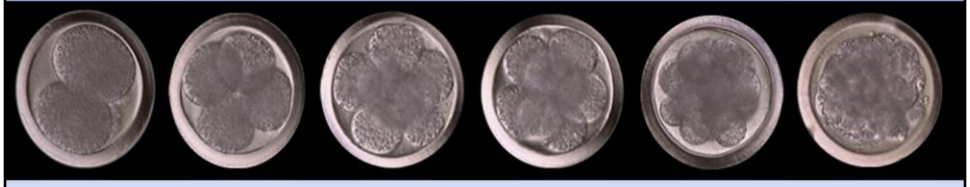
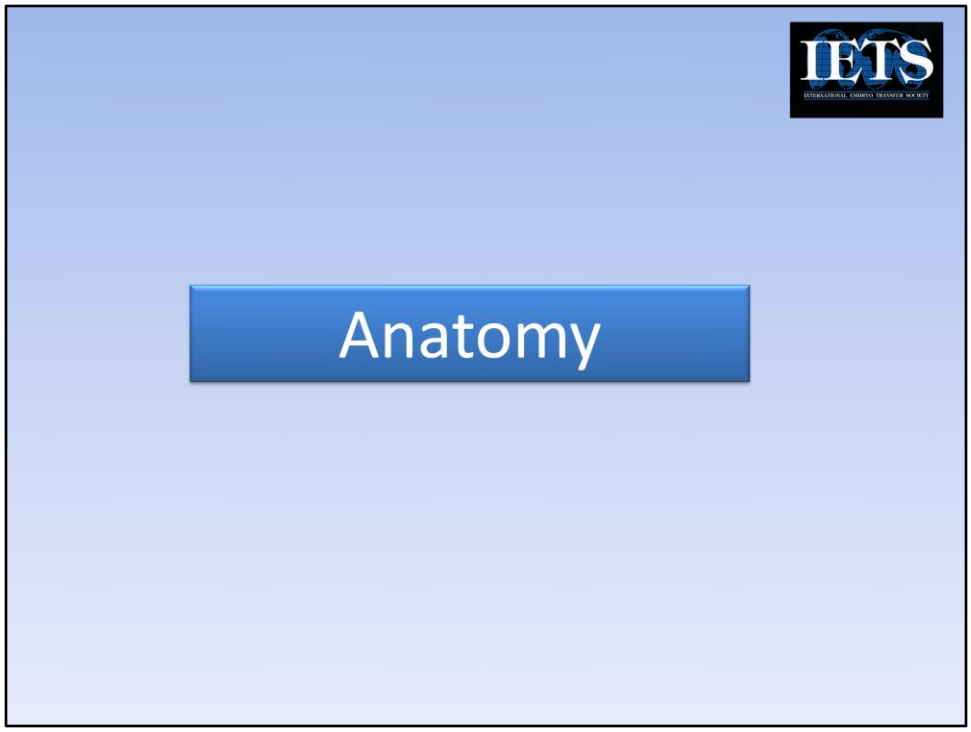
Foundation



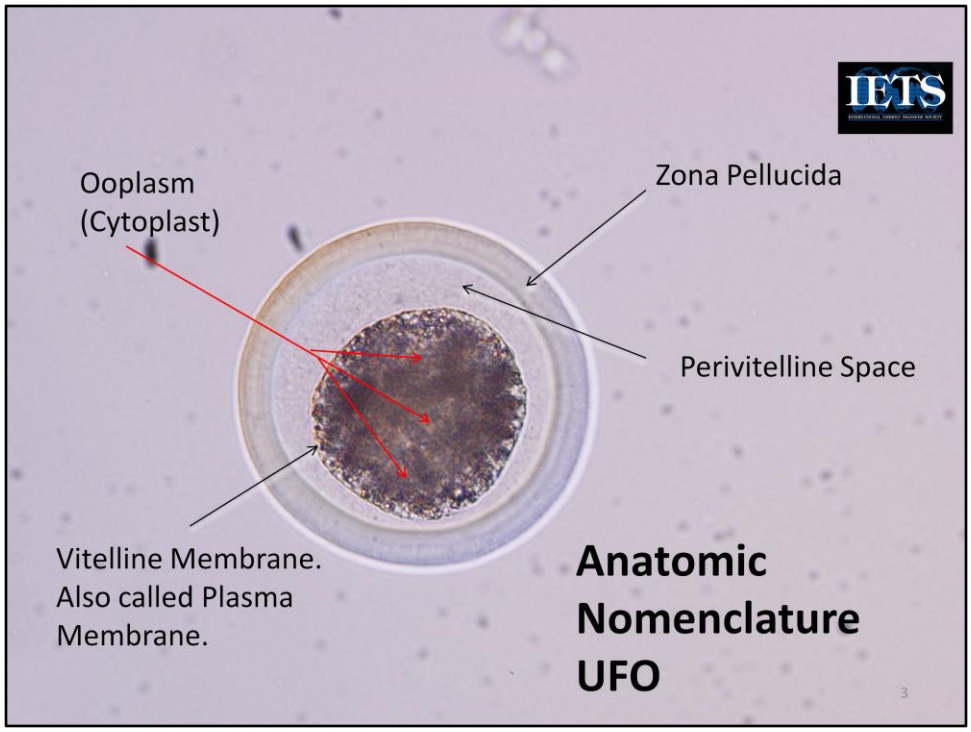
Bovine In Vivo Embryo Slide Set
Tutorial

Revised 2010

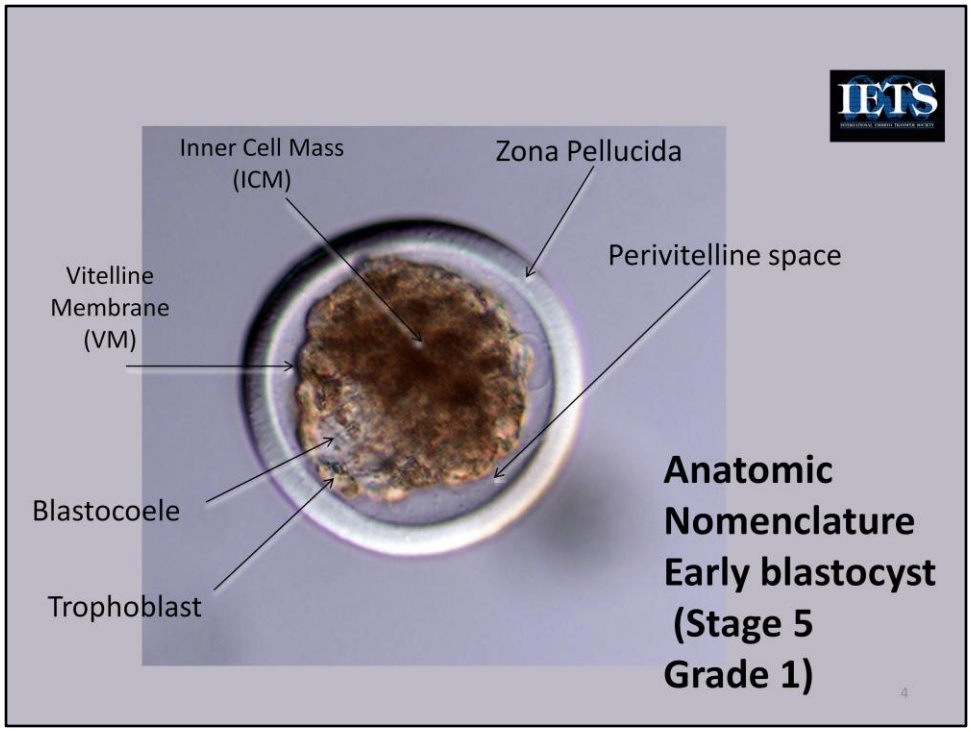


Most of the microphotographs in this tutorial were taken with either bright field (BF) or differential interference contrast (DIC) microscopy. At the bottom of each slide is a description of its contents. It begins with the magnification of the microscope at the time the picture was captured and the type of contrast used to view the slide. For example 100x DIC means the photograph was taken with a 10x objective and a 10x eyepiece and DIC was the contrast type. A glossary of terms for embryo nomenclature and grading criteria can be found in the IETS Manual. It's worth mentioning that the words cytoplast, cytoplasm, and ooplasm all mean the same thing.

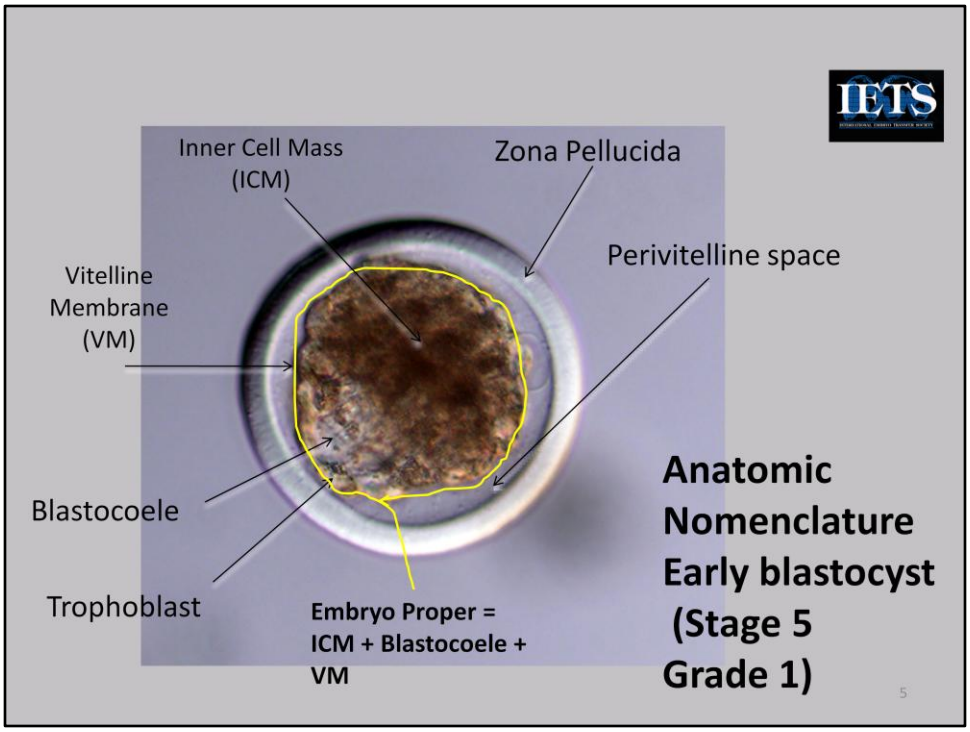
The definitions of stages and grades can be found in the IETS manual.



100x BF. Unfertilized ova (UFO) collected day 7 post estrus. The major key to differentiating this UFO from a morula is that the vitelline membrane (VM) of a UFO is smooth around the perimeter of the cytoplasm as opposed to a morula that has small bulges (blastomeres) protruding from the surface of the embryo proper. A UFO is a one cell entity, and a morula is multicellular. This ovum obviously has only one cell with a very smooth plasma membrane (VM).



100x DIC. Stage 5 Grade 1 embryo. Early blastocyst collected day 7 post onset of estrus. Notice the presence of blastomeres immediately inside the VM causing an irregular bulge, or bumpy edge to the VM. The inner cell mass plus the blastocoele and VM is collectively called the embryo proper. Compare the bulges on the VM of this early blastocyst to the very smooth non-bulging VM of the previous UFO.



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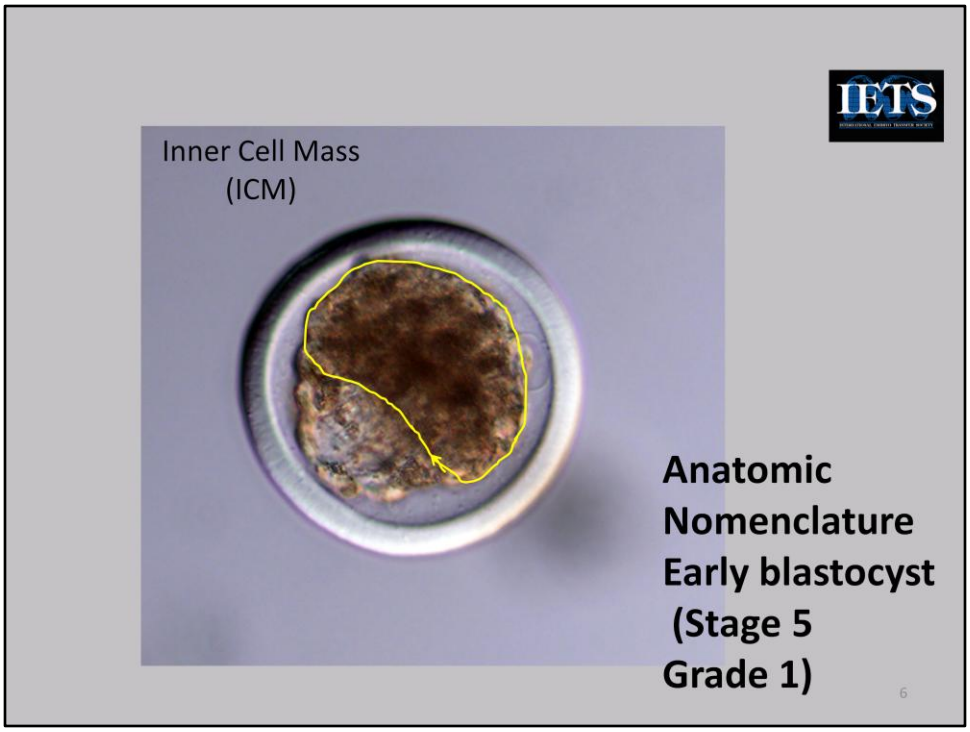
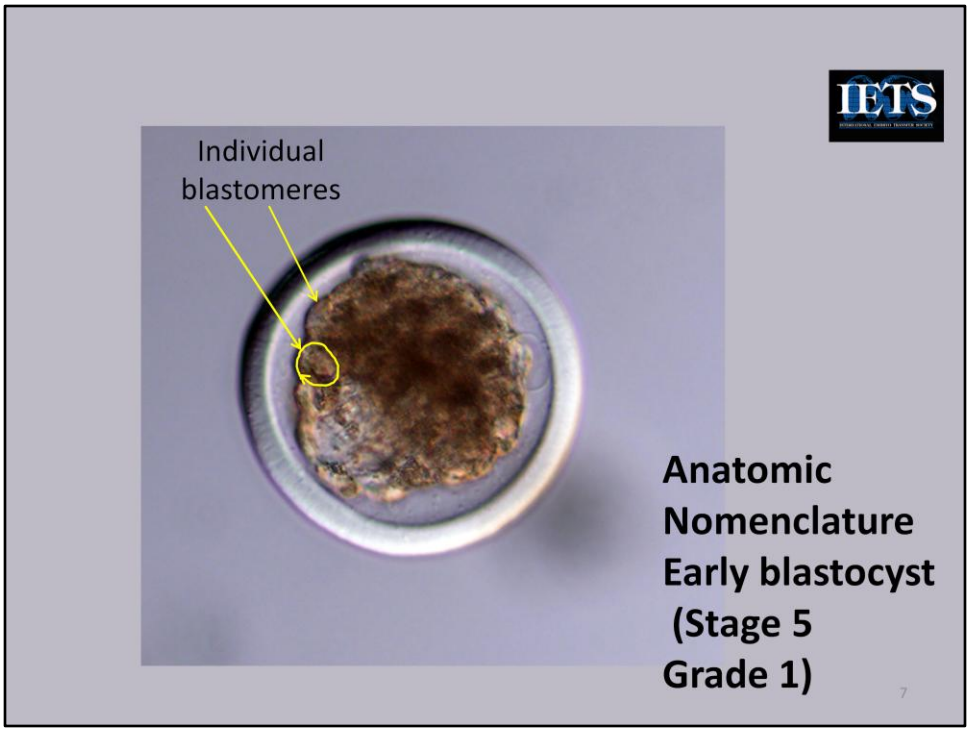
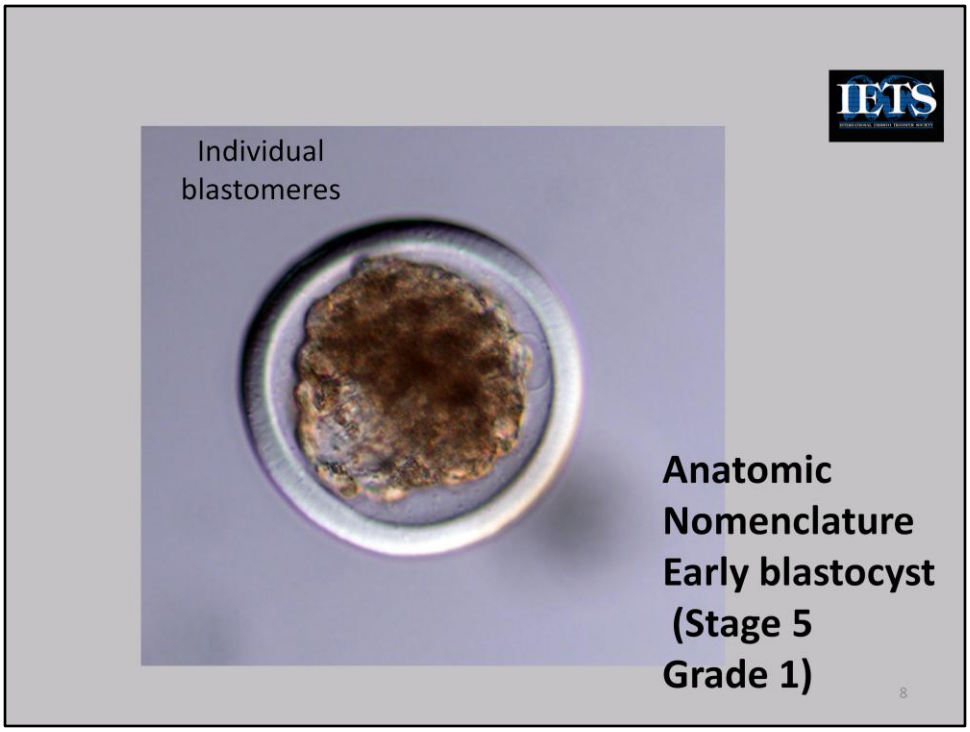


100x DIC. Same as previous slide, but ICM is outlined in yellow.

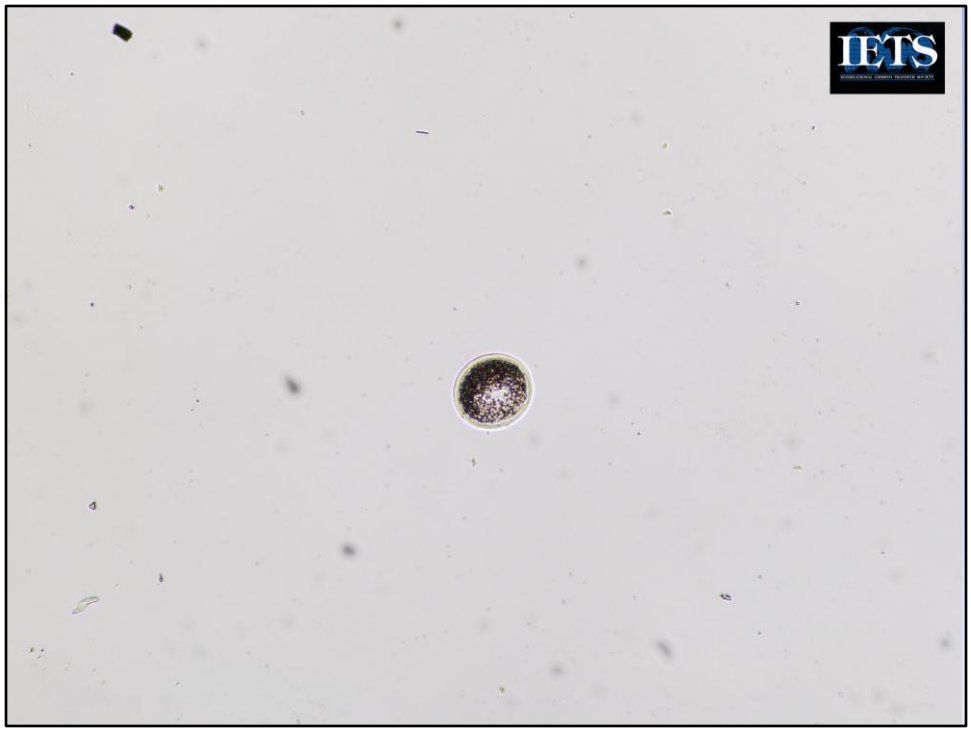


100x DIC. Same embryo as slide #4 and #5, but arrows point to individual blastomeres. Also, one fused blastomere is outlined in yellow.

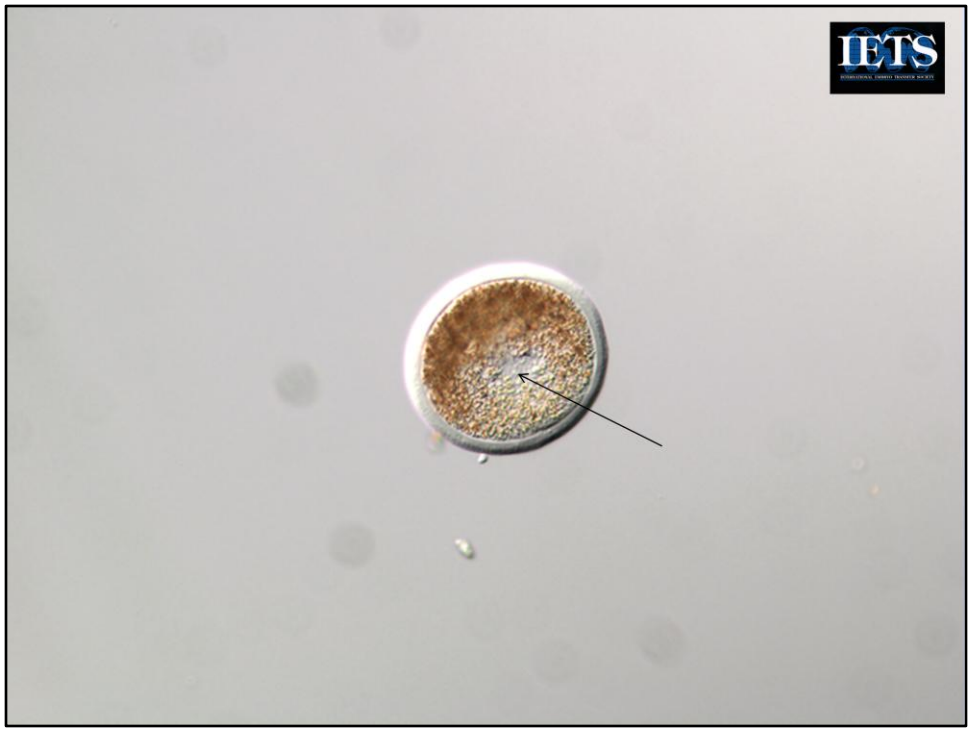


100x DIC. Same embryo as slide #4 and #5, but no animation.

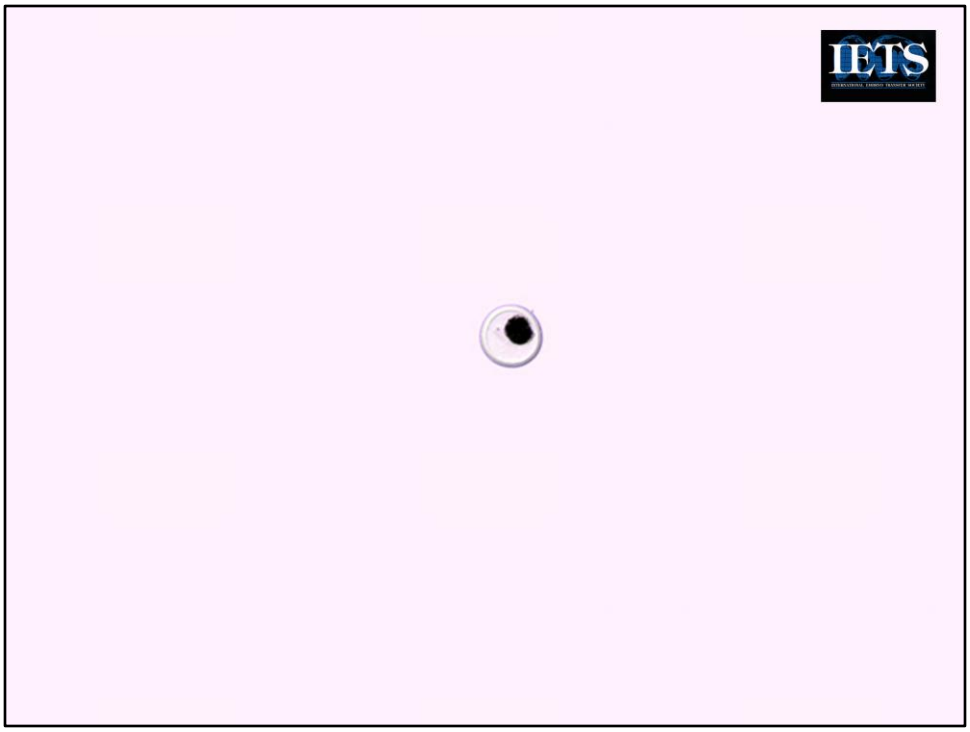
UFOs have many different physical appearances. A good portion of this tutorial is dedicated to UFOs to make sure students get a broad perspective of the variation often encountered with UFOs in commercial embryo transfer (ET).



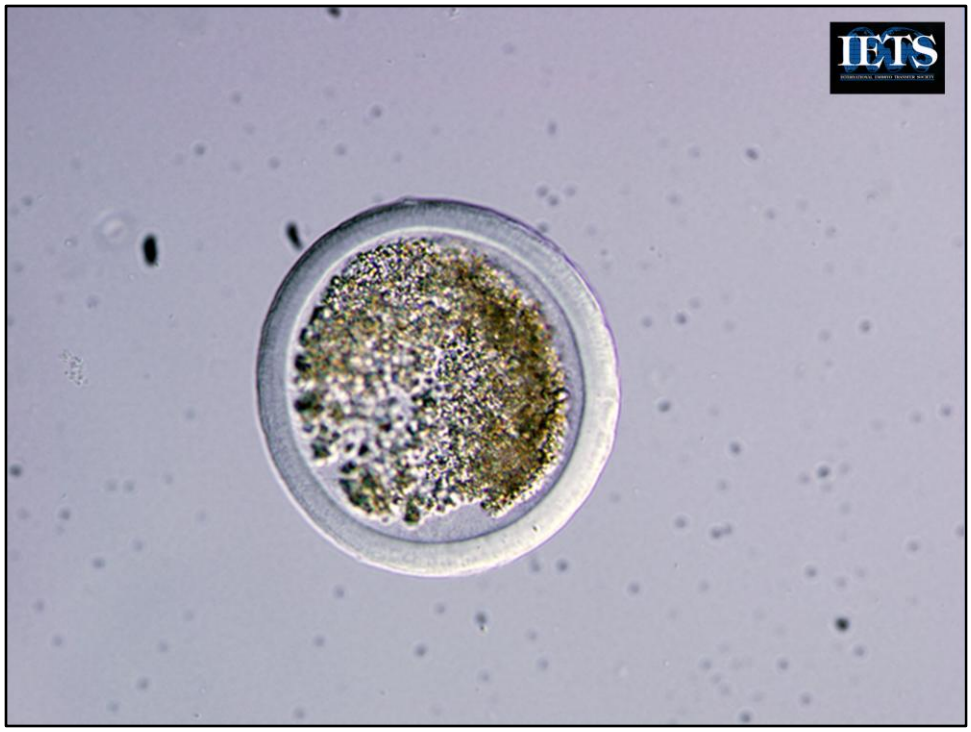
40x brightfield (BF). This is a UFO. The ooplasm is no longer condensed and encased inside an intact vitelline membrane. Consequently there is essentially no perivitelline space. The cytoplast (ooplasm) has began to degenerate and appears granular. These tiny granules shouldn't be confused with blastomeres.



