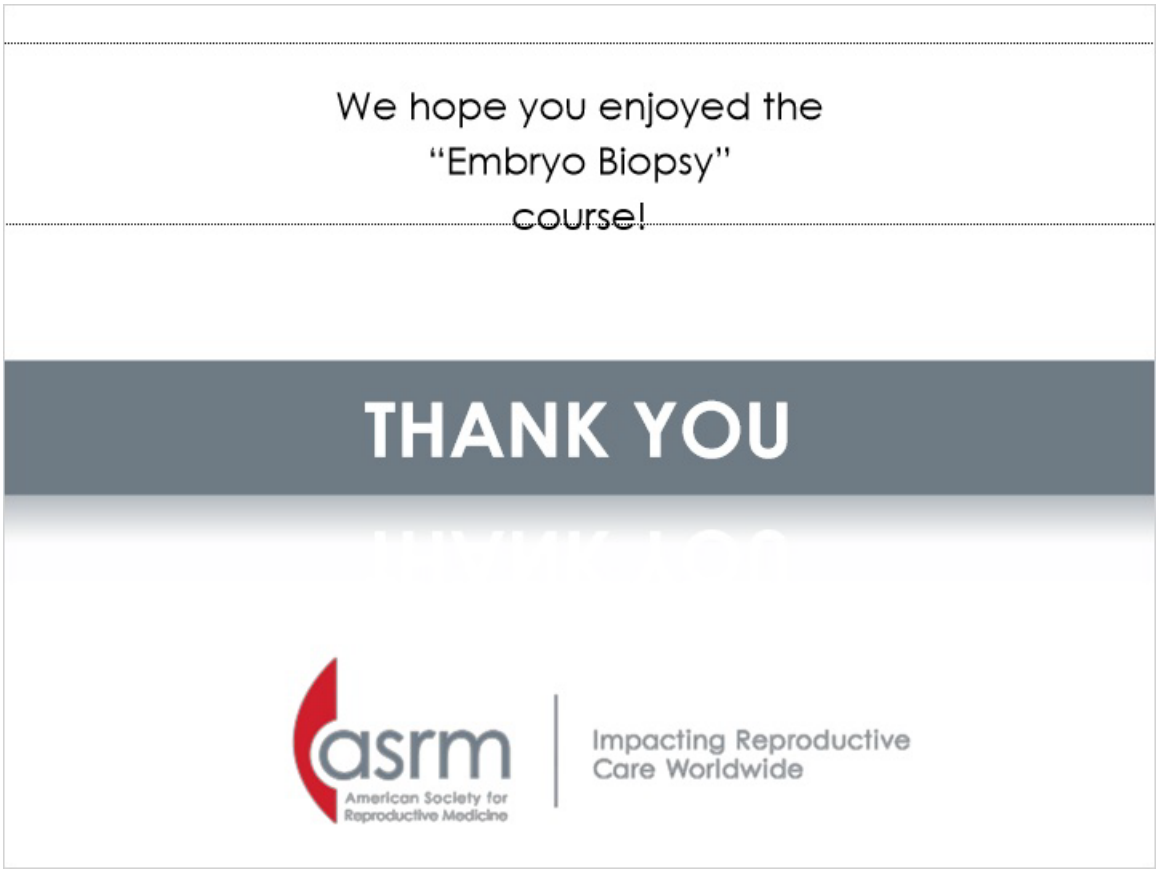
1.40 Embryo Biopsy Summary



Notes:

In summary, biopsy has been shown to be a safe and efficient technique to be able to sample material from the oocyte or embryo. Zona pellucida breaching via acidified medium or the laser appears to have similar success rates. Biopsy is a skill that requires good eye-hand coordination as do other micromanipulation techniques. Whether biopsy occurs at the polar body stage, cleavage stage, or the blastocyst stage depends upon several advantages and disadvantages that should be evaluated. The biopsied sample is then sent to a genetics reference laboratory to undergo preimplantation genetic testing.

1.41 Thank You



Notes:

Thank you for participating in this educational activity.