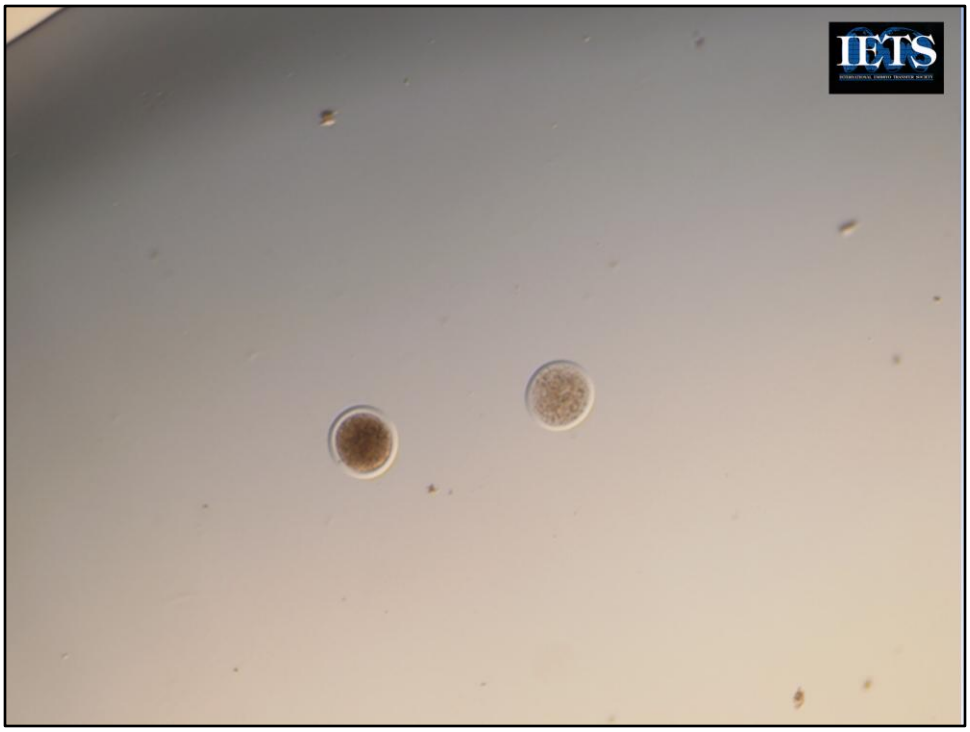
100x DIC. This is the exact same UFO as the previous slide. At this power a clear area (see arrow) can be seen which could be confused with a blastocoele cavity. However, this ova is a one celled structure which contains no blastomeres or trophoblast cells. If the Petri dish was shaken this UFO would appear flattened or non-spherical.



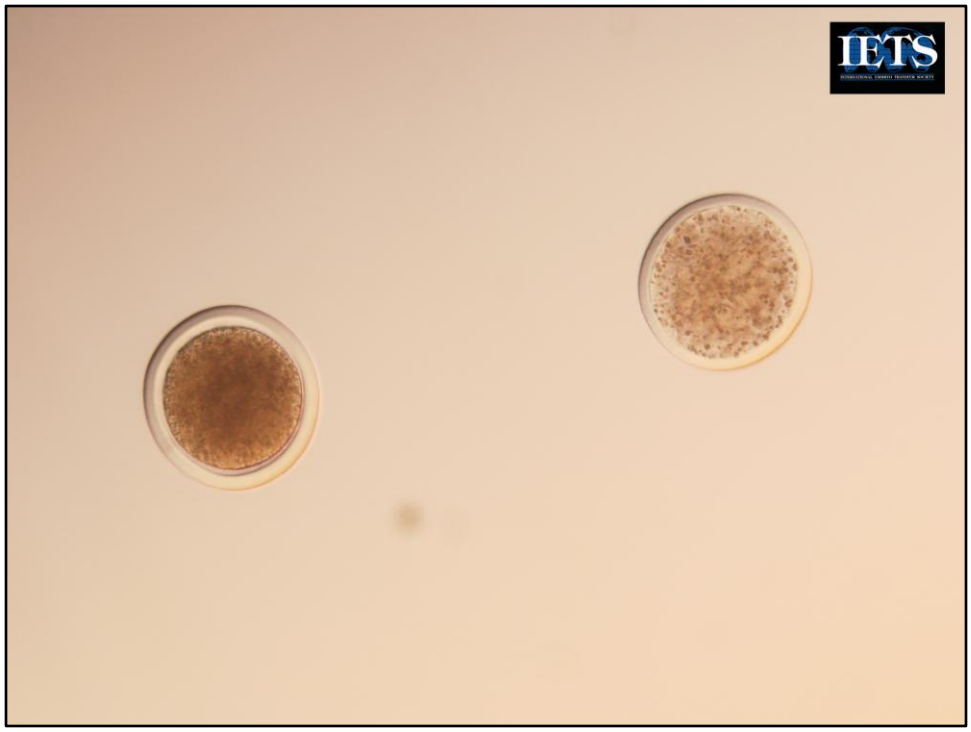
40x BF. This is another UFO. The ooplasm is not perfectly centered, but has drifted slightly to the 2 o'clock position (inconsequential). Notice the smoothness of the vitelline membrane surrounding the ooplasm. The cytoplast remains intact with no apparent degeneration.



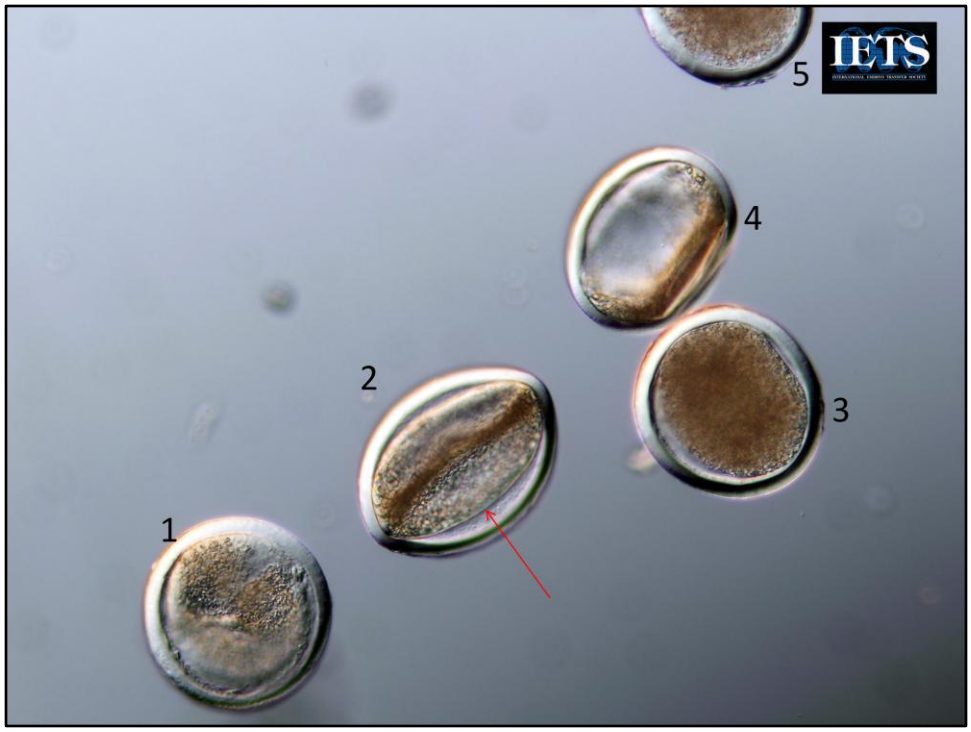
200x BF. UFO. The vitelline membrane has essentially degenerated and broken up. The cytoplasm has also begun to degenerate giving the ova a speckled or granular appearance. It's important to know that the cellular debris is not a bunch of tiny blastomeres. Healthy blastomeres would be much larger in an embryo collected at day 7 post estrus, and they would be fused with other blastomeres if they were alive.



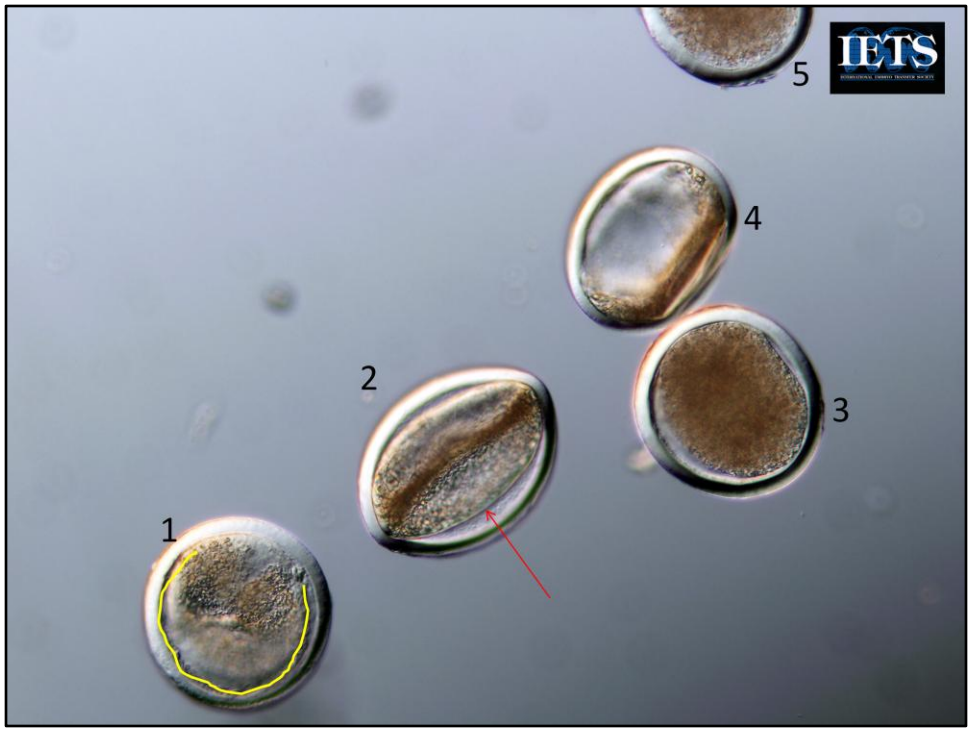
10x BF. Both are UFOs from the same donor female. The one on the left has a very healthy cytoplast with a very tight vitelline membrane surrounding it. The ova on the right has a degenerated ooplasm and vitelline membrane. Notice the speckled effect of the cytoplasm. Collected on day 7 post estrus, UFOs can have many shapes and appearences.



100X BF. Same slide as previous at higher magnification.



100x DIC. All five of these ova are unfertilized. Ovum #1 is somewhat unusual. From about 3 o'clock to 10 o'clock the cytoplasm has separated from the vitelline membrane {see yellow line on next slide}. This gives a false impression of a layer of trophoblast cells. The lower half of this ovum looks somewhat like a blastocoele. Coupled with the vitelline membrane attached to the zona this ovum could be confused with a blastocyst, but it is not fertilized.
Ovum #2 is a UFO with a flattened zona giving it a concave look. The red arrow is pointing to the vitelline membrane, which is very smooth but intact. There are no blastomeres present in this ovum. If this ovum was flipped or rolled when being evaluated under a microscope its cytoplasm would likely appear speckled or degenerate. Although the VM is intact in this UFO the cytoplasm has begun to degenerate and the flattening of the ovum has caused a dark ridge (ooplasm) to appear from about 2 o'clock the 7 o'clock.
Ovum #3 has only one cell (UFO) with a cytoplasm that almost completely fills the area inside the zona. There is only a tiny amount of perivitelline space from about 6 to 9 o'clock. The cytoplasm appears not to be degenerated in this ovum.
Ovum #4 can be confused with a late stage blastocyst, but it is unfertilized. This ovum is not spherical, but somewhat concave. Both ova #2 and #4 have an apparent ridge running from 2 to 7 o'clock. This is where the flattened zona has caused the degenerated cytoplasm to flatten also. These ova happen to be on their edge while being photographed giving the appearance of a ridge, which is actually condensed cytoplasm.
Ovum #5 is also a UFO, but only the bottom half of it is visible. It appears to look very similar to ovum #3.



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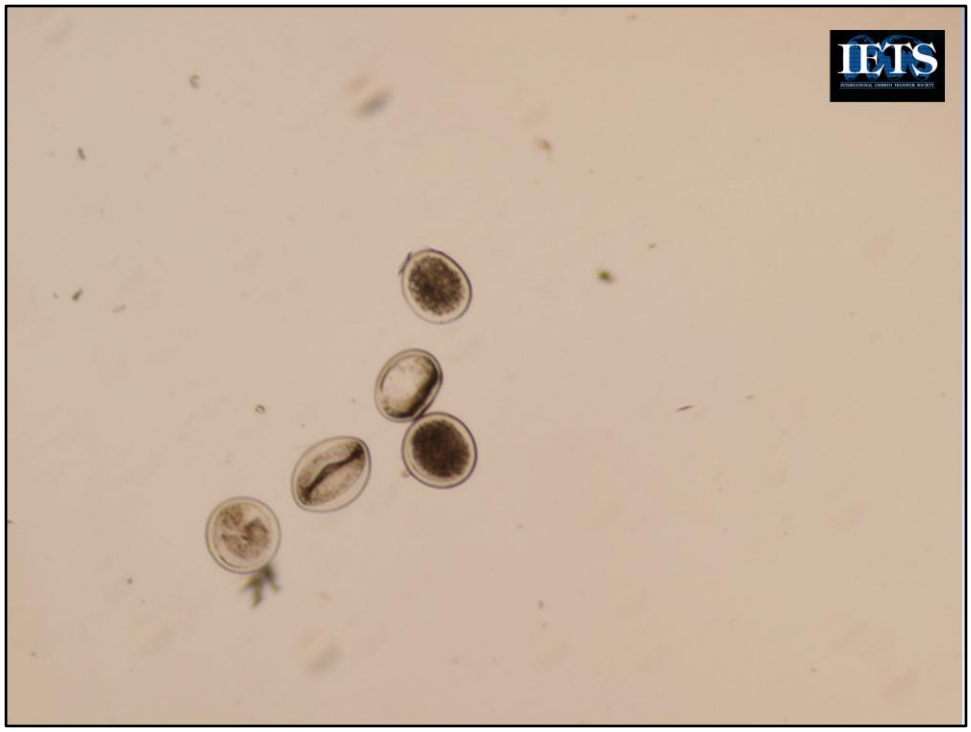
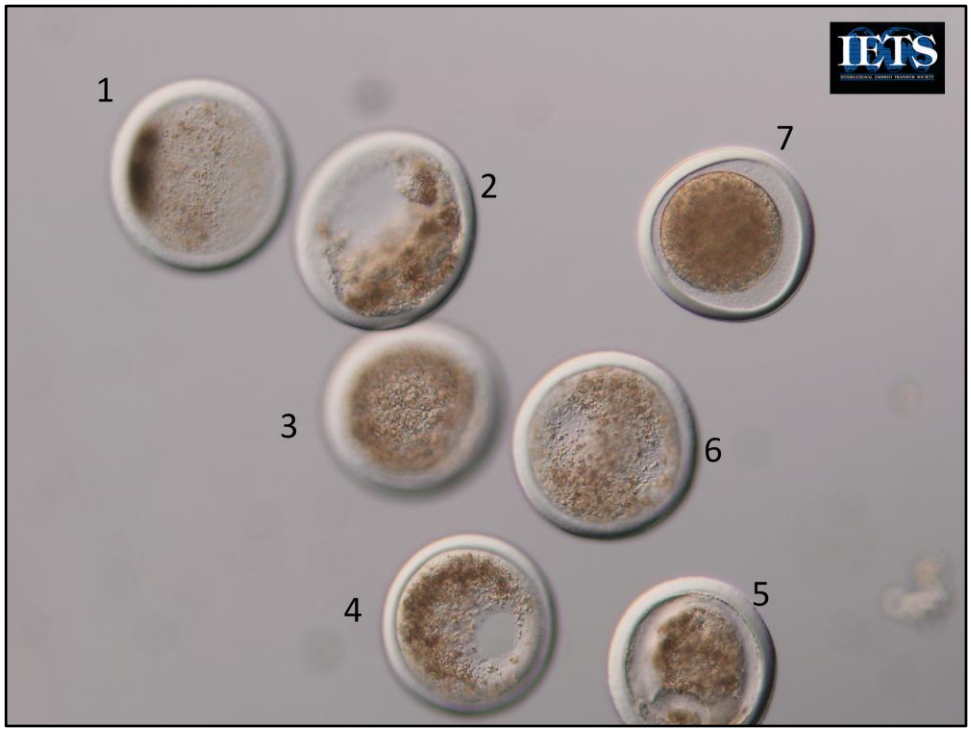
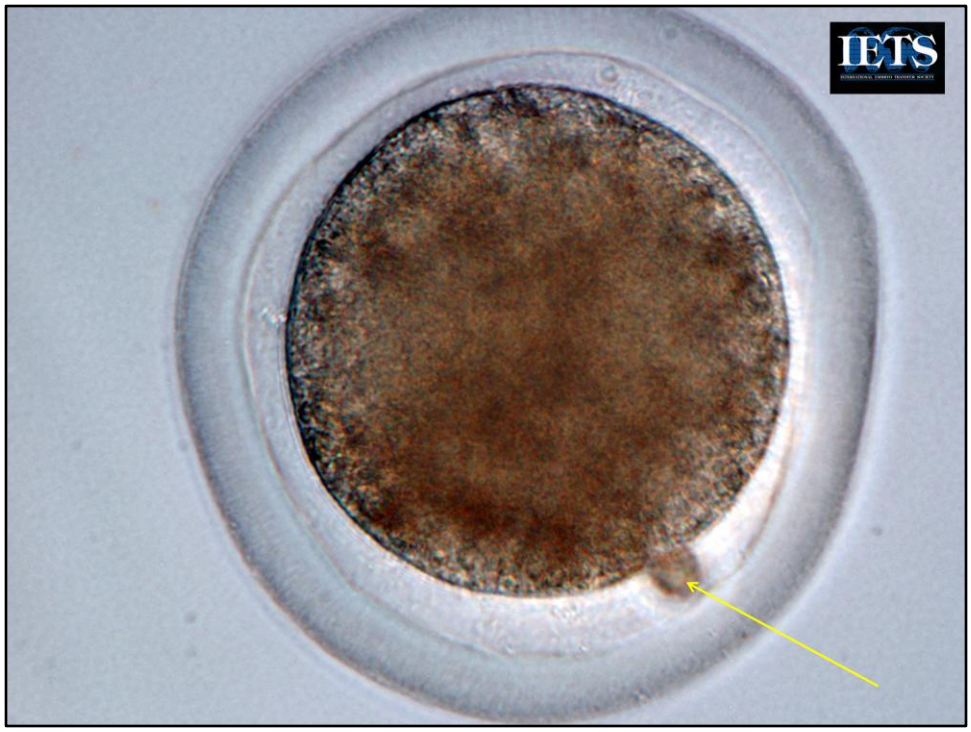


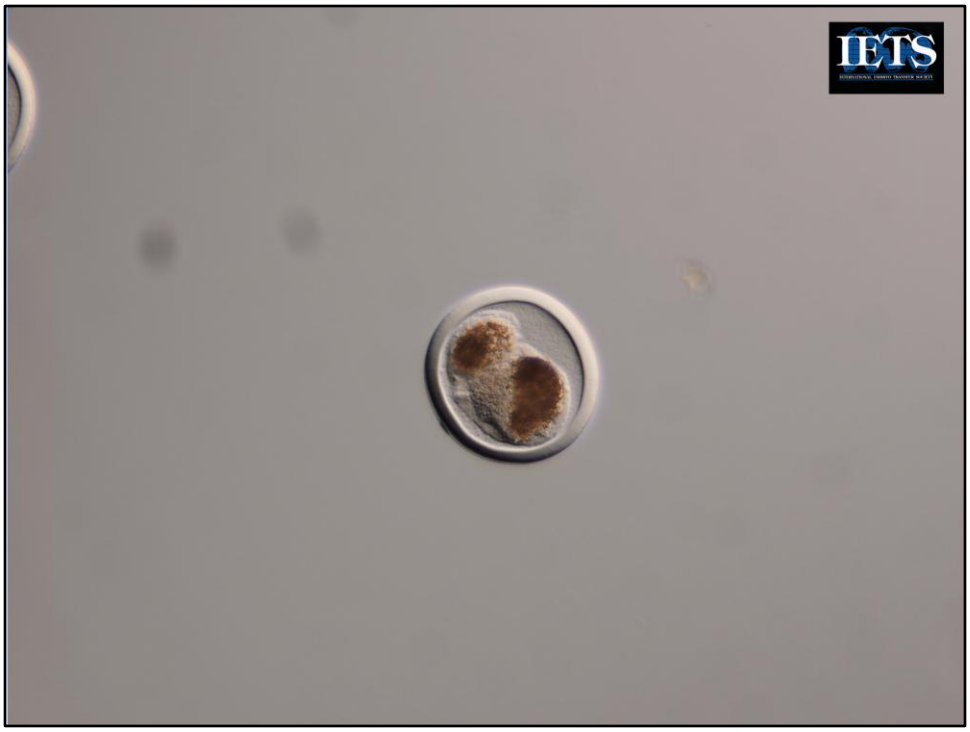
40X BF. Same slide as previous at a lower magnification.



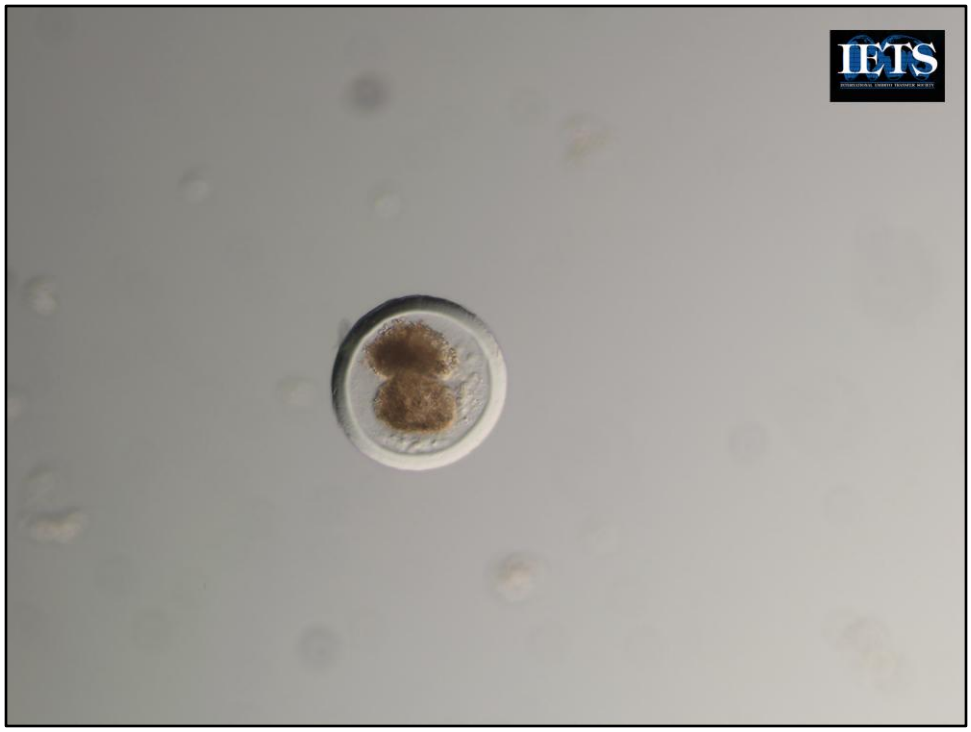
100x DIC. All 7 ova are UFOs. The cytoplast in #7 is dense and completely encased by an intact vitelline membrane. Notice how perfectly smooth the surrounding membrane is compared to a morula or early blastocyst that would have a bulging vitelline membrane due to numerous blastomeres. All other ova have some level of degeneration as illustrated by vitelline membrane breakdown and speckled ooplasm. Ovum #2 could be mistaken for a fertilized degenerated embryo, but it is unfertilized with a fragmented cytoplasm. There is some condensed chromatin in ovum #2 giving it the false appearance of having degenerated blastomeres. The diameter of ovum #6 is noticeably larger than ovum #3. The zona of ovum #6 is soft thereby causing it to lose its spherical shape and flatten out . However, it could simply be a larger oocyte.



400x DIC. UFO. The ooplasm is dense and encased by a healthy and intact VM. There is a polar body at 5 o'clock in the perivitelline space (arrow).

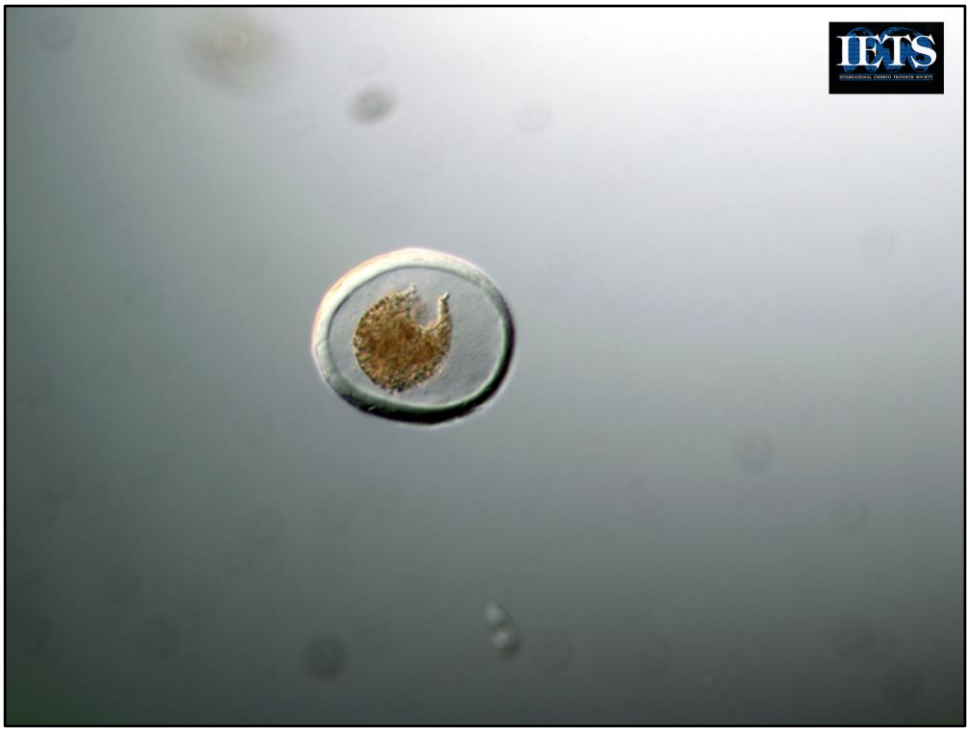


100x DIC. UFO. The cytoplasm appears to be in the process of fragmenting into two distinct pieces. There are two areas of condensed cytoplasm (dark brown areas), one twice the size as the other, but only one VM. Often, ova like this will exhibit a more complete fragmentation making it appear like a two cell fertilized degenerate ovum. In cases like that it can be very difficult to distinguish between fragmented UFOs and fertilized two cell degenerates unless the ovum is exposed to a DNA stain so nuclei can be counted.

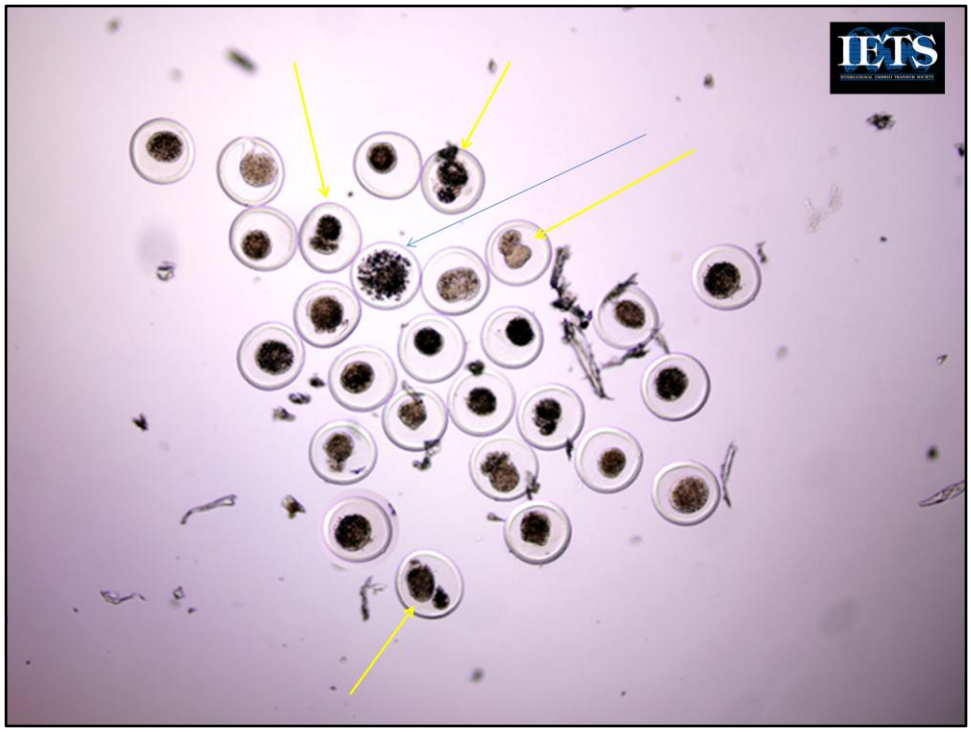


100x DIC. This is either a UFO that has fragmented into two fairly equal halves, or it is a degenerate ovum. In commercial embryo transfer, it could be considered academic whether or not an ovum is a fragmented UFO or a fertilized degenerate since neither will generate offspring.

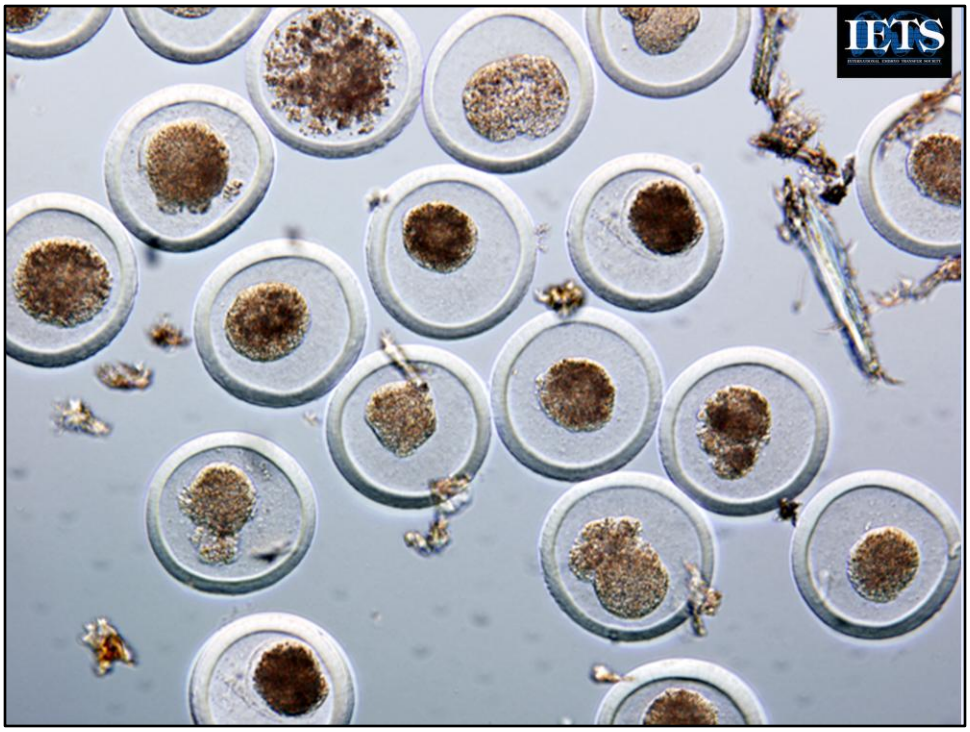
100x DIC. This UFO illustrates degeneration and fragmentation of the cytoplast.



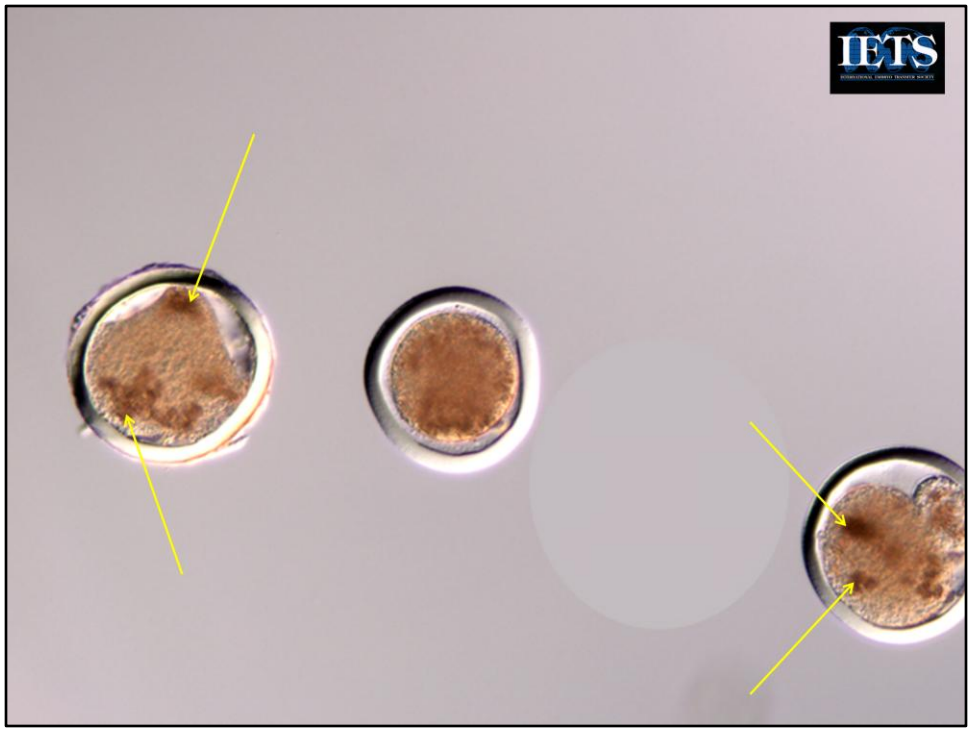
100x DIC. UFO. There is a break in the VM at 1 o'clock. This ovum is in the beginning phases of ctyoplasmic degeneration.



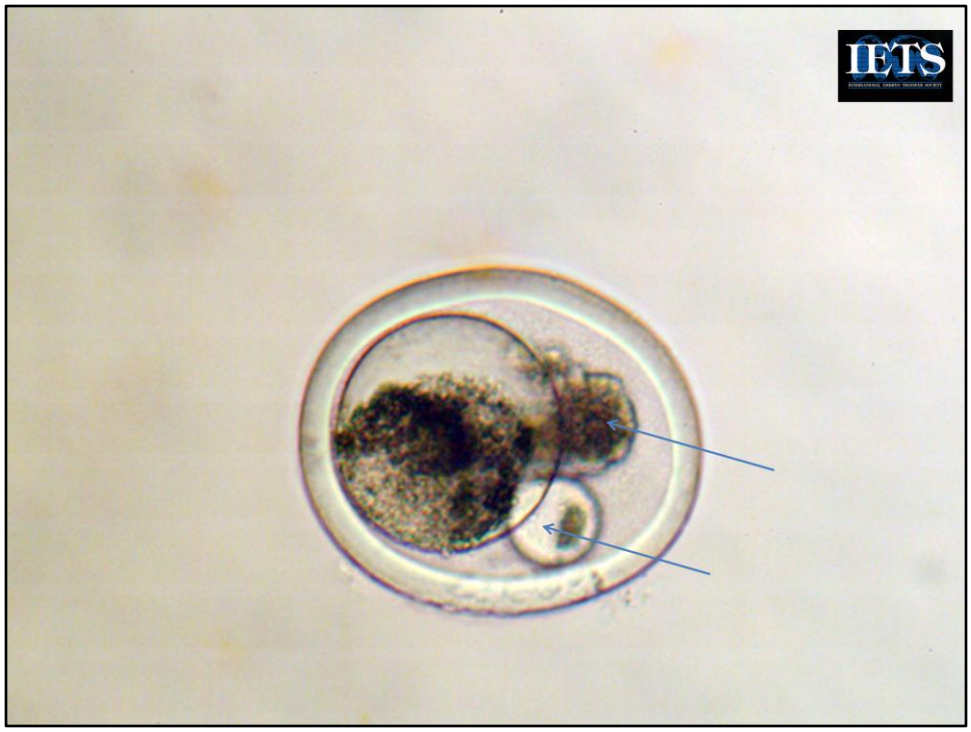
40x BF. All these ova are UFOs. A few have fragmented cytoplast (ooplasm) (yellow arrows), and one has a degenerated cytoplast (blue arrow).



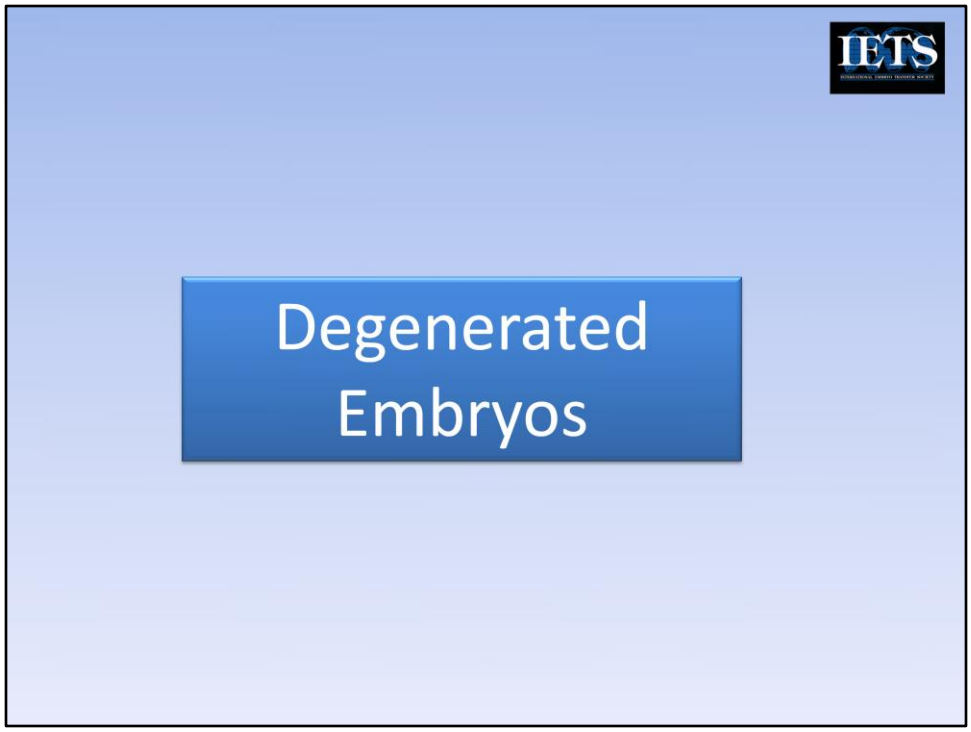
100x DIC. Same slide as previous, but higher magnification.



100x DIC. Three UFOs.The ooplasm of these three UFOs, especially the outer two, has condensing chromatin which is manifested by the dark spots (arrows). UFOs can have many different looks. It's important to be able to differentiate between a UFO and a viable embryo.

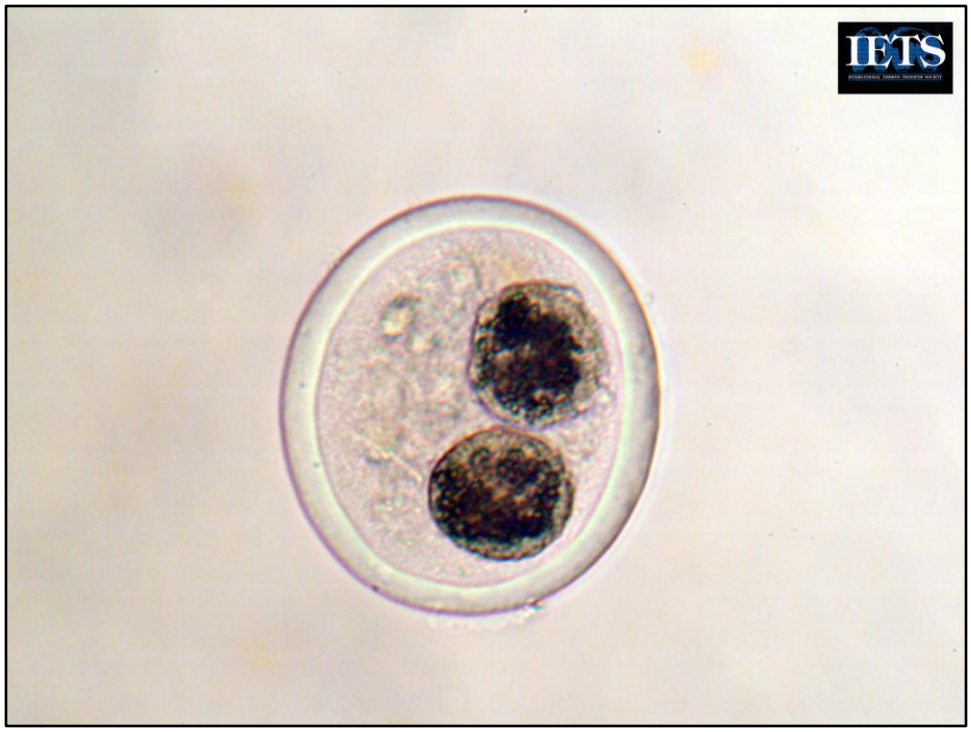


200x BF. UFO. Although there appears to be 3 separate cells in this ovum it is unfertilized and fragmented. The largest cell mass has a very distinct plasma membrane. The ooplasm has detached from the VM from 9 to 5 o'clock, and has retracted away from it. The smoothness of the VM is a key to differentiating the UFO from a blastocyst. Some of the ooplasm (arrows) has leaked out of the VM creating a false multicellular look.



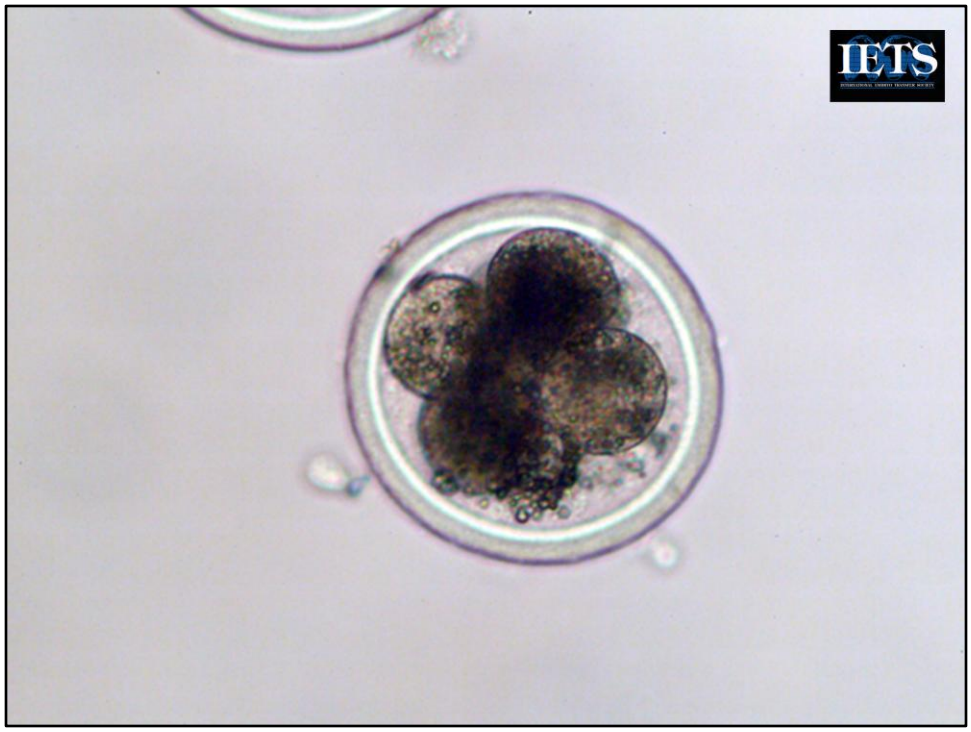
Degenerated embryos are ova that have been fertilized and undergone cleavage, but are considered dead.

200x DIC. This two cell degenerated embryo was collected day 7 post onset of estrus. The two blastomeres are very large, oval shaped, and apparently healthy, yet dead. If this had been collected from the oviduct 48 hours post onset of estrus it would be considered a healthy two celled embryo.

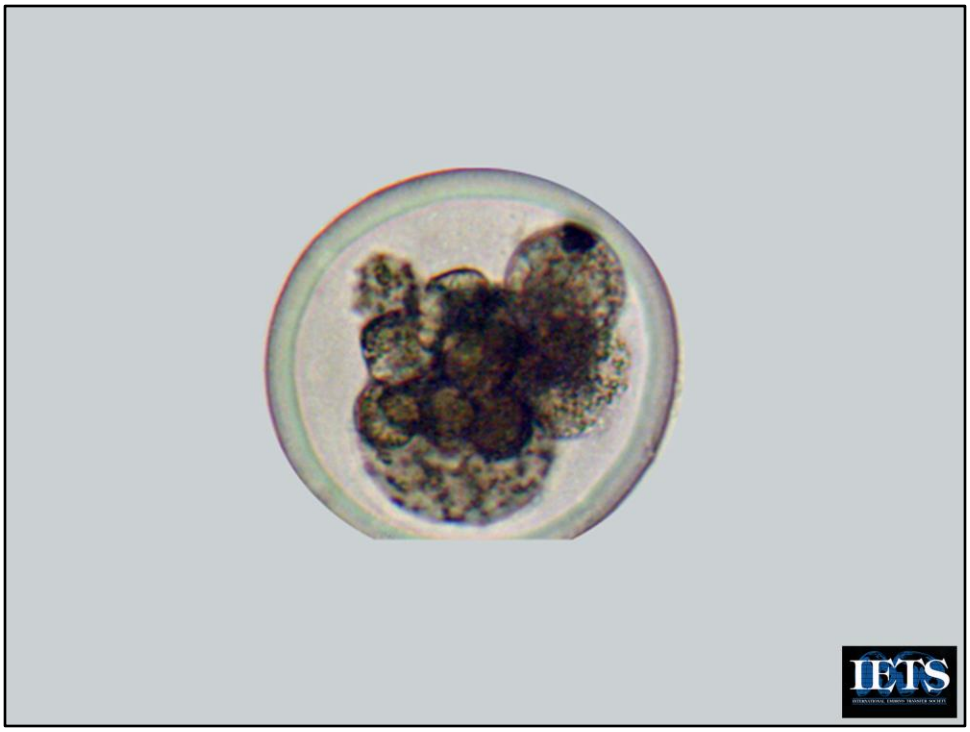


200x BF. 2 cell degenerated embryo. Without DNA staining it would be difficult to differentiate this from a fragmented UFO. However, these two cells are equal in size and, after staining, showed two nuclei. Compare the size of the blastomeres of this two cell with that from the previous slide.

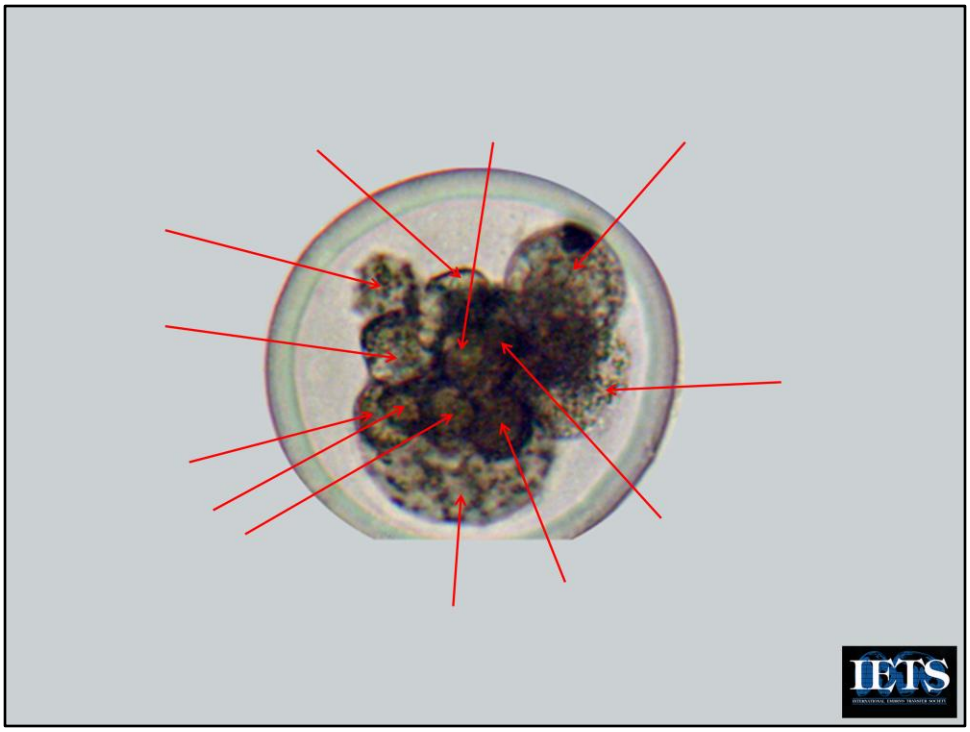
100x. 3 cell degenerated embryo. Whether this is a fragmented UFO or a degenerated embryo is difficult to discern without nuclear staining. Either way, an embryo like this collected 7 days post estrus is dead.



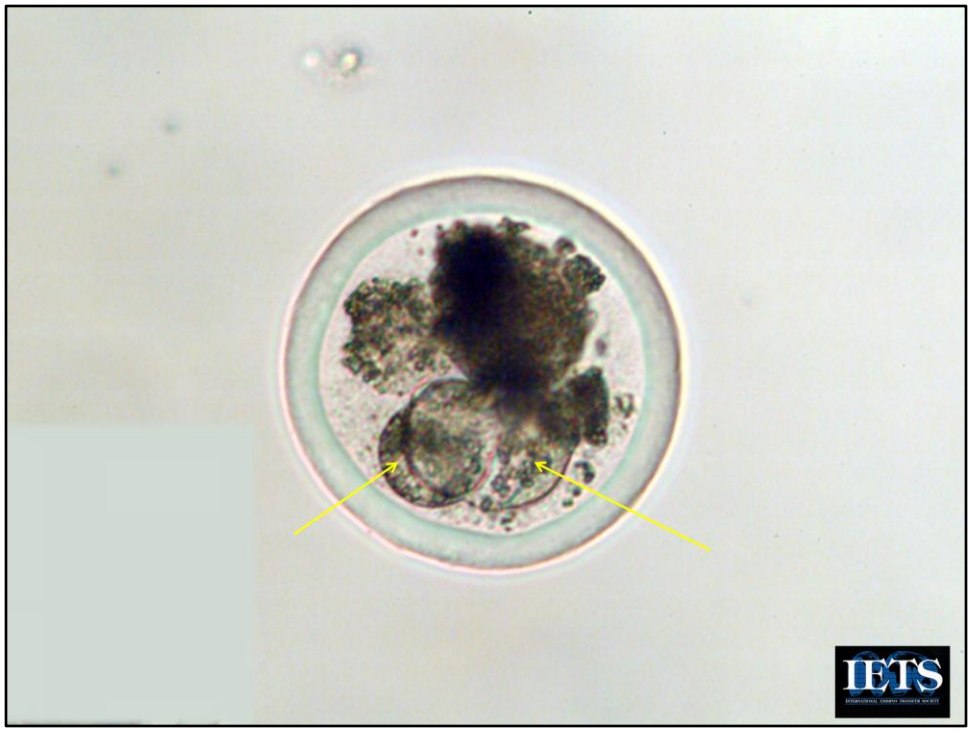
200x BF. 4 cell degenerated embryo There are 4 distinct cells/blastomeres in this embryo. Collected at day 7, this embryo is considered dead.



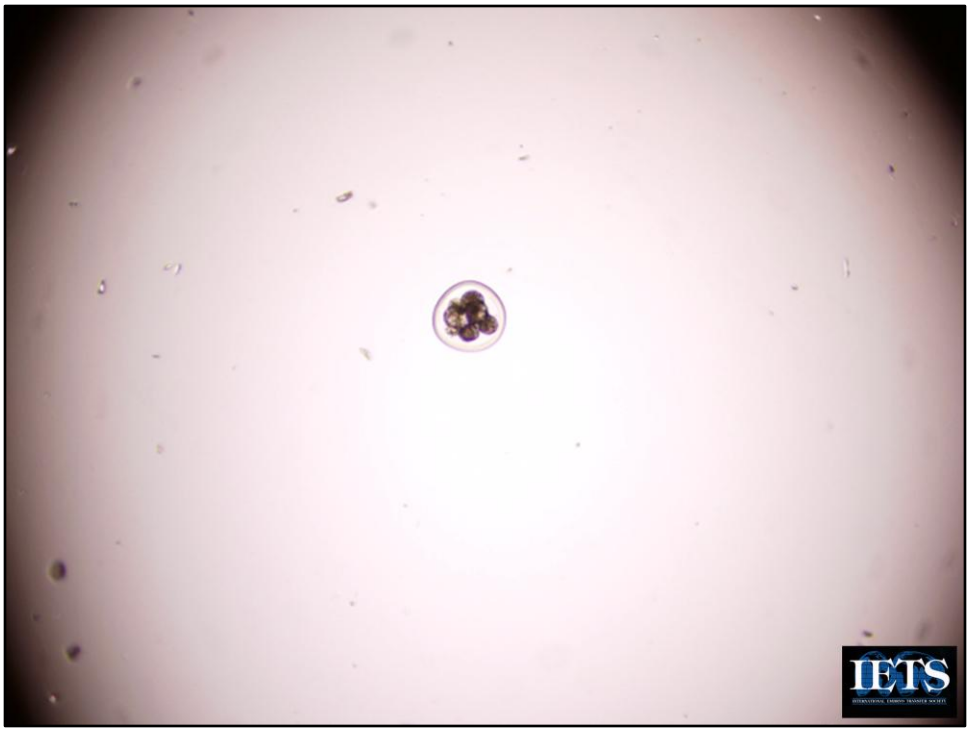
200x BF. Degenerated embryo. This is a multicellular embryo with blastomeres of varying sizes. A blastomere can be considered dead at day 7 post estrus if it is isolated (not fused with another cell) and visible as a small sphere. A healthy blastomere should be fused with one or more blastomeres creating a small mass of intercommunicating cells. It is helpful for an embryologist to observe an embryo like this in three dimension instead of one dimension like in this microphotograph. That can be done by shaking or lightly swirling the Petri dish causing the embryo to "roll" allowing a more thorough visual perspective. When rolled, this embryo had mostly separated unfused cells that would be considered dead or non-viable. There are a few cells (blastomeres) in the middle of the mass that makes it extremely difficult to diagnose whether or not they are alive or functional. As a rule, embryos like this one should be discarded. However, a small percentage of embryos like this will produce a live calf if transferred fresh, but only if a few of the blastomeres are fused and alive. Rolling the embryo is the key to proper diagnosis. The following slide of the same embryo has arrows pointing to the individual blastomeres.



200x BF. Degenerated embryo. It should be noted that a few of the blastomeres in this embryo are much larger than others. These cells likely died during early cleavage, for instance at the 4 cell stage. There are other cells behind the middle mass in a different plane. Those cells should be observed before a final diagnosis is made.

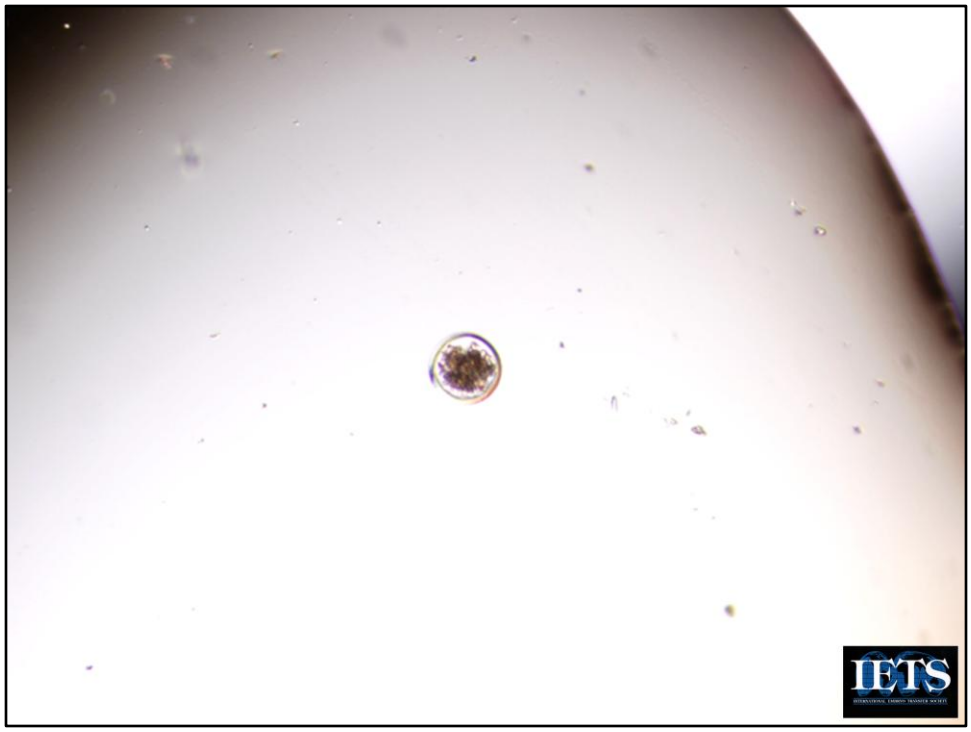


200x BF. 4 Cell Degenerated embryo. Two of the four blastomeres have an intact VM (see animation). The two blastomeres at the top are degenerating and dispersing their ooplasm into the perivitelline space.

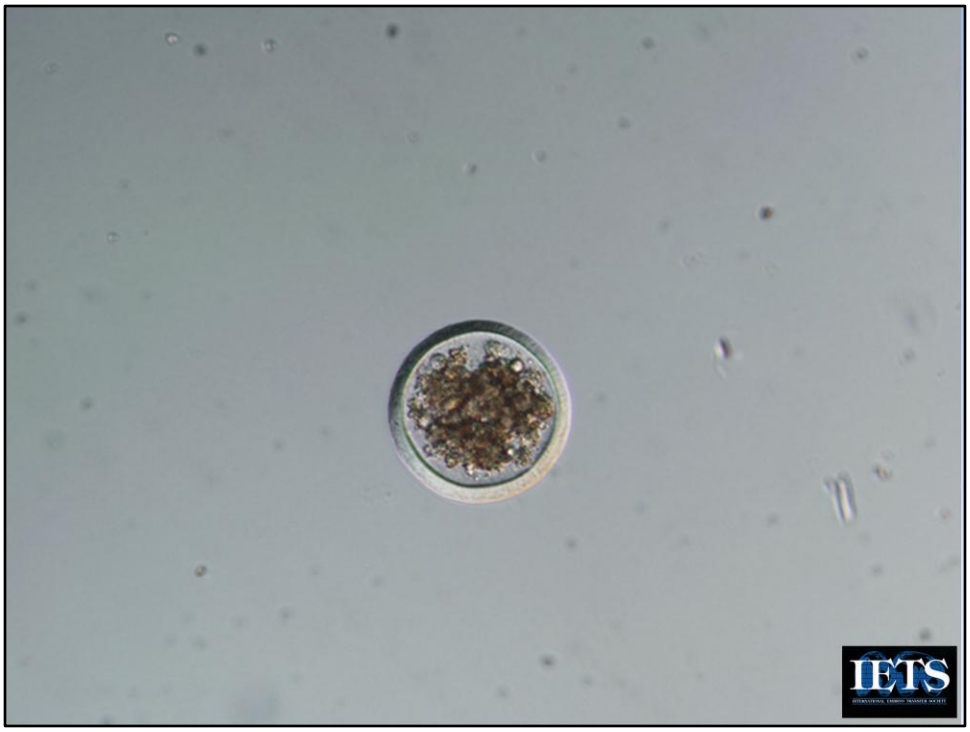


40x BF. 8 cell degenerated embryo.

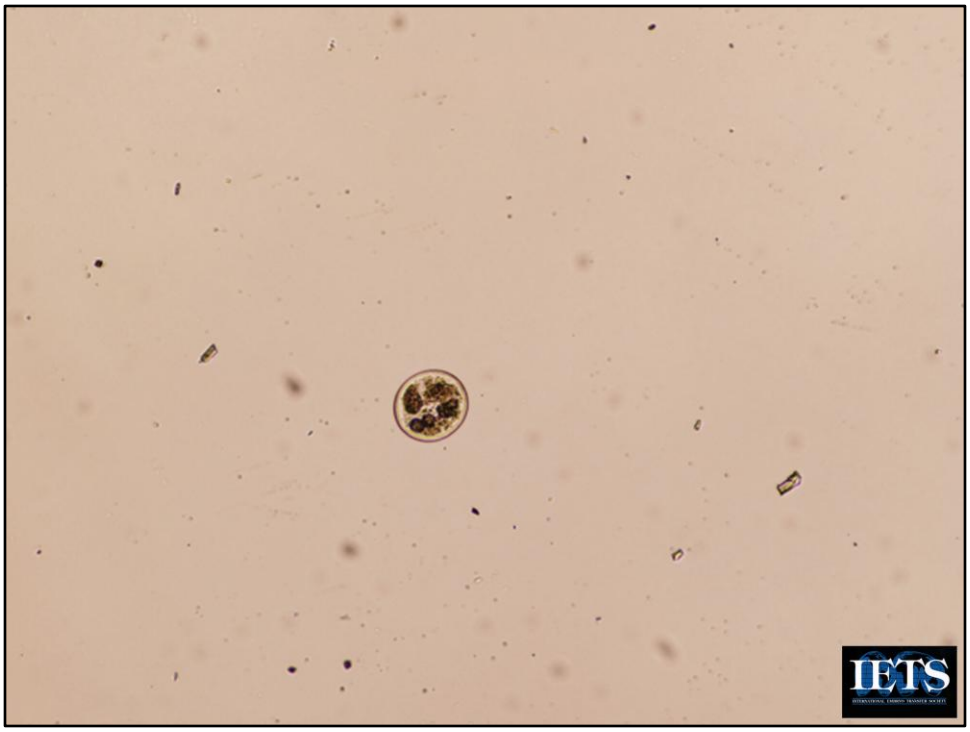
100x DIC. 8 cell degenerated embryo. Note that none of the cells are fused.



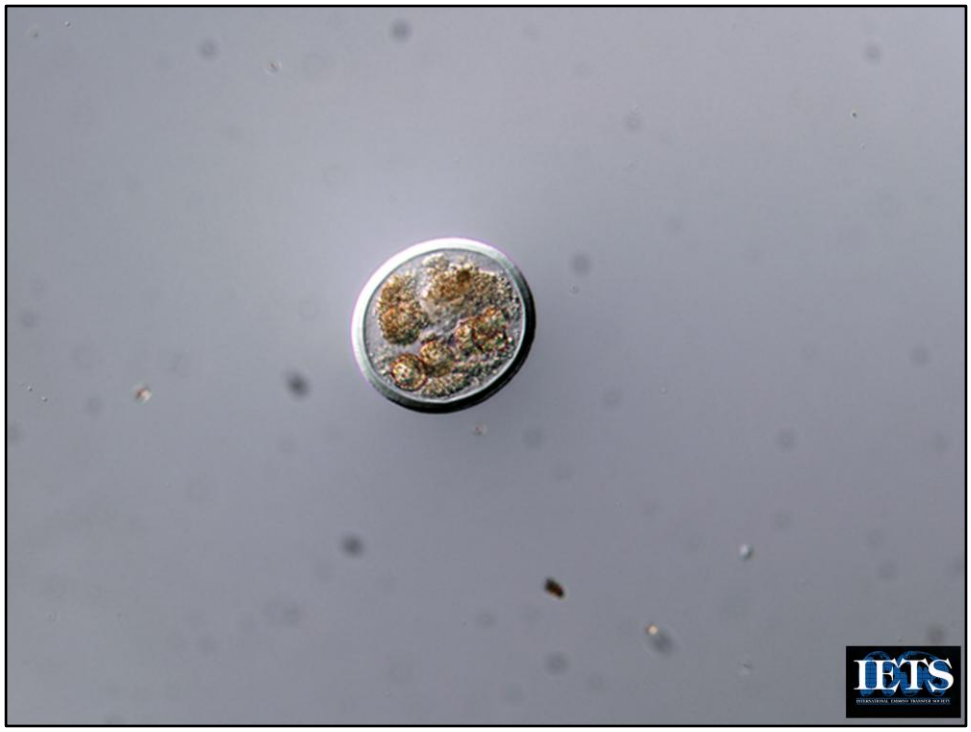
40x BF. Multi-cell degenerated embryo. At this magnification it is somewhat difficult to make an accurate diagnosis without rolling the embryo. It's possible that some of the cells in this embryo are fused. If so, this embryo would be diagnosed as stage 3 (early morula) grade 3 (poor quality).



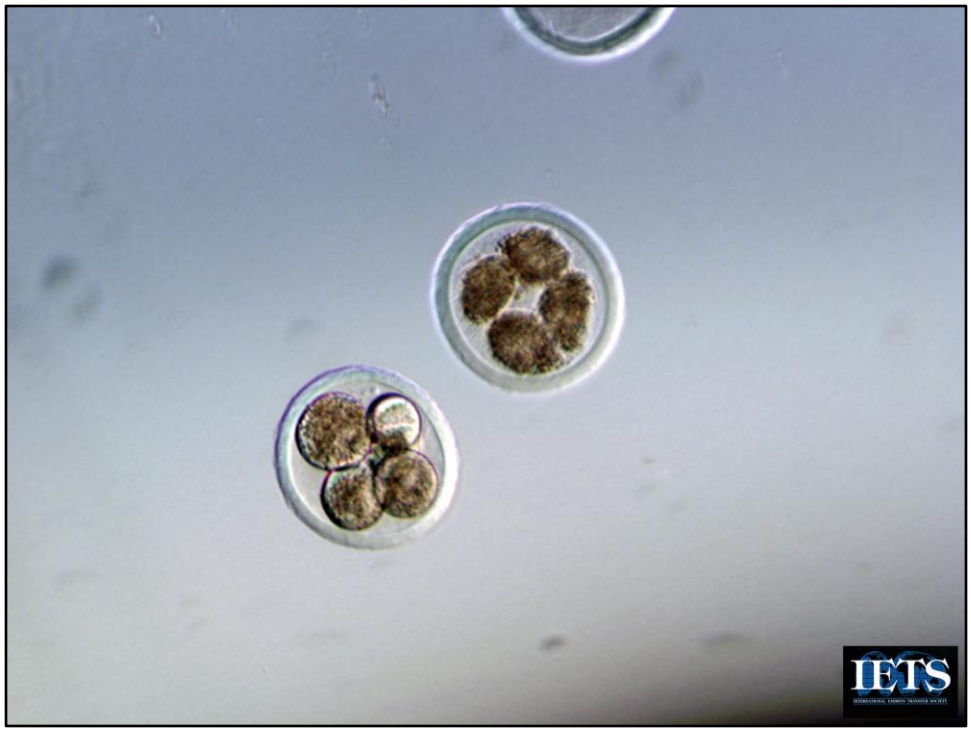
100x DIC. Multi-cell degenerated embryo. Same slide as previous embryo. This embryo does not have an intact VM. The cells at perimeter are very small and unfused.



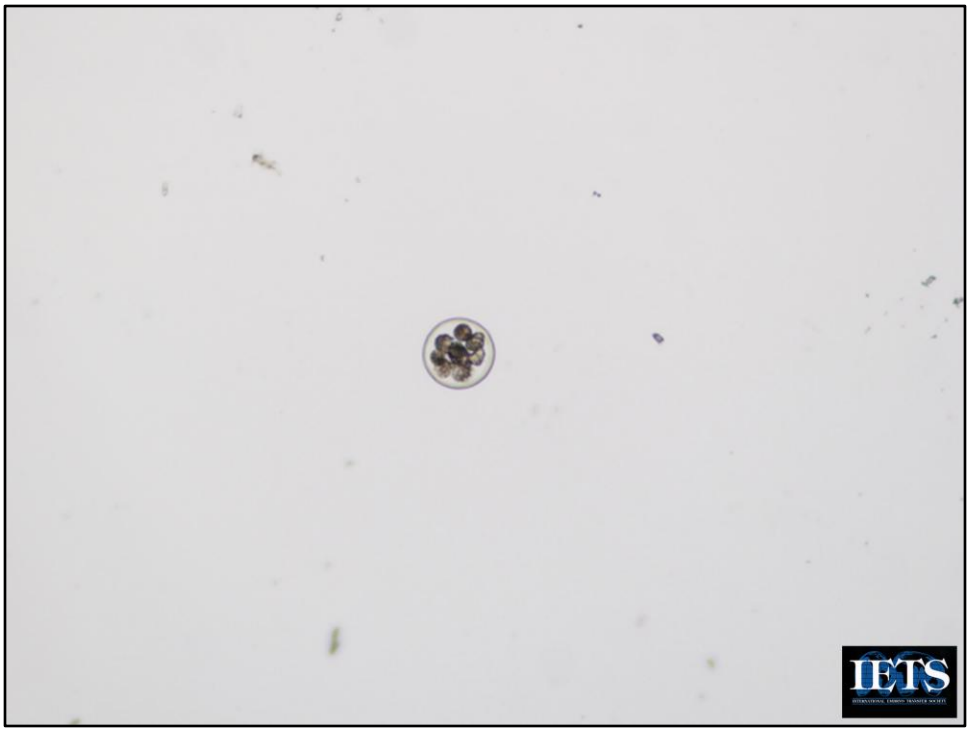
40x BF. Degenerated embryo. This embryo does not have an intact VM surrounding two or more blastomeres. It is also possible that this ova is a UFO with a fragmented cytoplast. This embryo likely died at the 8 cell stage if it was fertilized.



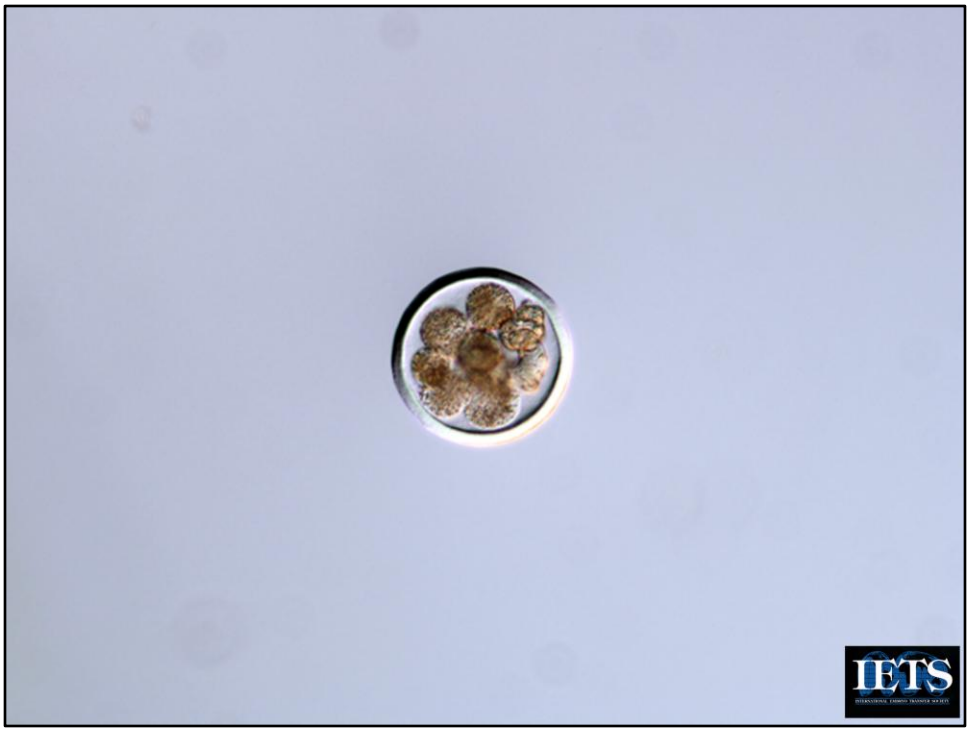
100x DIC. Same embryo as previous slide at higher magnification. There is no evidence of any fused healthy cells in this degenerated embryo.



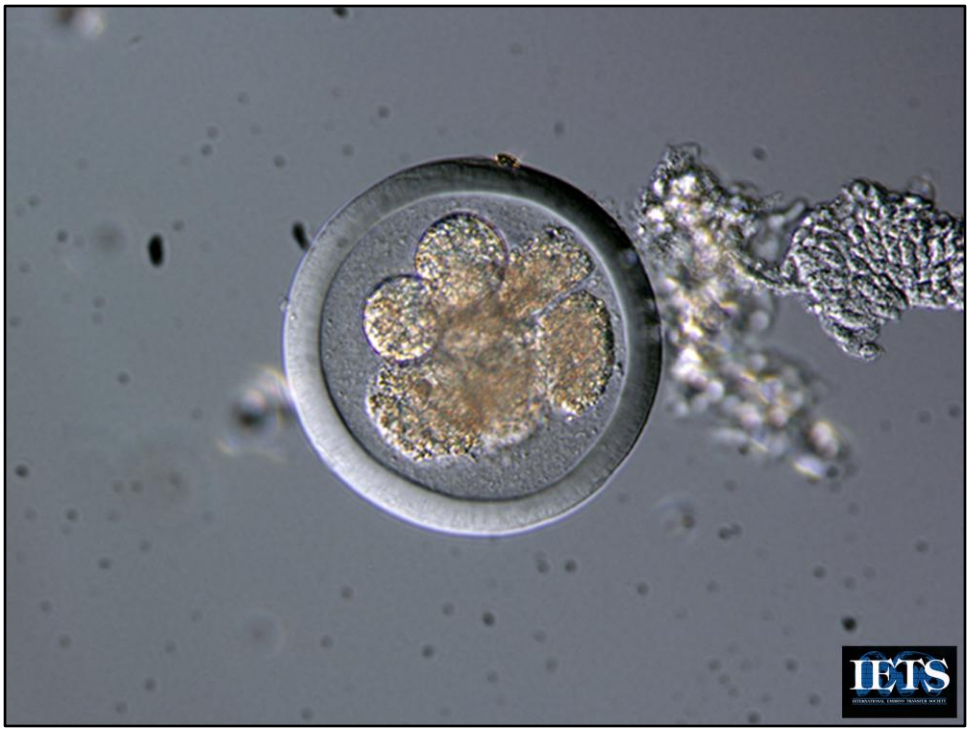
100x DIC. This slide has two 4 cell degenerated embryos recovered from the same donor collection (flush). The lower left embryo is most likely fertilized and degenerated. Notice that each blastomere has an intact plasma membrane completely surrounding it. However, the upper embryo could be either degenerated or a one cell (UFO) with ooplasm fragmented into 4 equal pieces. Either way, both embryos are dead.



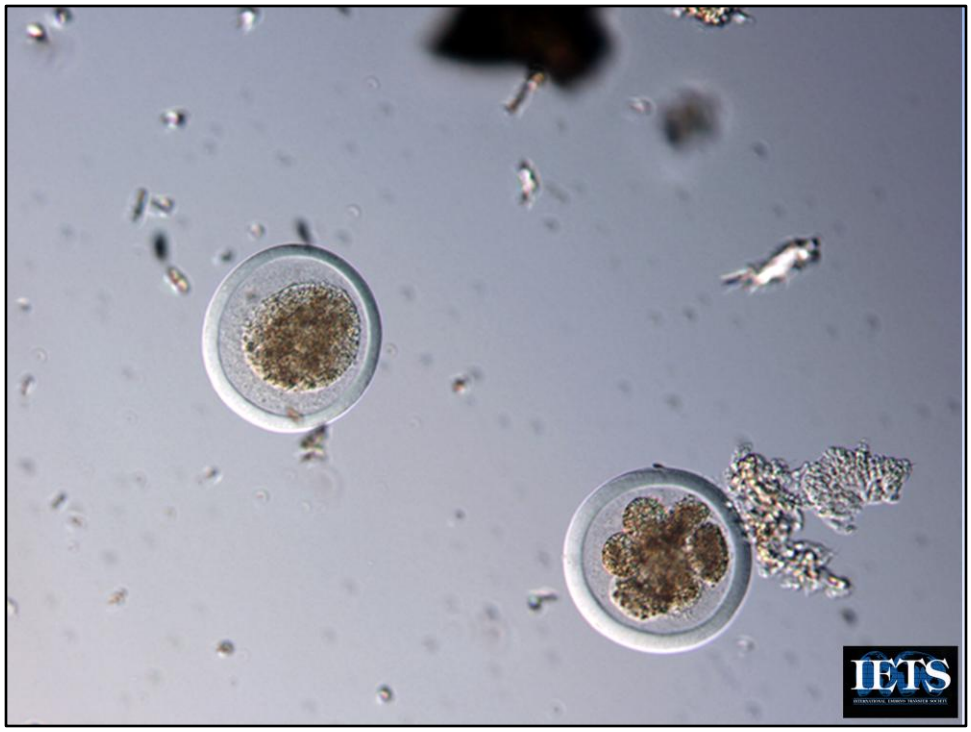
40x BF. 8 cell degenerated embryo. Notice how none of the blastomeres are fused. The perimeter of each blastomere is visible.



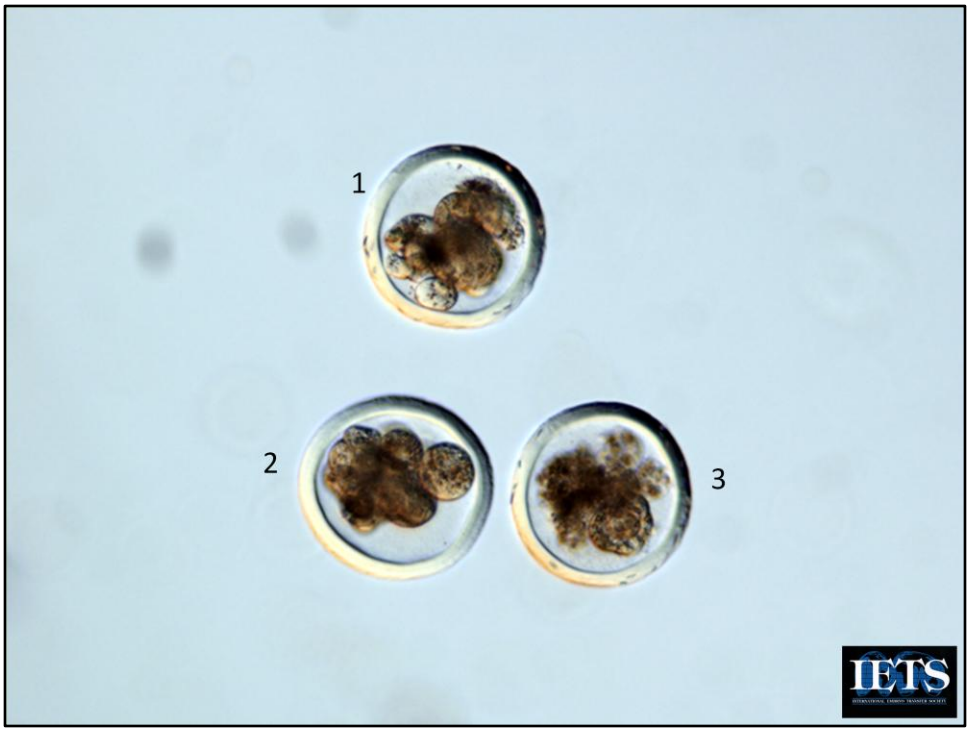
100x DIC. Same 8 cell degenerated embryo as previous slide at higher magnification.



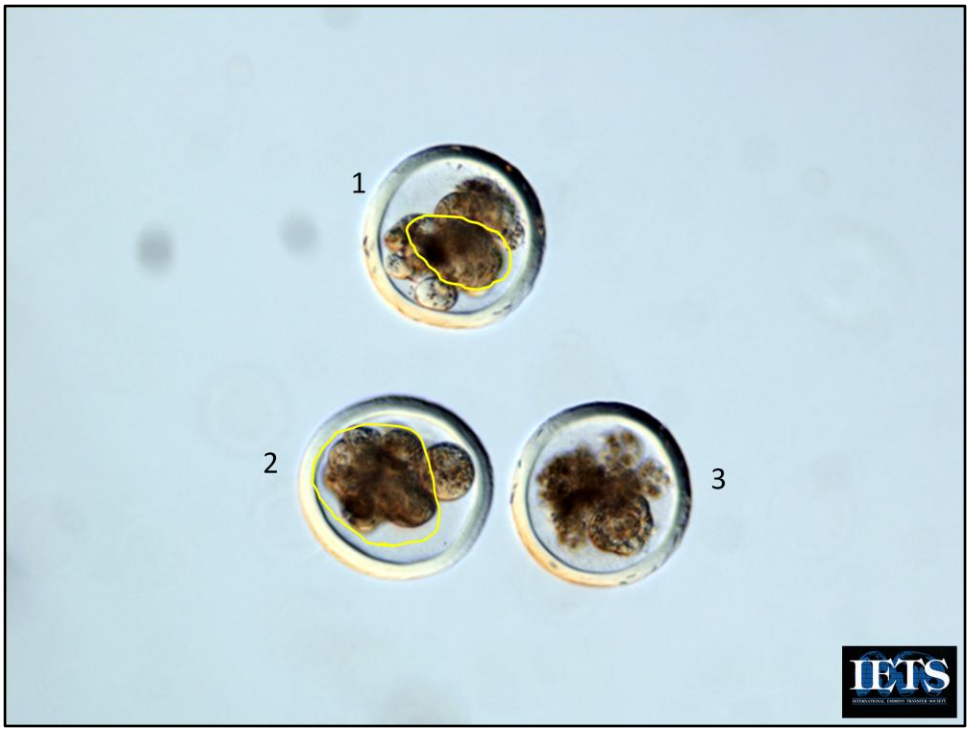
200x BF. 8 cell degenerated. All 8 cells can't be seen in this plane, but rolling the ovum allows the blastomeres on the back side to be visible. The mass to the immediate right of this degenerated embryo is uterine endometrial cells that are sticking to the zona.



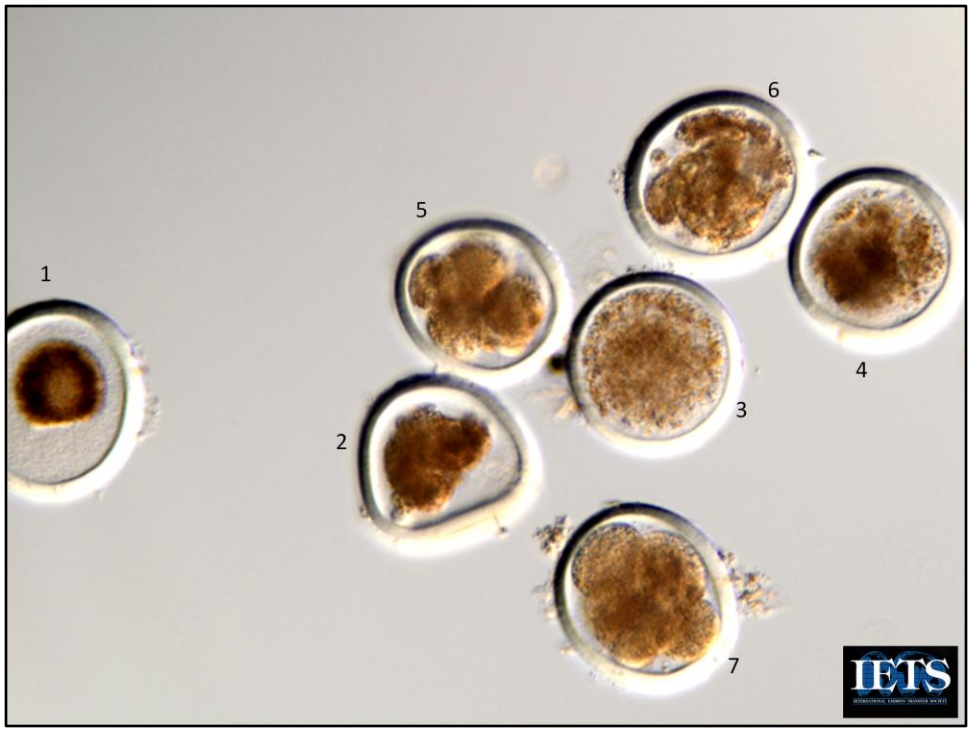
100x DIC. The ovum on the left is a UFO. The one on the right is an 8 cell degenerated embryo.



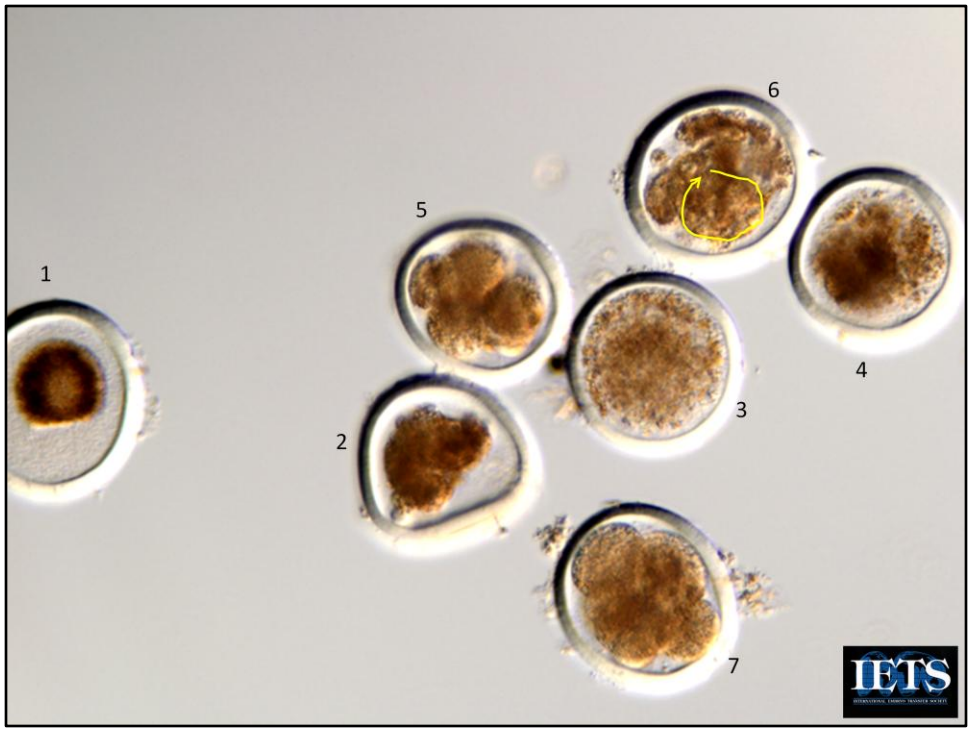
100x DIC. At first glance, these ova/embryos appear to all be degenerated. However, embryo #1 possibly has a few fused blastomeres (see animation on next slide). There are a couple of extruded dead blastomeres at 7 to 8 o'clock. #2 is fertilized with one round extruded blastomere at 3 o'clock. The remaining mass has a few fused cells (blastomeres) that give this embryo a chance to survive if transferred fresh. Considering that this embryo was collected 7 days post estrus, it is well behind in development. It appears that there are 4 to 6 fused cells in this mass. #3 is a fertilized degenerated embryo. It has about 4 cells. One interesting note about #1 and #3 is the sperm entrapped in the zona pellucida.



100x DIC. Same slide as previous with animation. At first glance, these ova/embryos appear to all be degenerated. However, embryo #1 possibly has a few fused blastomeres (see animation). There are a couple of extruded dead blastomeres at 7 to 8 o'clock. #2 is fertilized with one round extruded blastomere at 3 o'clock. The remaining mass has a few fused cells (see animation) that give this embryo a chance to survive if transferred fresh. Considering that this embryo was collected 7 days post estrus, it is well behind in development. It appears that there are 4 to 6 fused cells in this mass. #3 is a fertilized degenerated embryo. It has about 4 cells. One interesting note about #1 and #3 is the sperm entrapped in the zona pellucida.



100x DIC. #1 is a UFO with a condensed ooplasm and intact VM. #2 is a UFO with a slightly irregular shaped cytoplast/ooplasm. Its zona is not perfectly spherical. #3 is a UFO with a degenerated VM and degenerated dispersed cytoplast. #4 is a UFO with a partially degenerated VM and ooplasm. #5 is a fertilized 4 cell degenerate. #6 is Stage 3, Grade 3. It has a few fused blastomeres at 6 o'clock (yellow circle next slide) and several small blastomeres beginning to undergo degeneration. #7 is another 4 cell degenerated embryo.



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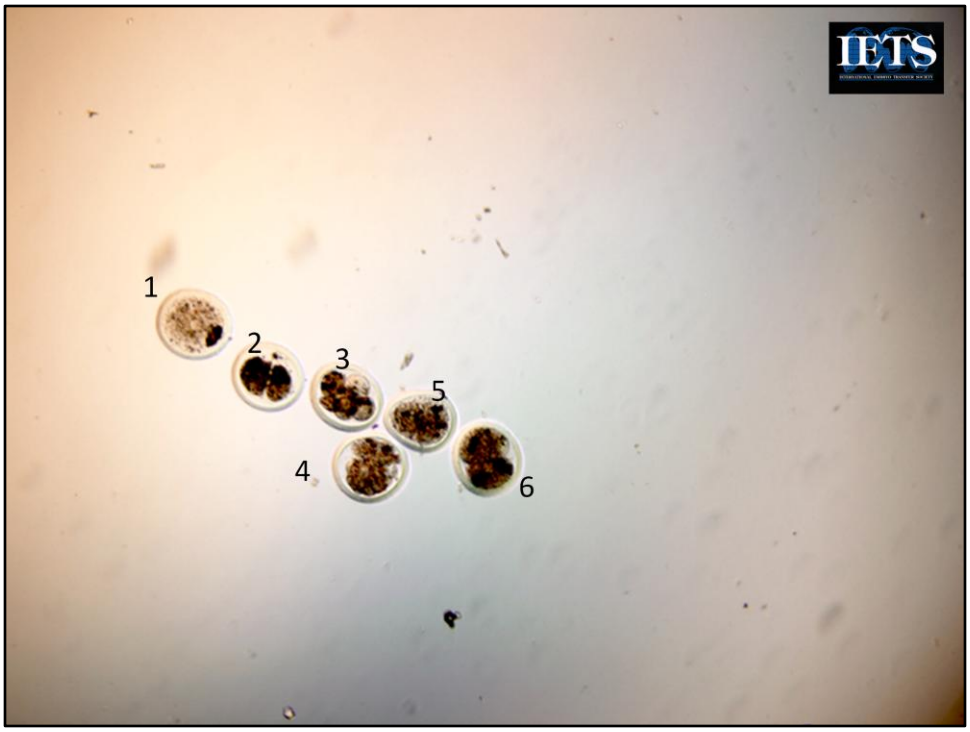
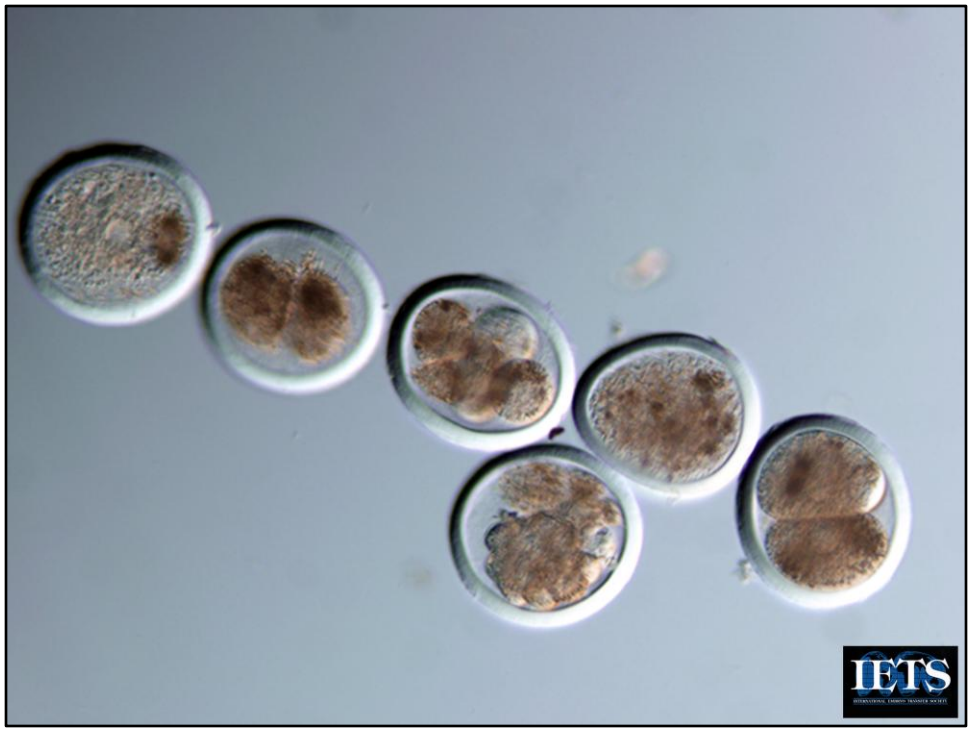
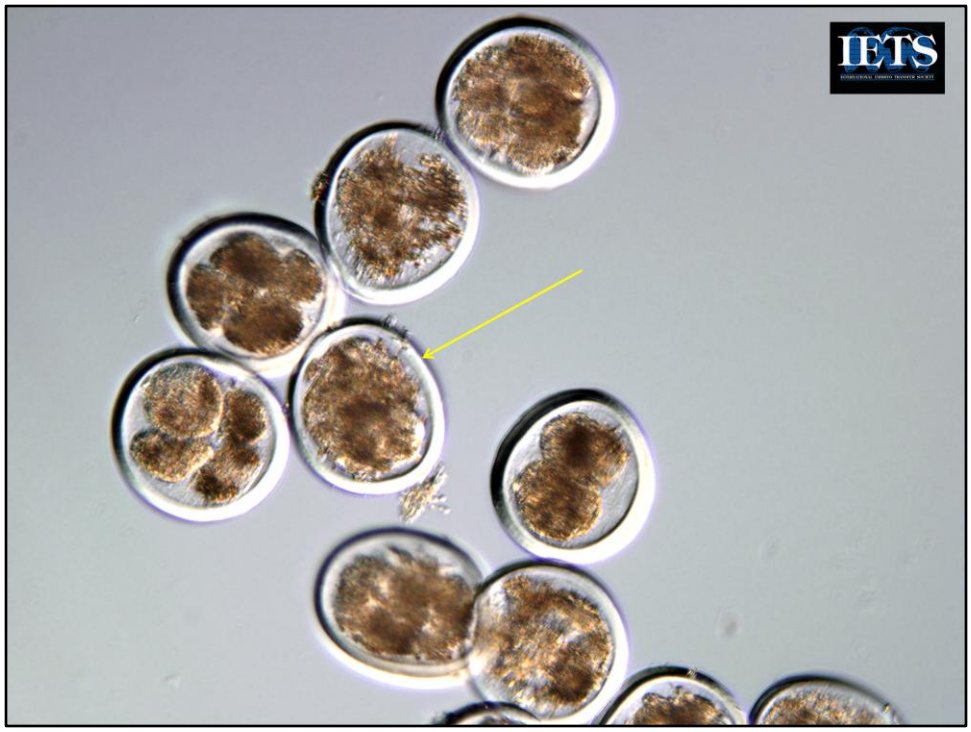


40x BF. #1 is a UFO with a dispersed degenerated cytoplast. #2 is a UFO with a fragmented cytoplast. #3 is an 8 cell degenerated embryo. #4 is a morula Stage 4, Grade 2 . The mass on the lower half consists of healthy fused blastomeres. The healthy mass makes up slightly over half of the total cell mass in this embryo. The extruded cells at the top are dead. #5 is a UFO with a degenerated ooplasm. #6 is a 2 cell degenerated embryo.

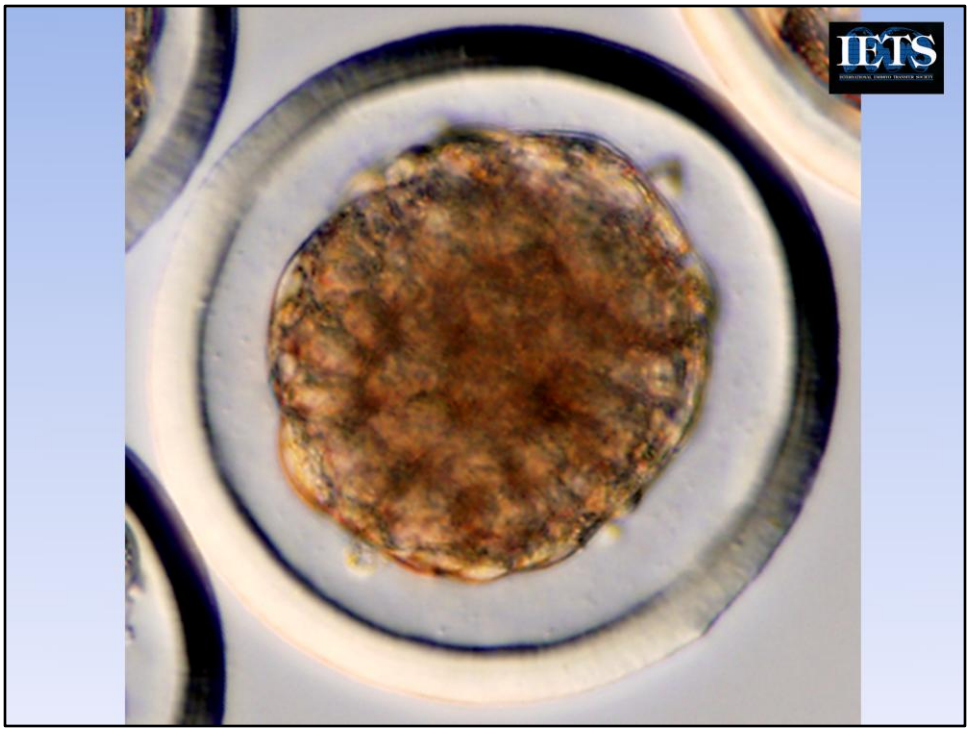


100x DIC of previous slide. #1 is a UFO with a dispersed degenerated cytoplast. #2 is a UFO with a fragmented cytoplast. #3 is an 8 cell degenerated embryo. #4 is a morula Stage 4, Grade 2 . The mass on the lower half consists of healthy fused blastomeres. The healthy mass makes up slightly over half of the total cell mass in this embryo. The extruded cells at the top are dead. #5 is a UFO with a degenerated ooplasm. #6 is a 2 cell degenerated embryo.

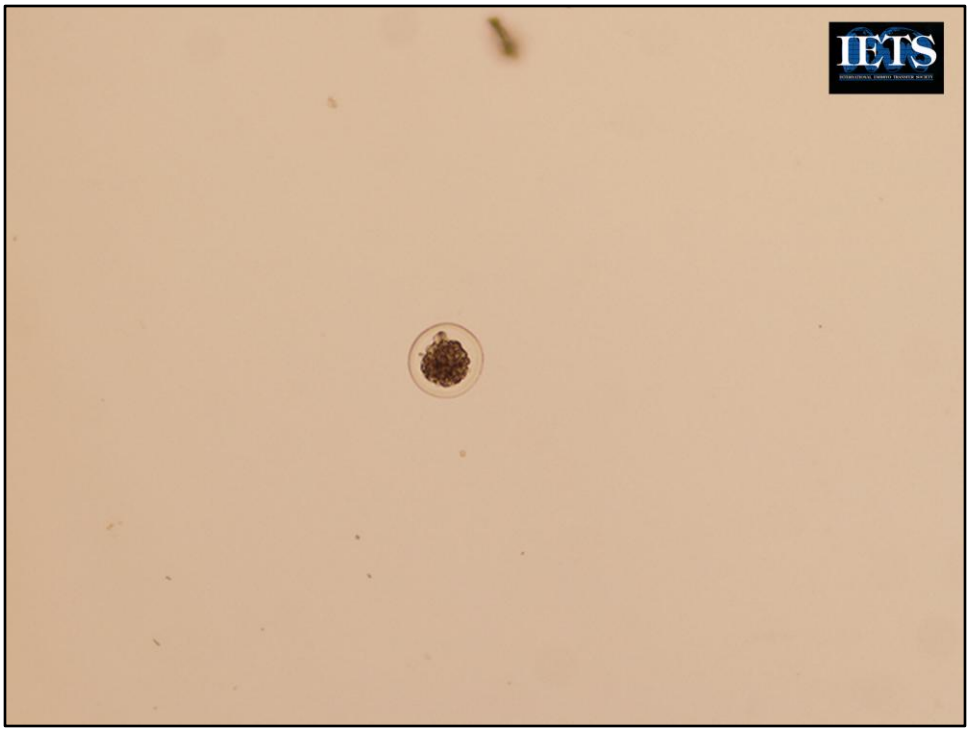


100x DIC. With the possible exception of one embryo (arrow), this group of embryos are all fertilized degenerated. The embryo with the arrow touching it is a viable embryo. It needs to be rolled for further verification, but in this plane it appears to be a Stage 4, Grade 2.

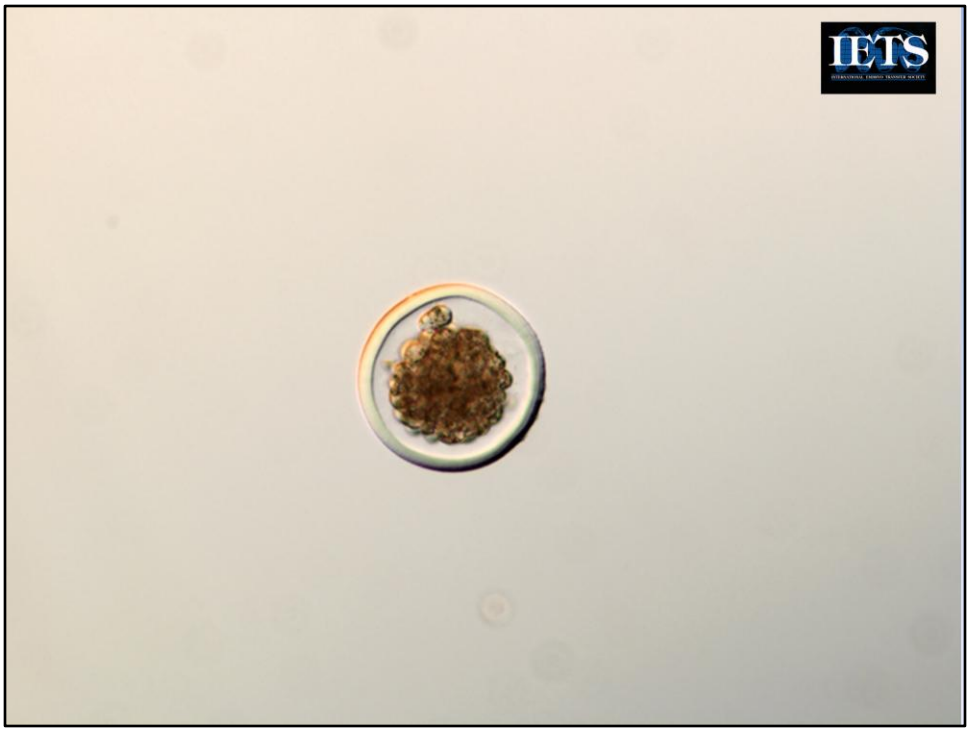
Viable Embryos



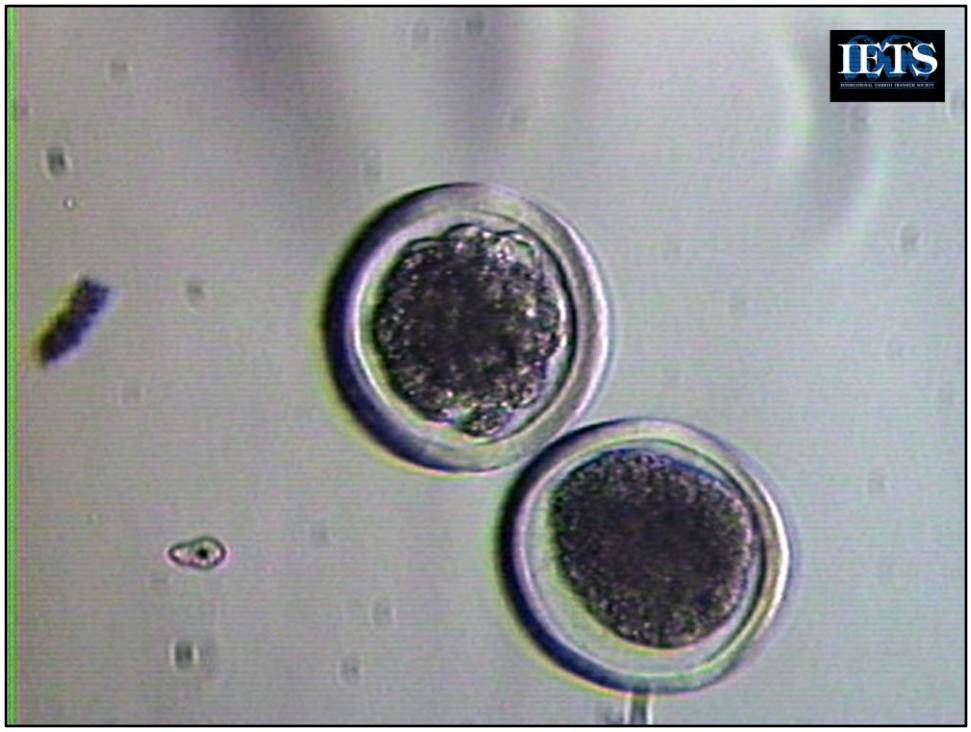
400x DIC. Stage 4, Grade 1. This is an anatomically near perfect day 7 morula. There are very few, if any, extruded blastomeres outside the VM. The differentiating factor between this and a UFO is the bulging of the blastomeres in this embryo causing the VM to be irregular, yet contiguous and visible. Close inspection of the embryo proper allows one to see a large mass of fused blastomeres.



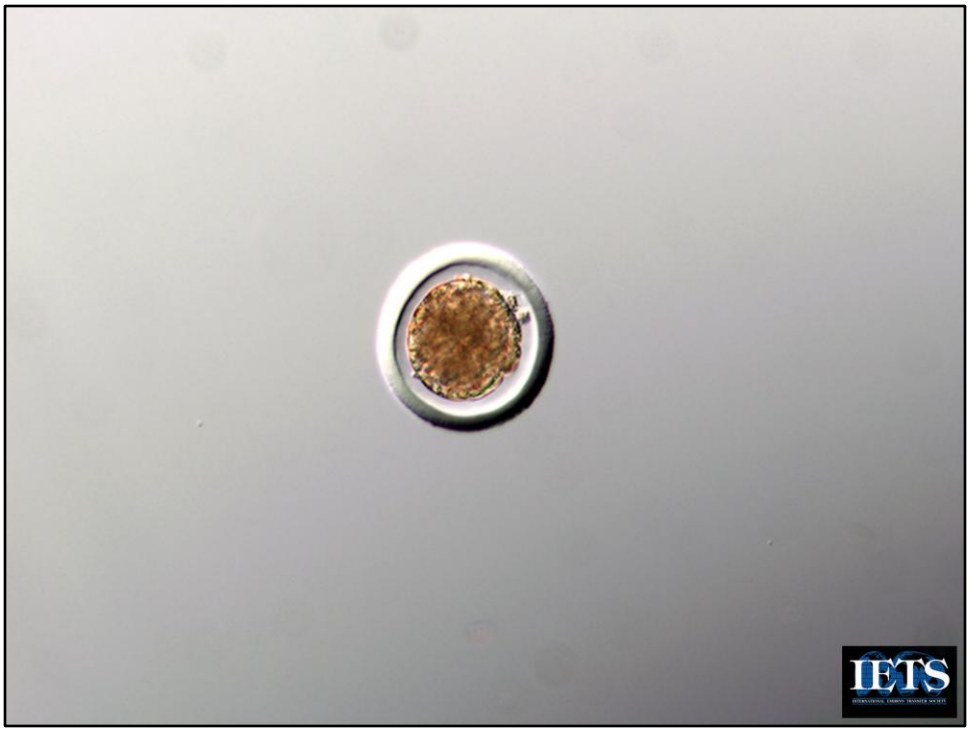
40x BF. Stage 4, Grade 1. This morula has one extruded blastomere at 12 o'clock. It 's an excellent quality embryo. It can be transferred fresh or will freeze/thaw with excellent results.



100x DIC. This is the same embryo as the previous slide at higher magnification. Notice the irregular surface of the VM caused by the small bulging blastomeres.

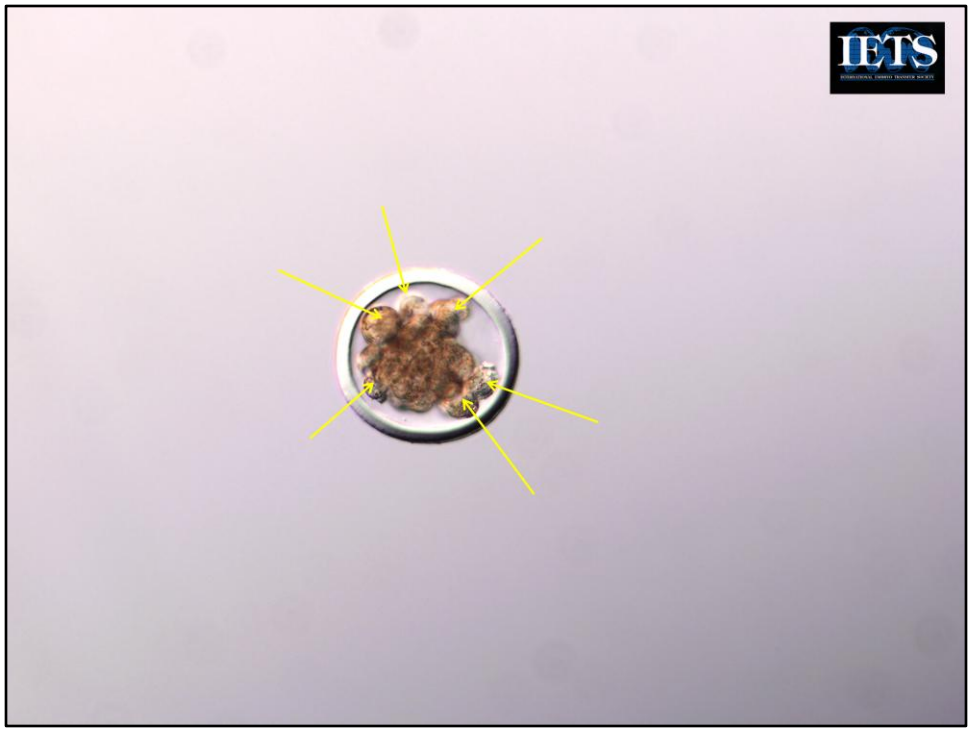


200x BF. The top embryo is a morula (Stage 4, Grade 1), and the bottom ovum is a UFO. Notice the small blastomeres protruding from the VM in the top embryo, and the smooth VM surrounding the ooplasm in the UFO.

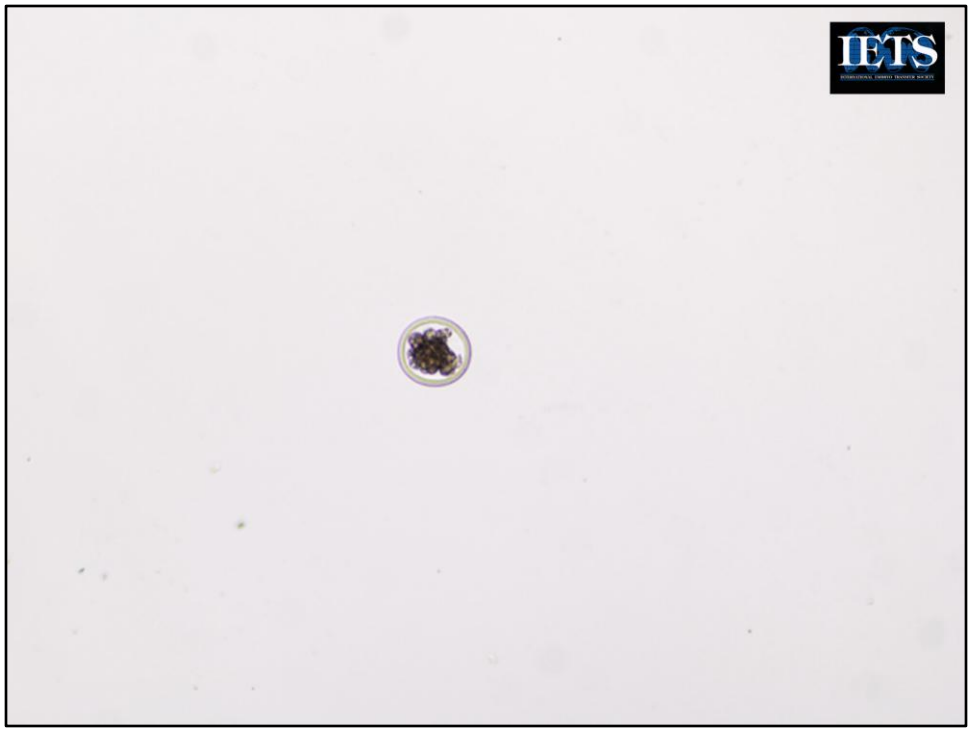


100x DIC. Stage 4, Grade 1. The VM on this near perfect morula is almost as smooth as that of a UFO. Morula like this can be confused with being a UFO. The key is to see the blastomeres with an occasional bulge into the VM. This one has a slight bulge at about 4 o'clock. It also has two tiny extruded blastomeres at 2 o'clock.

40x BF. Stage 4, Grade 2. This embryo has several extruded blastomeres that downgrade it from a Grade 1 to a Grade 2.



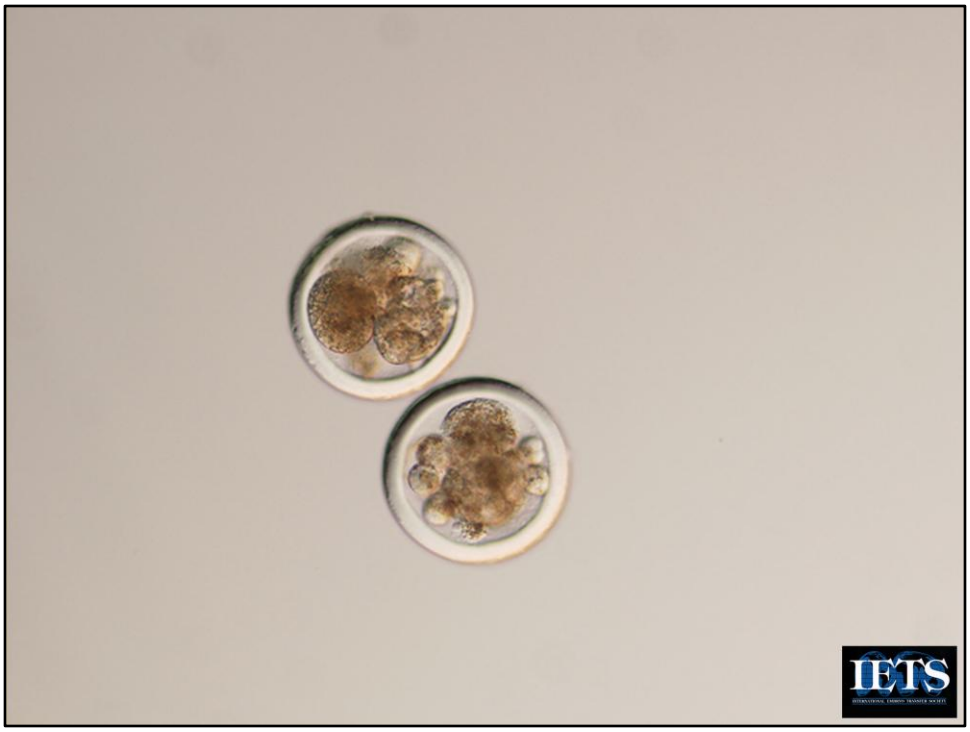
100x DIC. Stage 4, Grade 3. This morula has several extruded unfused cells (arrows) in this plane, plus a few others as it was observed while rolling. The healthy fused mass in the middle represents about half of the total blastomeres.



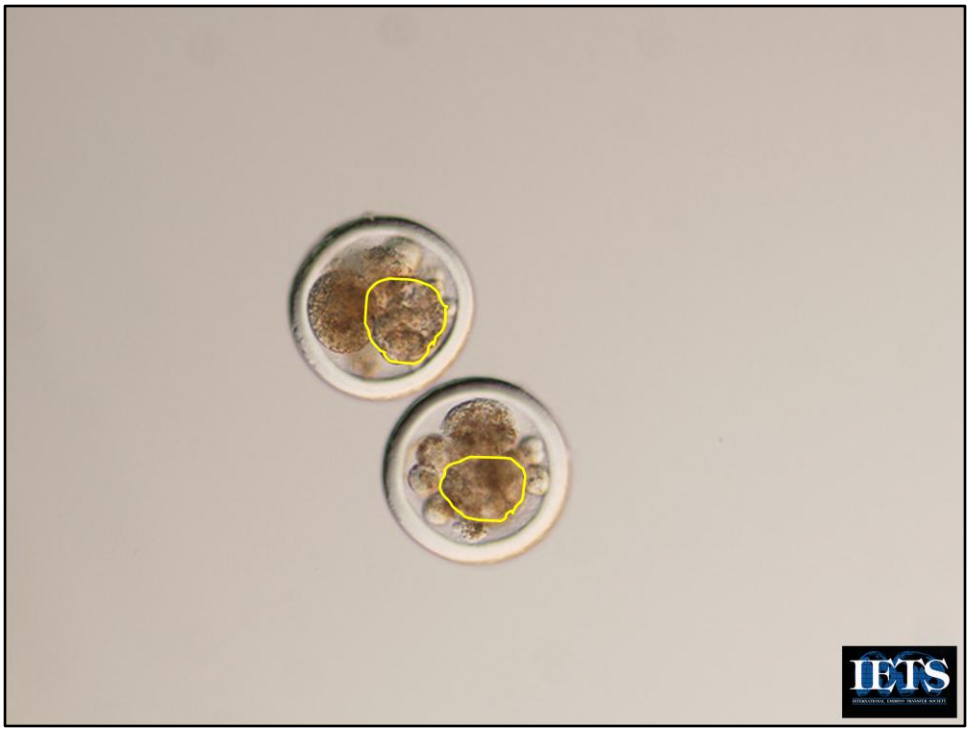
40x BF. Same embryo as previous slide at lower magnification.

40x BF. Stage 3 (early morula), Grade 1. There are only two dead extruded cells in this morula. This embryo was collected 6.5 days post onset of estrus.

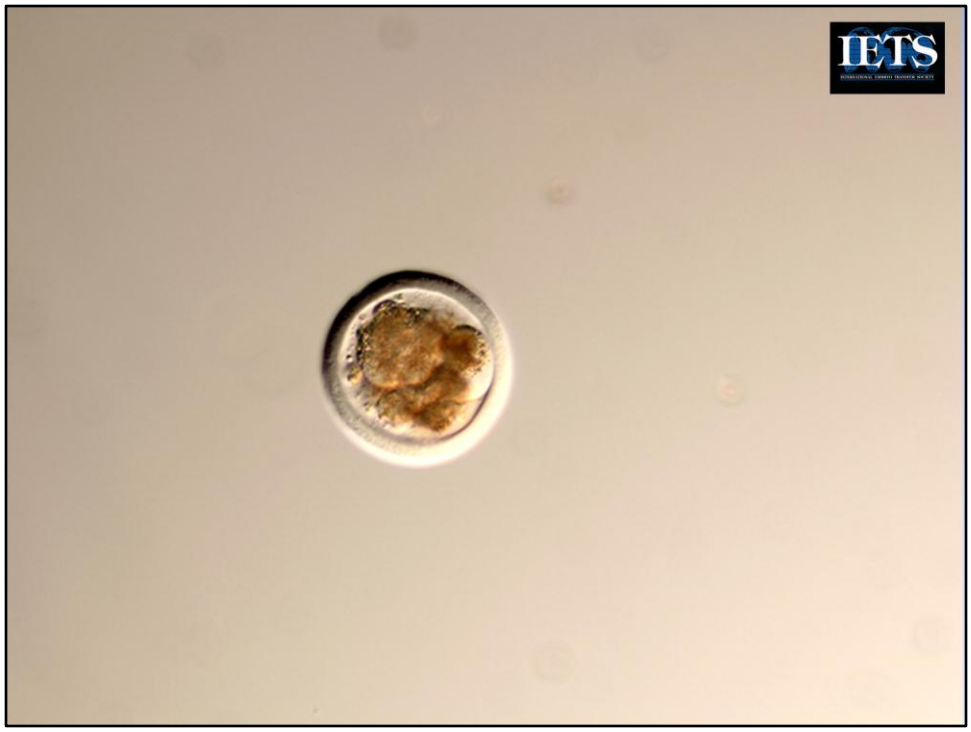
100x DIC. Same embryo as previous slide.



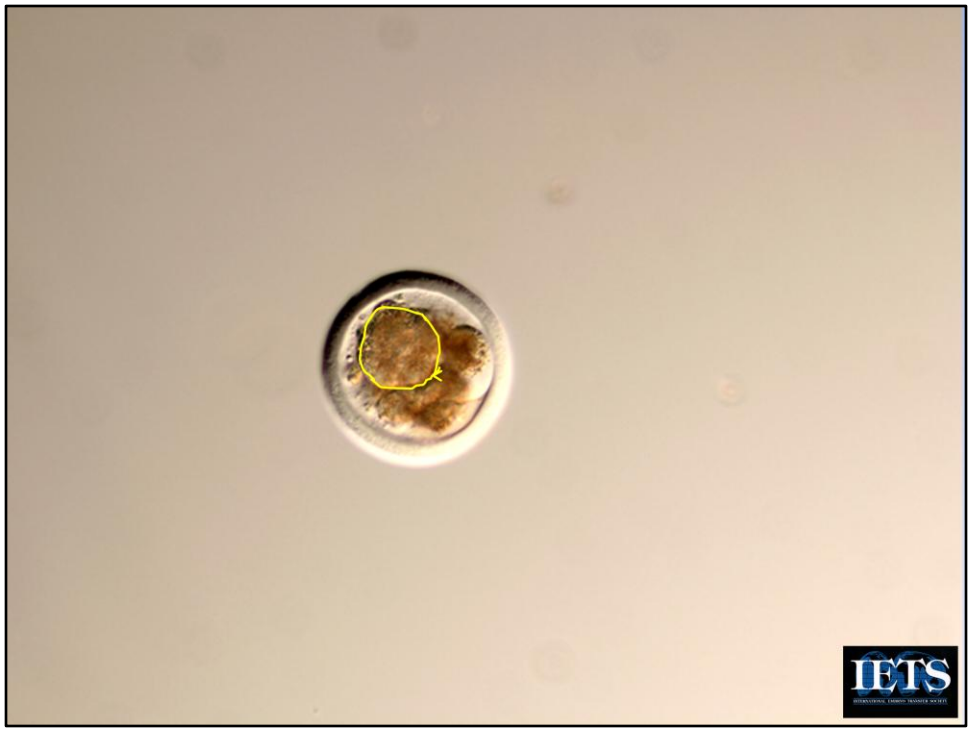
100x DIC. Stage 3, Grade 3. Both of these embryos were collected 7.0 days post estrus. They are both classified as early morulas. The animations encircle the healthy masses (see next slide). The uppermost embryo has a more well defined mass of fused blastomeres than does the lower embryo. The remaining cells (outside the fused masses) are extruded blastomeres. A cell would be considered extruded, or unfused/dead, if 360 degrees of its outer diameter (plasma membrane) can be seen.



100x DIC. Stage 3, Grade 3. Both of these embryos were collected 7.0 days post estrus. They are both classified as early morulas. The animations encircle the healthy masses. The uppermost embryo has a more well defined mass of fused blastomeres than does the lower embryo. The remaining cells (outside the fused masses) are extruded blastomeres. A cell would be considered extruded, or unfused/dead, if 360 degrees of its outer diameter (plasma membrane) can be seen.



100x DIC. Stage 3, Grade 3. The healthy mass of this early stage morula has approximately 6 to 8 fused blastomeres. This embryo was collected 7.5 days post estrus and should have > 60 cells. The healthy mass is surrounded by the yellow line (see next slide).



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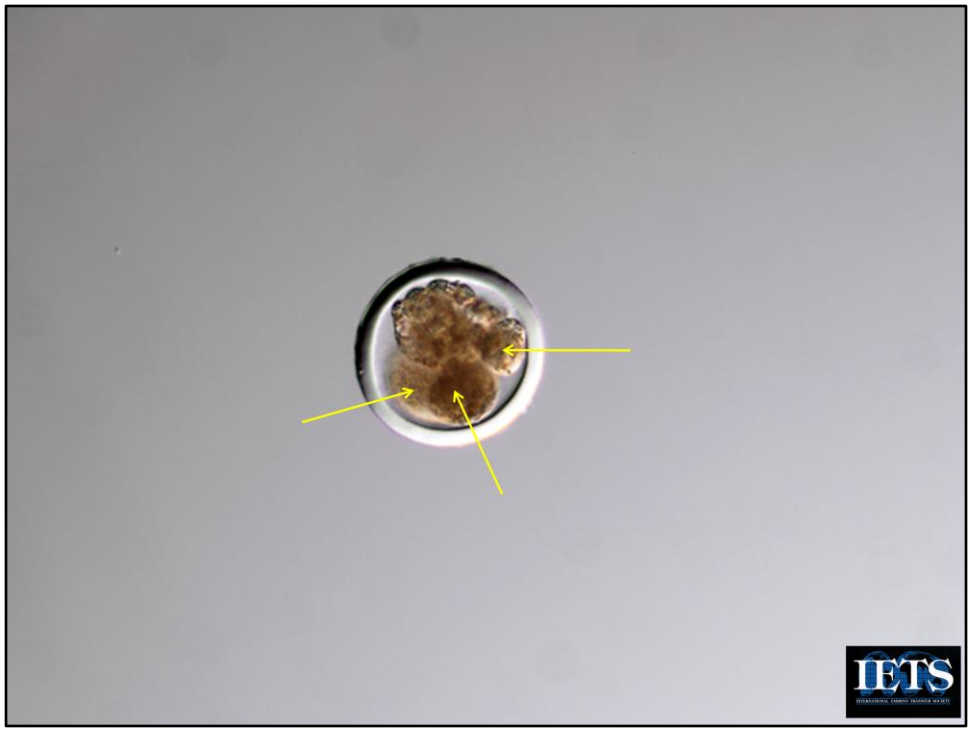
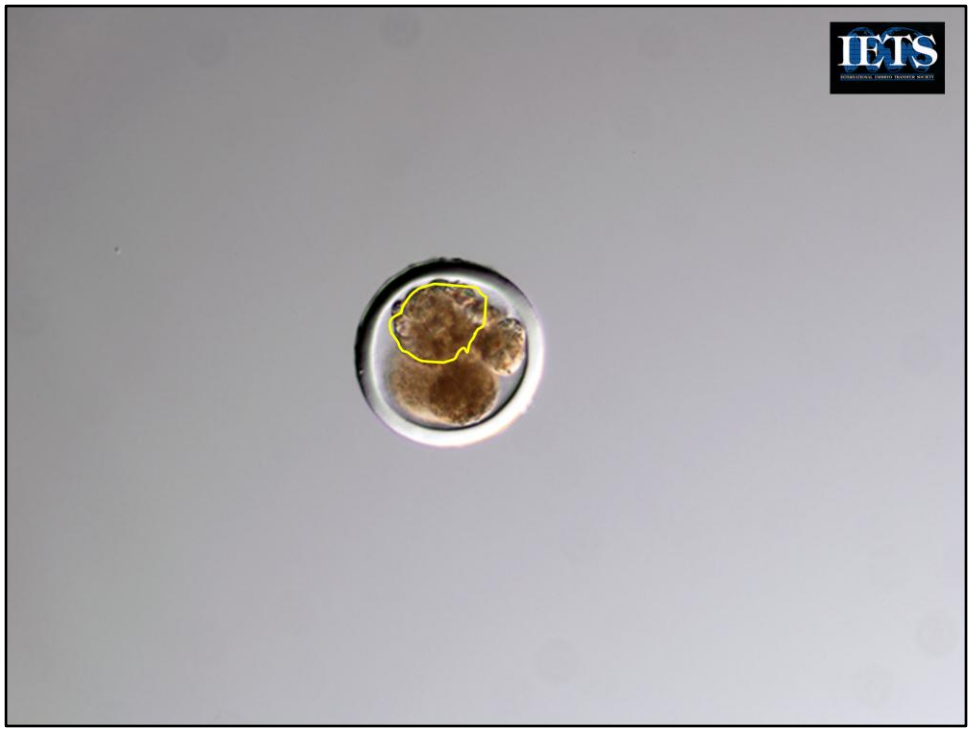
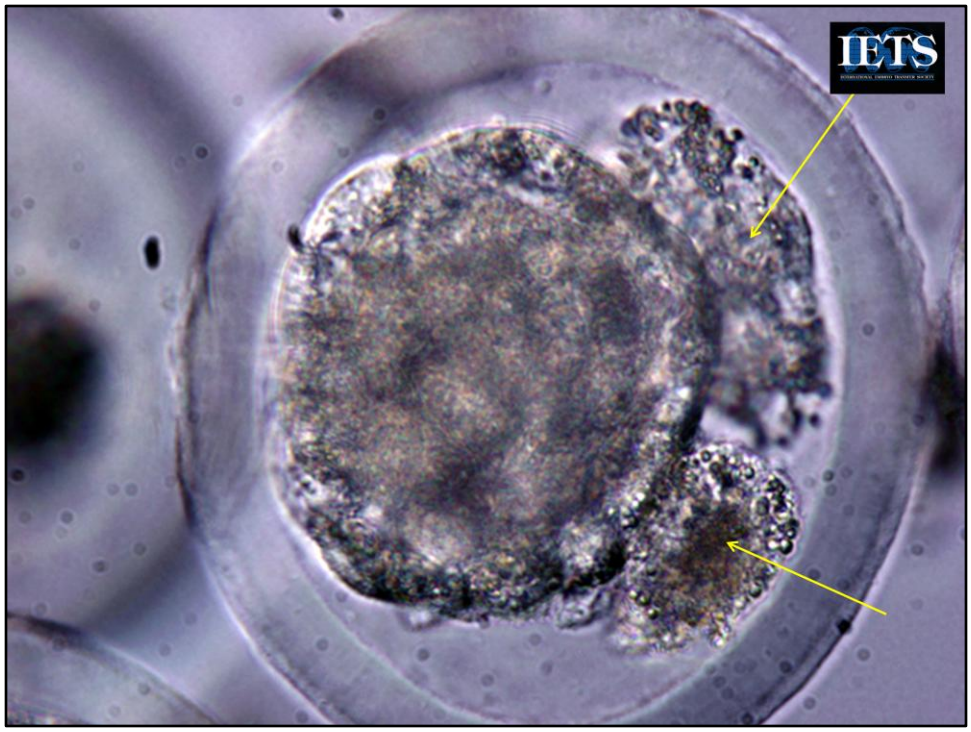


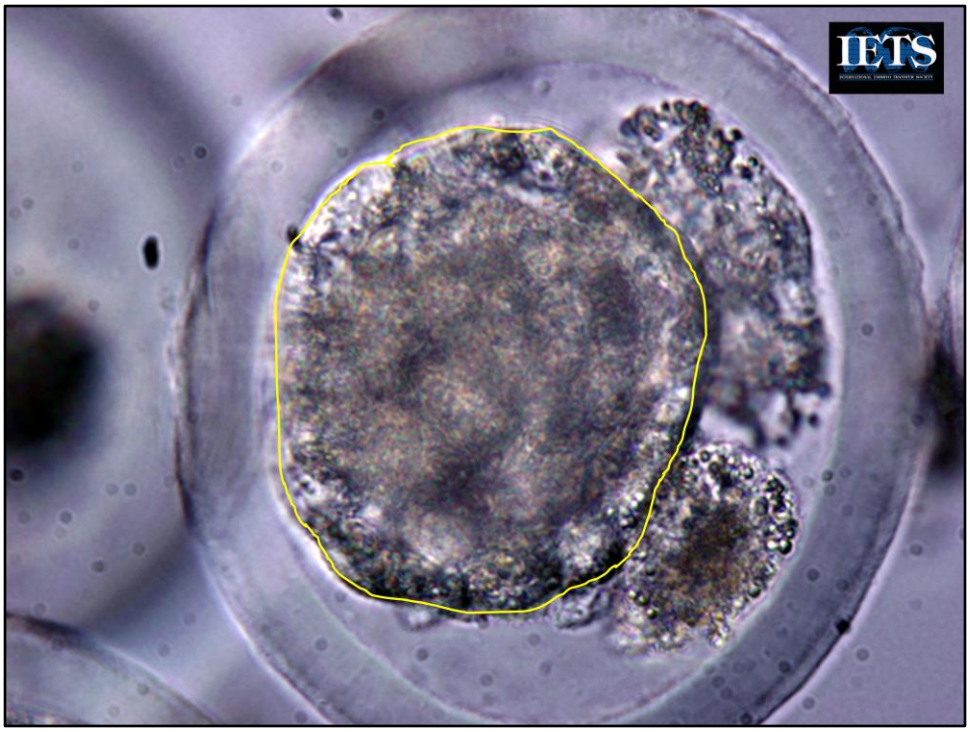
100x DIC. Stage 4, Quality 3. This embryo was collected 7.0 days post onset of estrus. There are three large extruded blastomeres (see yellow arrows) in this plane. Rolling revealed two more dead blastomeres on the opposite side of this embryo. Slightly less than 50% of the embryo is healthy mass (see blue circle next slide).



100x DIC. Stage 4, Grade 3. This embryo was collected 7.0 days post onset of estrus. There are three large extruded blastomeres (see arrows) in this plane. Rolling revealed two more dead blastomeres on the opposite side of this embryo. Slightly less than 50% of the embryo is healthy mass (see yellow circle).



400x BF. Stage 4, Grade 2. Arguably, this embryo could be considered a grade 1 instead of a grade 2. The main mass (circled – see next slide) has a very well defined VM covering a healthy group of well fused blastomeres. In the visible plane there are two large extruded blastomeres. Rolling the embryo revealed two more smaller ones on the back side. This is a good example of the subjectivity involved in grading embryos.



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400x BF. Stage 4, Grade 2. Although the embryo proper (outlined in yellow – see next slide) of this embryo is very good quality, the zona is oblong. This constitutes a 1 step downgrade according to the IETS manual.

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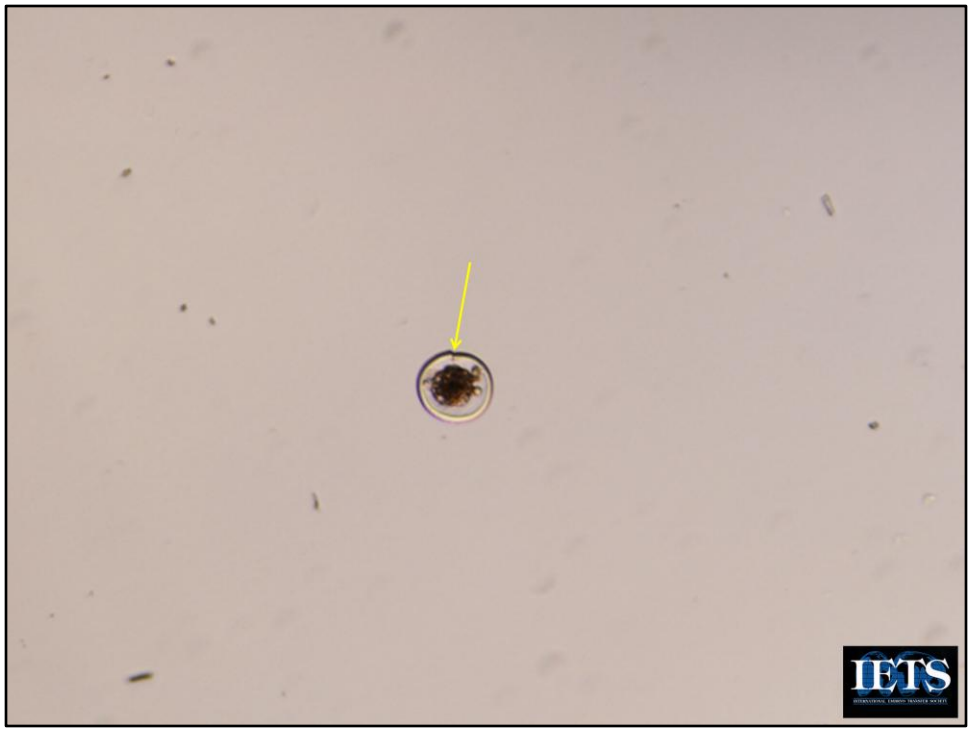
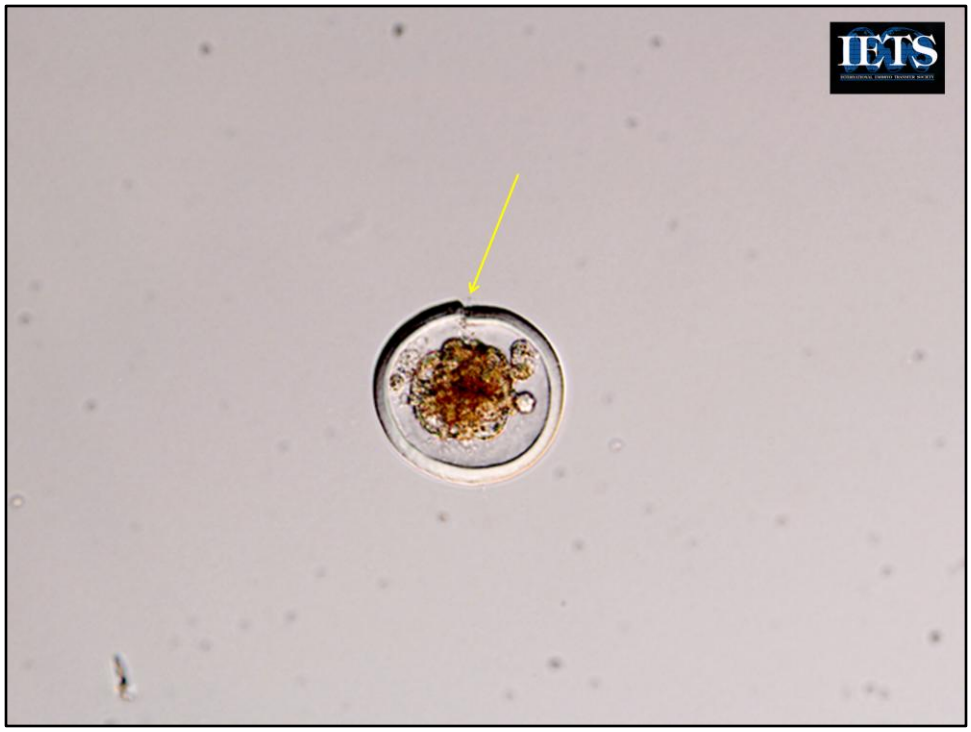
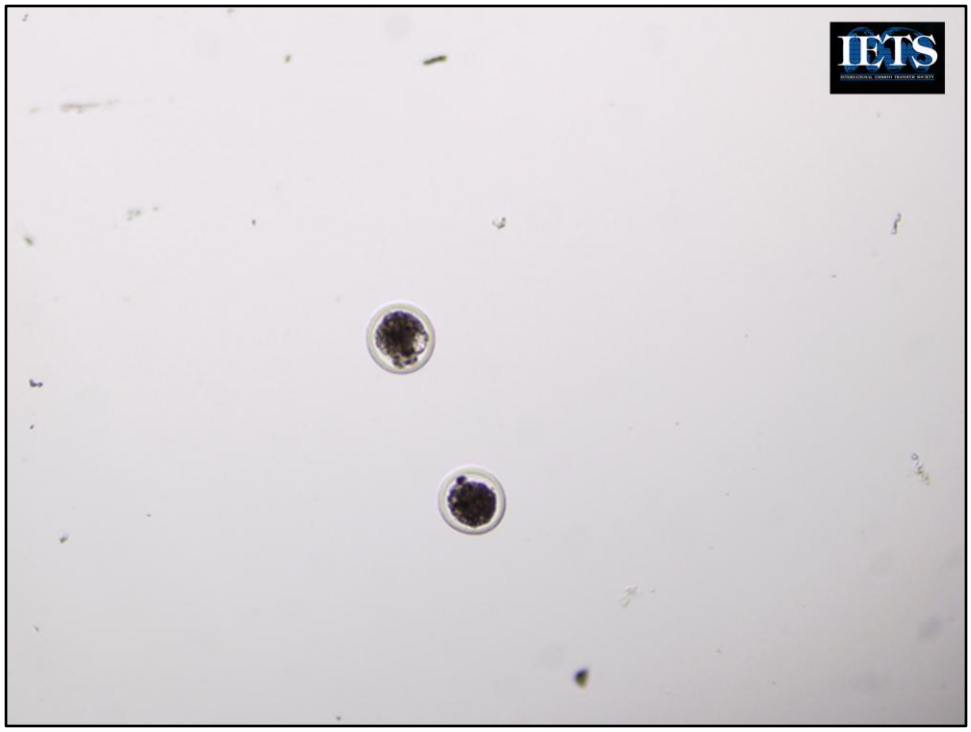


40x BF. Stage 4 Grade 1 with cracked zona. In this plane there are three extruded cells and a very good quality embryo proper. However, the cracked zona would prevent this embryo from being exported. Arrow points to crack.

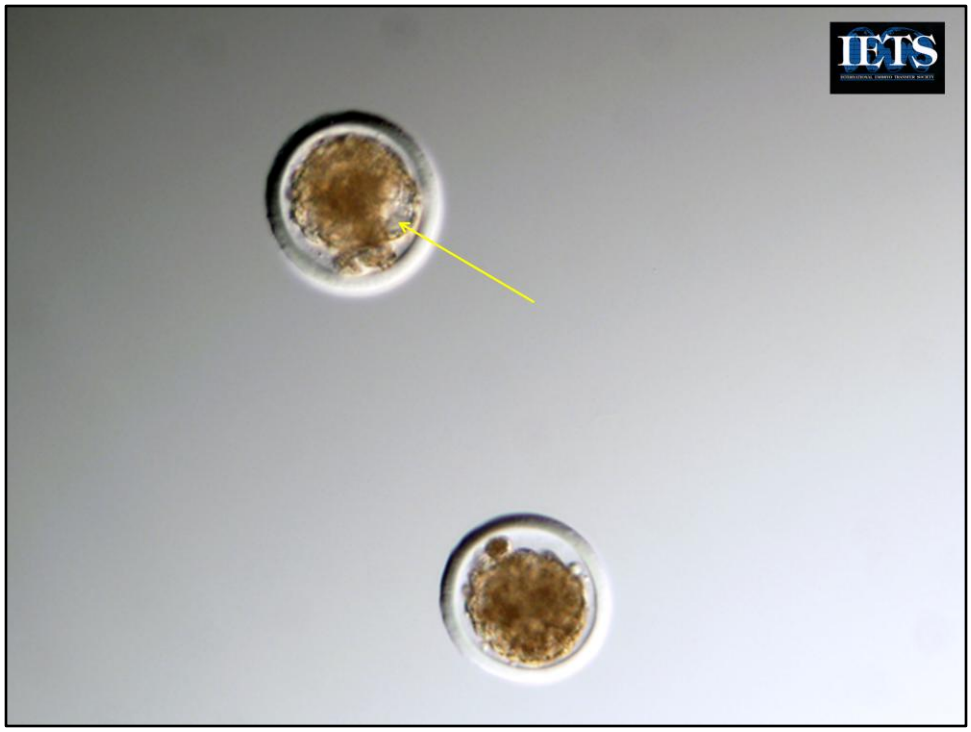


100x DIC. Same slide as previous. Arrow points to crack.

400x DIC. Stage 4, Grade 3 with a cracked zona. There is a crack in the zona at 5 o'clock allowing about one third of the embryo proper to escape. This could have been caused by hydrostatic forces during the collection procedure, or by a weakened zona. There are still several extruded blastomeres inside the perivitelline space. The crack in this embryo would exclude it as a candidate for export.

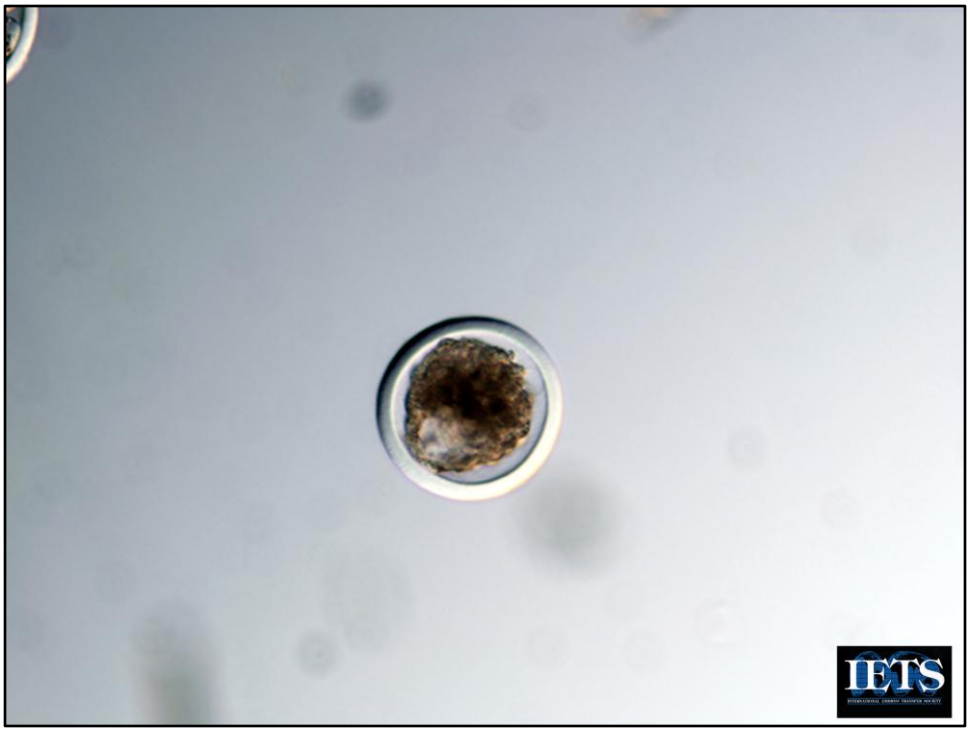


40x BF. Top embryo Stage 5, Grade 1. Bottom embryo Stage 4 Grade 1.

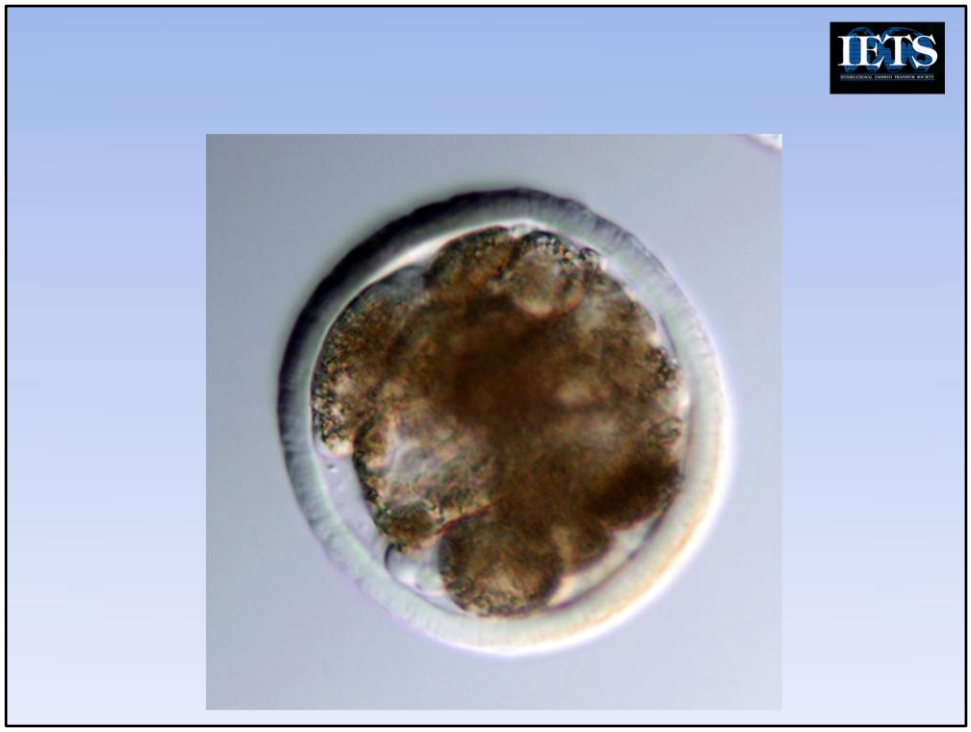


100x DIC. Same slide as above at higher magnification. Notice the blastocoele beginning to form at 4 o'clock (arrow) making this embryo an early stage blastocyst.

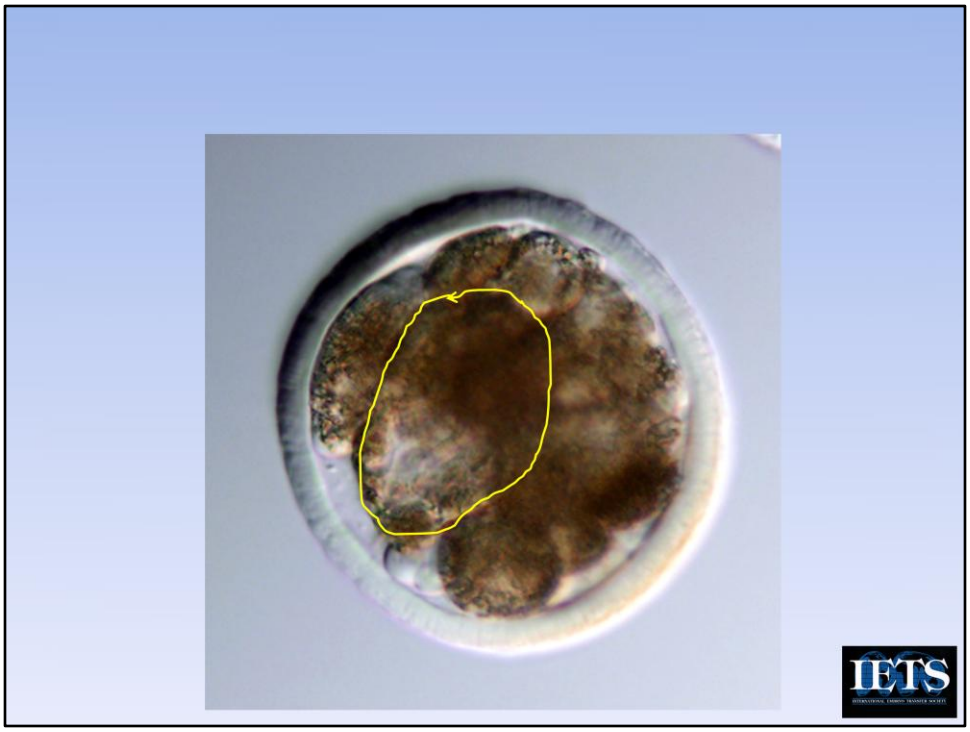
200x BF. Stage 5 Grade 1. The arrow points to the “hollow” early developing blastocoele cavity.



100x DIC. Stage 5, Grade 1.

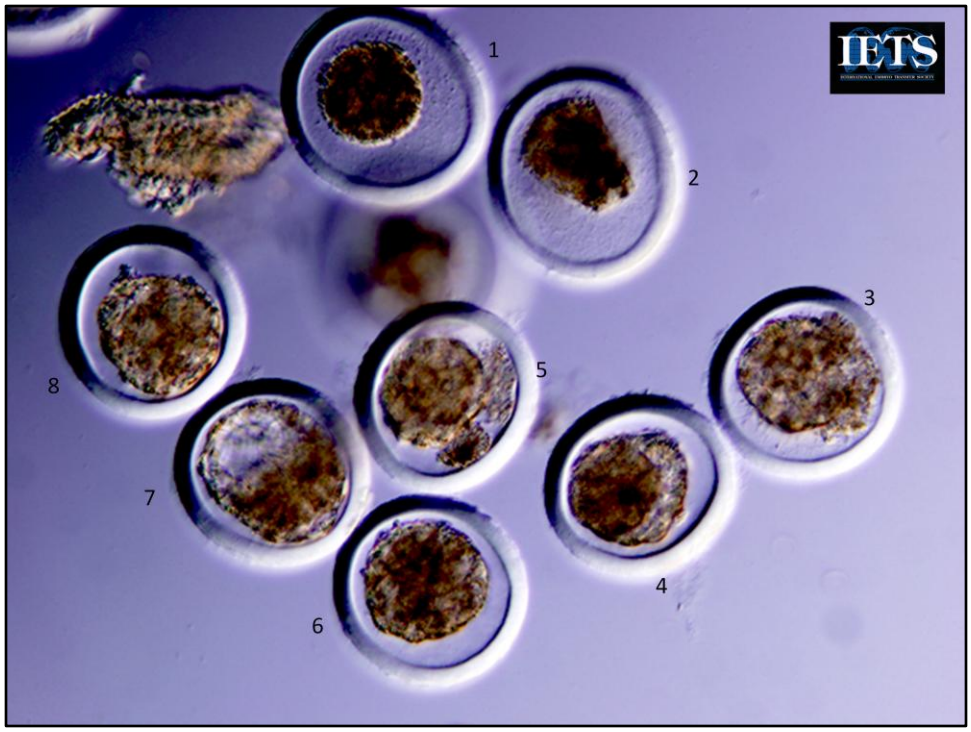


400x DIC. Stage 5, Grade 3. The viable cells in this embryo have differentiated and formed a blastocoele (lower left part of encircled cell mass – see next slide – hollow cavity). The remainder of the cells are unfused and dead.

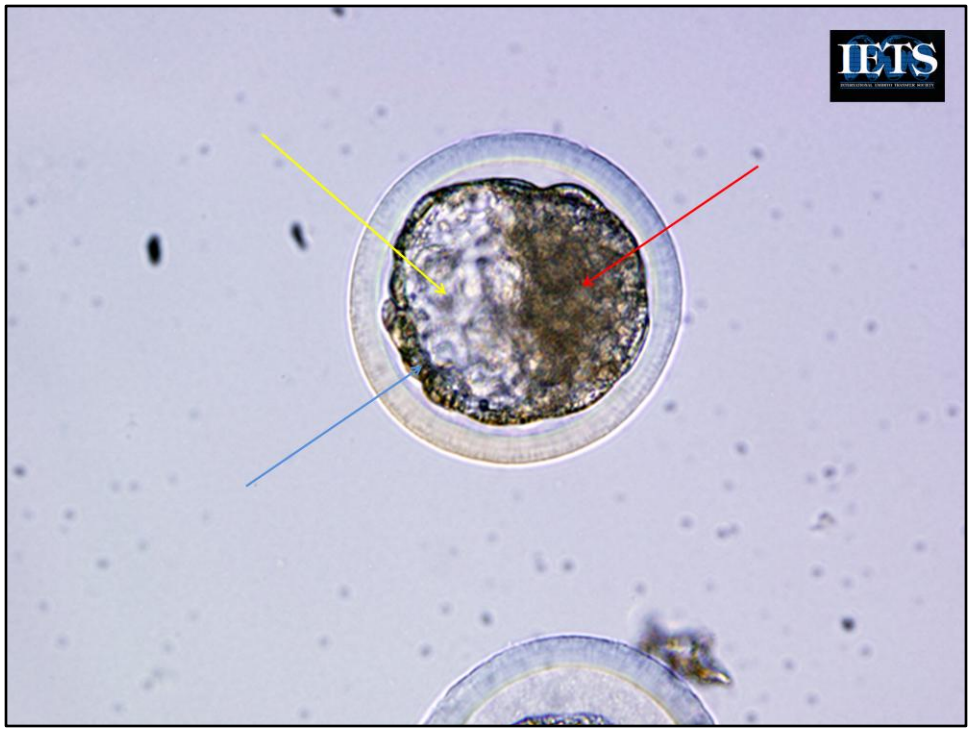


400x DIC. Stage 5, Grade 3. The viable cells in this embryo have differentiated and formed a blastocoele (lower left part of encircled cell mass – hollow cavity). The remainder of the cells are unfused and dead.

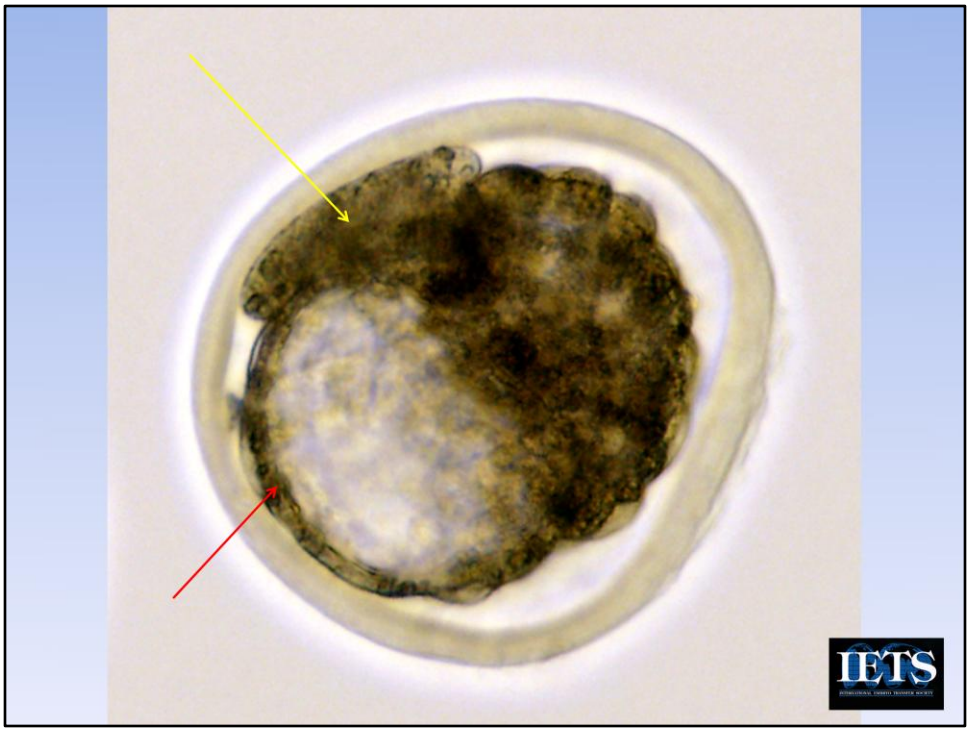
100x DIC. Both embryos are Stage 5, Grade 1. The blastocoele on the left embryo is approaching 50% of the total mass of the embryo proper and could be considered a Stage 6, Grade 1.



100x DIC. #1 & #2 = UFOs. #3 = Stage 4, Grade 2. There are extruded dead cells from 1 to 5 o'clock. Also, the VM along that same border is indiscernible and has a "frayed" appearance, thus allowing dead blastomeres and cellular debris to exit into the perivitelline space. #4 = Stage 5, Grade 1. The blastocoele cavity runs from 12 to 6 o'clock and represents about one third of the volume of the embryo proper. #5 = Stage 4, Grade 2. There are extruded cells along the right and ventral borders of the embryo proper. #6 = Stage 4, Grade 1. There are no flaws in this embryo in this plane. #7 = Stage 5, Grade 1. The blastocoele cavity represents slightly less than 50% of the embryo proper making it just shy of a stage 6. #8 = Stage 4, Grade 1. This is another flawless embryo with a VM that has only slight irregularities.

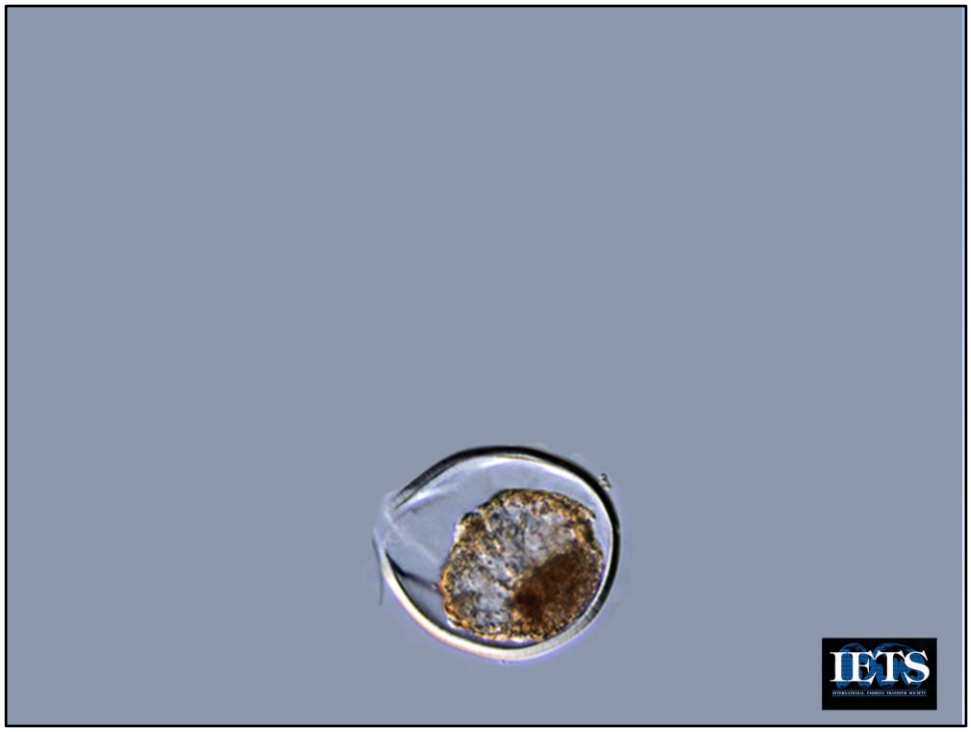


200x DIC. Stage 6, Grade 1. The yellow arrow points to the blastocoele cavity. The lighter colored blastocoele has slightly more total volume than the inner cell mass. That is the standard for classifying the embryo as a late stage blastocyst (stage 6). The blue arrow points to the trophoblast. The red arrow points to the inner cell mass.

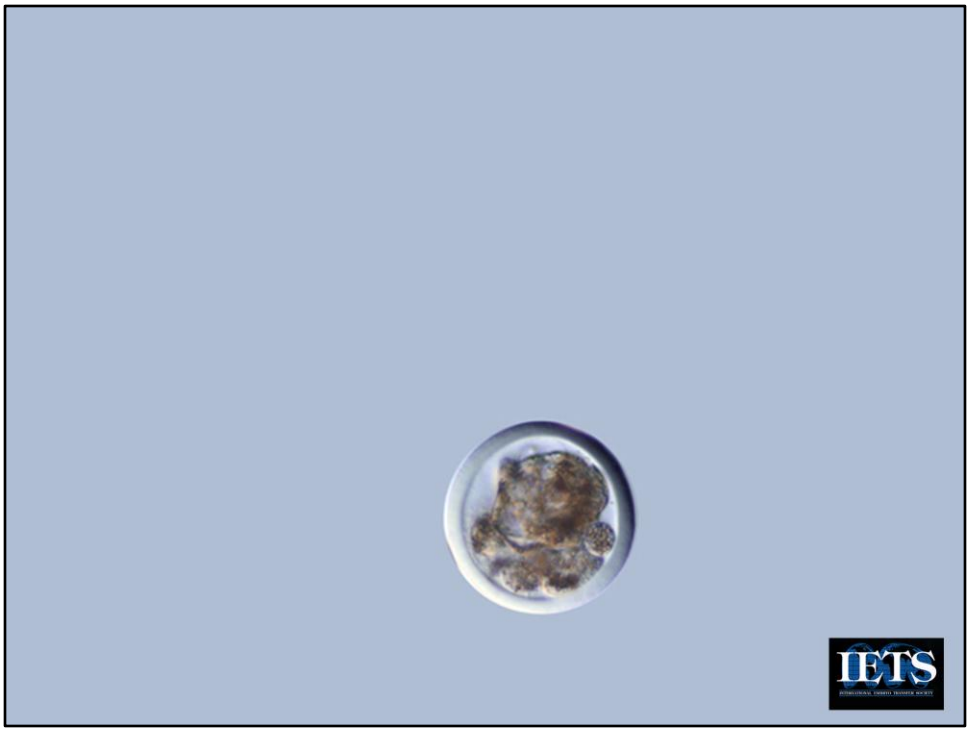


400x BF. Stage 6, Grade 1. There is one large extruded blastomere at 10 to 11 o'clock (see yellow arrow). It has been flattened due to the growth and expansion of the embryo proper filling the perivitelline space. The red arrow points out the distinct trophoblast cells, which are the first embryonic cells to visibly differentiate into a specific cell type (placenta).

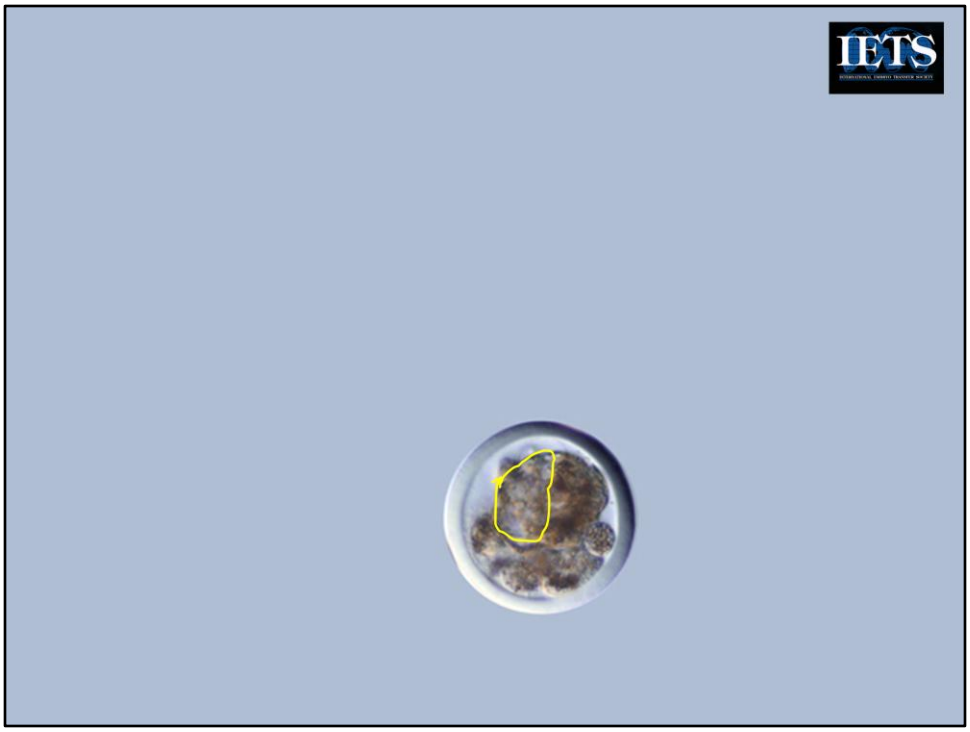
100x DIC. Stage 6, Grade 1. The blastocoele is about twice the size of the inner cell mass. The embryo proper has grown to the point that the VM now contacts the zona, thus eliminating a visible perivitelline space. However, the zona has not begun to expand geometrically and thin, thus this embryo is classified as a Stage 6 instead of Stage 7 (expanded blastocyst). One important point should be noted about embryos that have no perivitelline space – many of these embryos will be classified as Grade 1's due to the fact that any extruded blastomeres are flattened between the VM and the zona, and are, therefore often difficult to see.



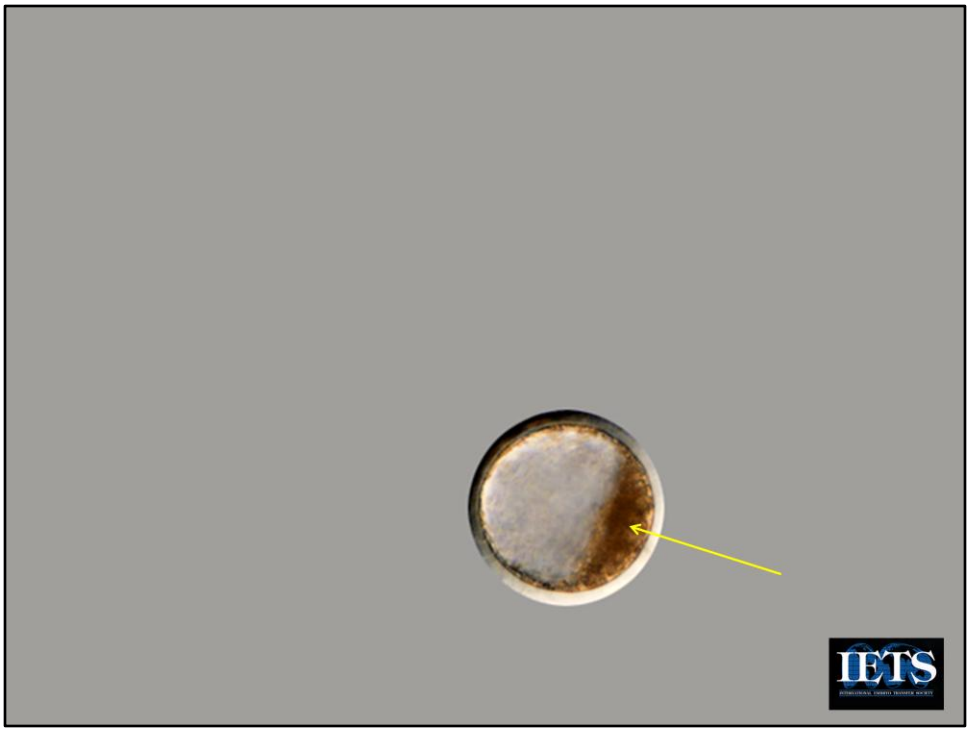
100x DIC. Stage 6, Grade 2. Although the embryo proper has no imperfections, the zona is severely cracked and torn at 9 o'clock resulting in a down Grade from a 1 to a 2. This embryo would not qualify for export.



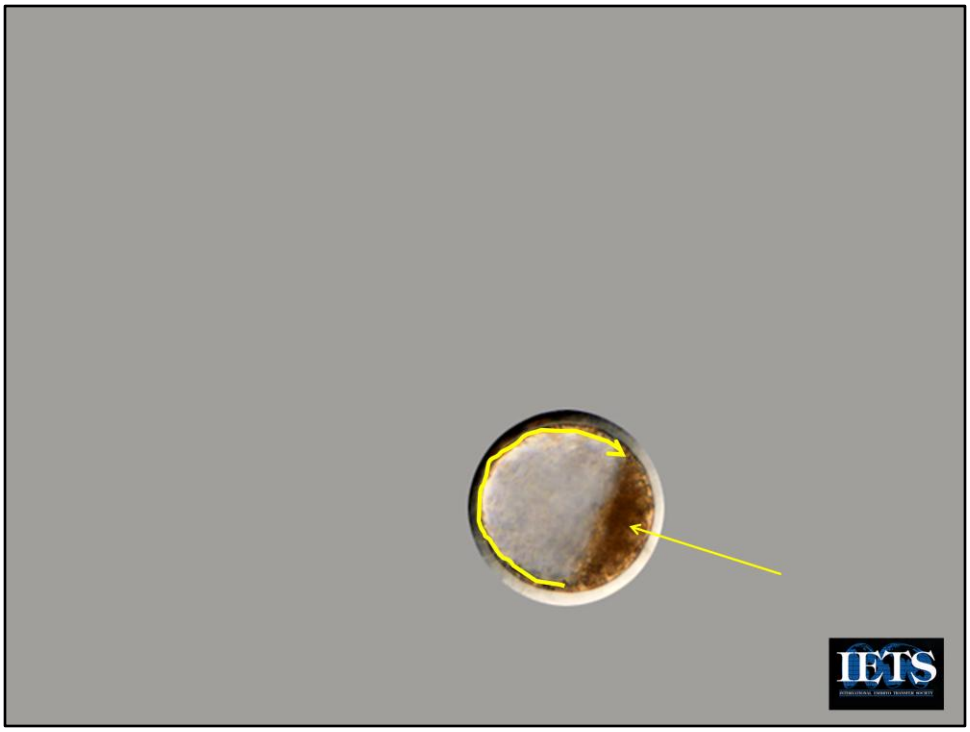
100x DIC. Stage 6 (arguably a stage 5), Grade 3. When this embryo was rolled during evaluation it was easier to see that the blastocoele (circle – see next slide) was slightly larger than the inner cell mass. Also, about half of the total cell mass was dead making the embryo a Grade 3.



100x DIC. Stage 6 (arguably a stage 5), Grade 3. When this embryo was rolled during evaluation it was easier to see that the blastocoele (circle) was slightly larger than the inner cell mass. Also, about half of the total cell mass was dead making the embryo a Grade 3.

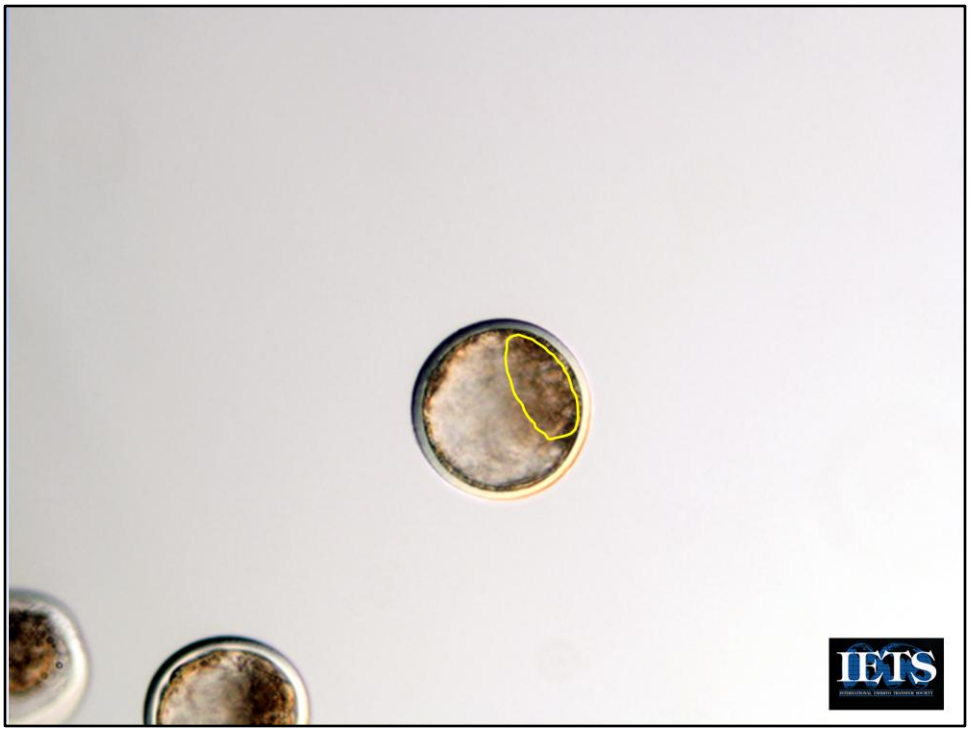


100x DIC. Stage 7, Grade 1. The blastocoele represents about 70% of the embryo proper, and the inner cell mass (yellow arrow) is relatively smaller (about 30% of the embryo proper). The embryo proper has expanded all the way to the zona, and the zona is stretching/thinning to accommodate the growth. A thin layer of trophoblast extends from the corners of the inner cell mass and extends along the entire outer border of the blastocoele and approximates the inner border of the zona (yellow arc – see next slide).

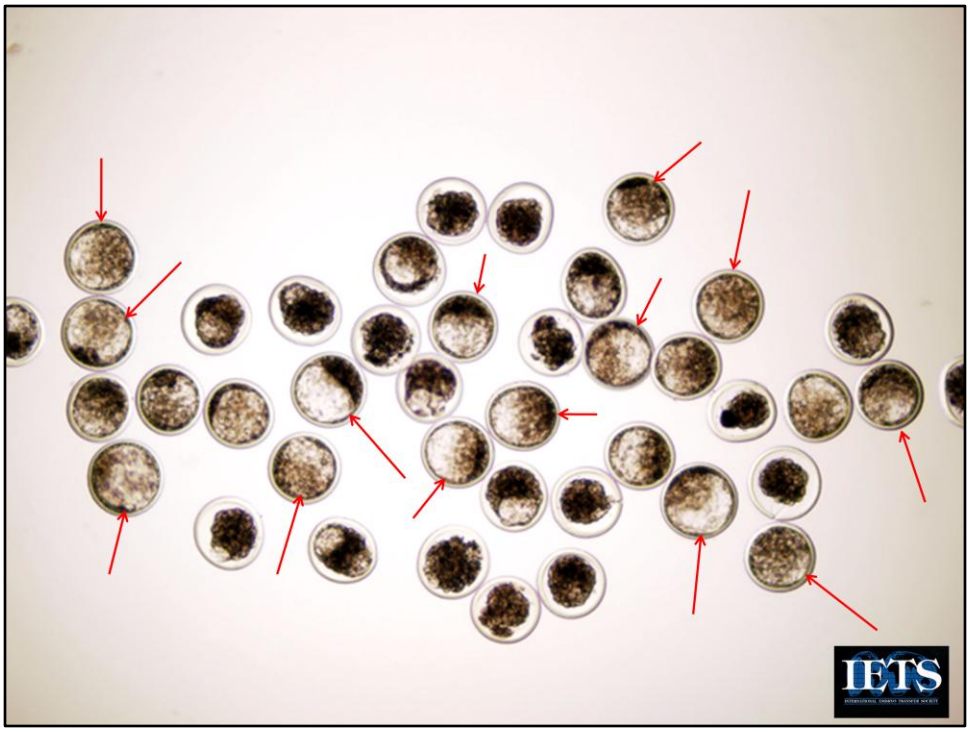


100x DIC. Stage 7, Grade 1. The blastocoele represents about 70% of the embryo proper, and the inner cell mass (yellow arrow) is relatively smaller (about 30% of the embryo proper. The embryo proper has expanded all the way to the zona, and the zona is stretching/thinning to accommodate the growth. A thin layer of trophoblast extends from the corners of the inner cell mass and extends along the entire outer border of the blastocoele and approximates the inner border of the zona (yellow arc).

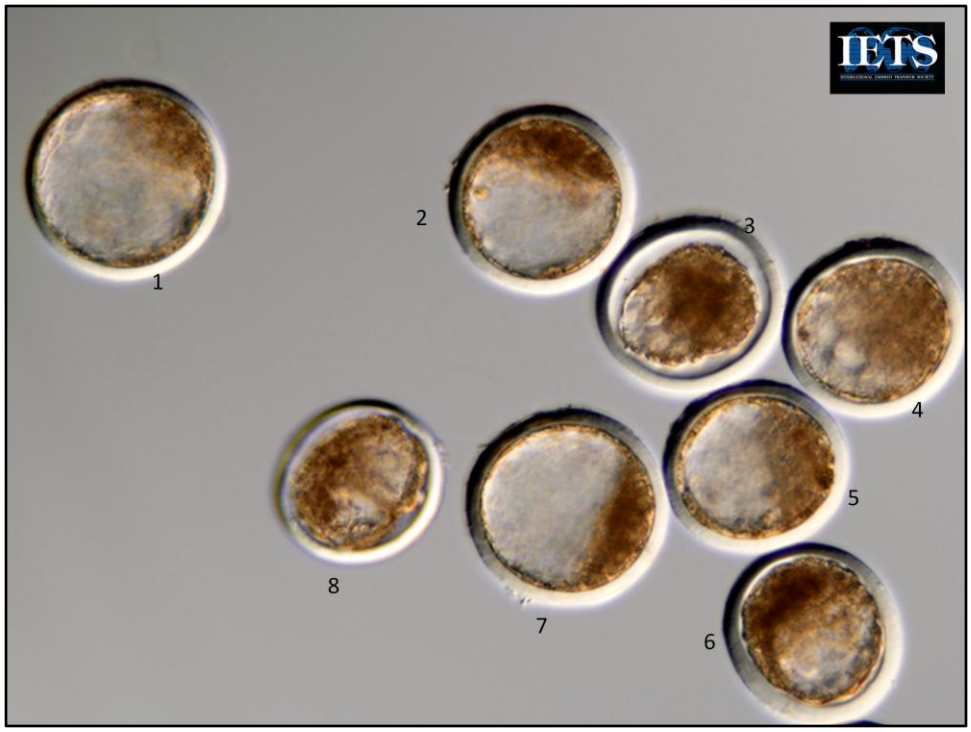
100x DIC. Stage 7, Grade 1. The trophoblast in this embryo is more visible between the zona and blastocoele. The inner cell mass is encircled by the yellow animation – see next slide.



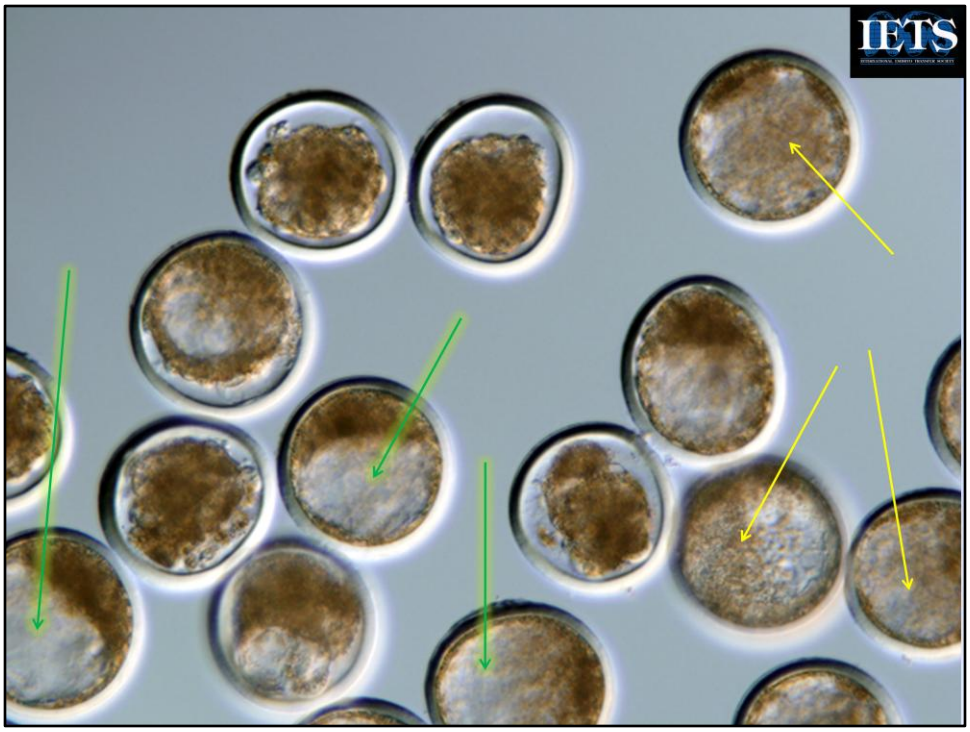
100x DIC. Stage 7, Grade 1. The trophoblast in this embryo is more visible between the zona and blastocoele. The inner cell mass is encircled by the yellow animation.



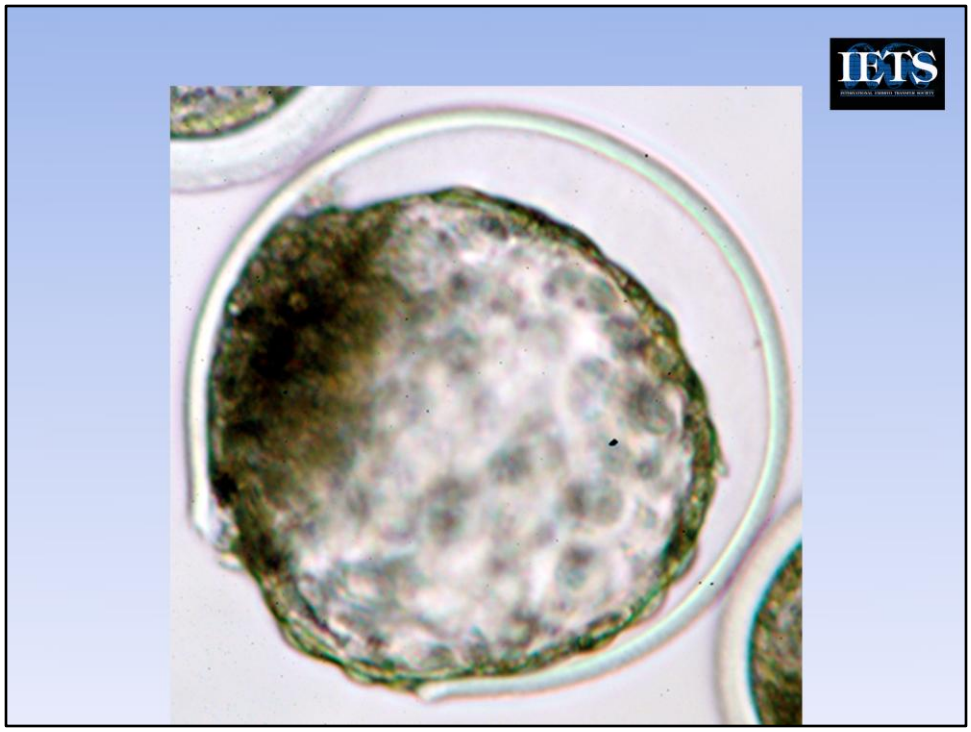
40x BF. Embryos collected from one superovulated donor 8.0 days post onset of estrus. Arrows point to the Stage 7 embryos. Notice the increased diameter and thinning of their zonas. The judgment between an advanced Stage 6 and a beginning Stage 7 is subjective.



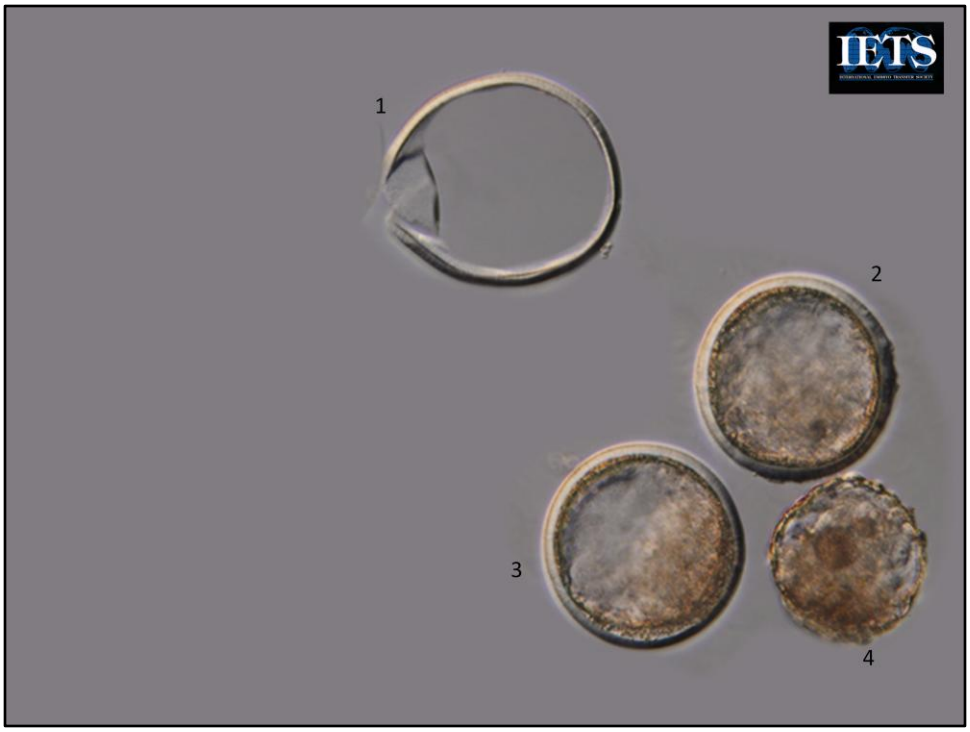
100x DIC. #1 = Stage 7, Grade 1 (7-1). #2 = 6-1, #3 = 5-1, #4 = 6-1. #5 = 6-1, #6 = 6-1, #7 = 7-1 (notice diameter compared to bordering embryos), #8 = 5-1.



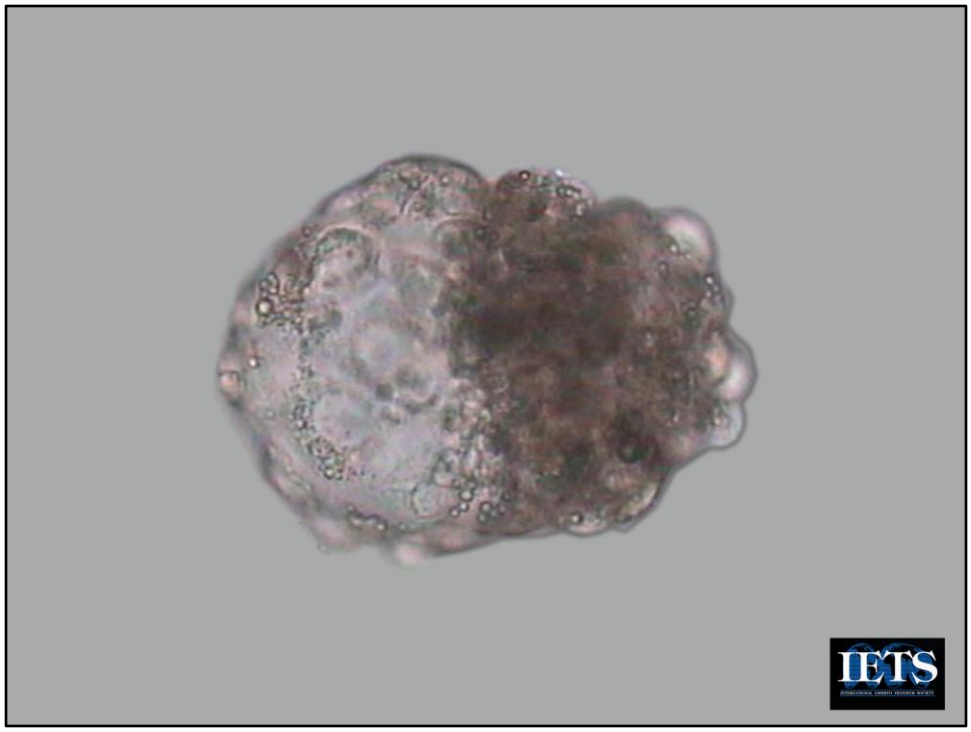
100x DIC. The three embryos with yellow arrows are Stage 7, Grade 2s. Notice the brown colored granular appearance of the blastocoele cavities of those embryos compared to the expanded blastocysts with translucent (green arrows) in this microphotograph. The granulation is an artifact of cellular debris along with dead and degenerated cells trapped tightly between the trophoblast and the zona. The green arrows point to Grade 1 embryos.



400x BF. Hatching blastocyst, Stage 8, Grade 1. The zona has cracked (6 to 8 o'clock) under the pressure of growth by the blastocyst.

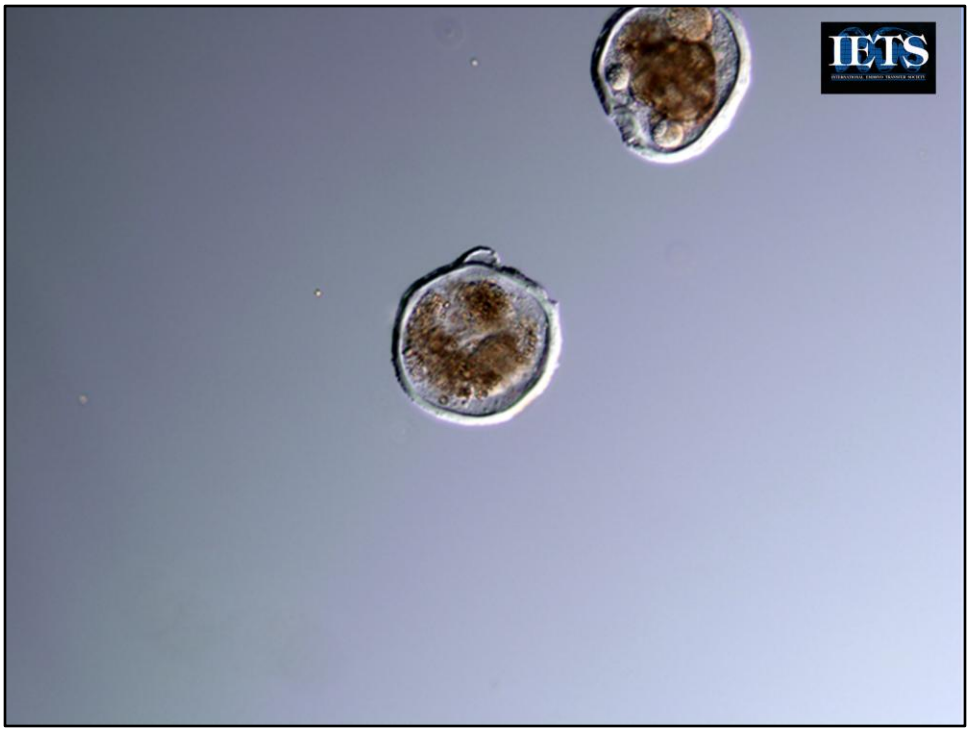


100x DIC. #1 is an empty zona. See tear at 9 o'clock. Notice how the zona wall is thinner in #1 as compared to #2 and #3 (both stage 7, grade 1). #4 is a hatched blastocyst (Stage 8, Grade 1) without a zona. #1 is likely the zona that belongs to #4.

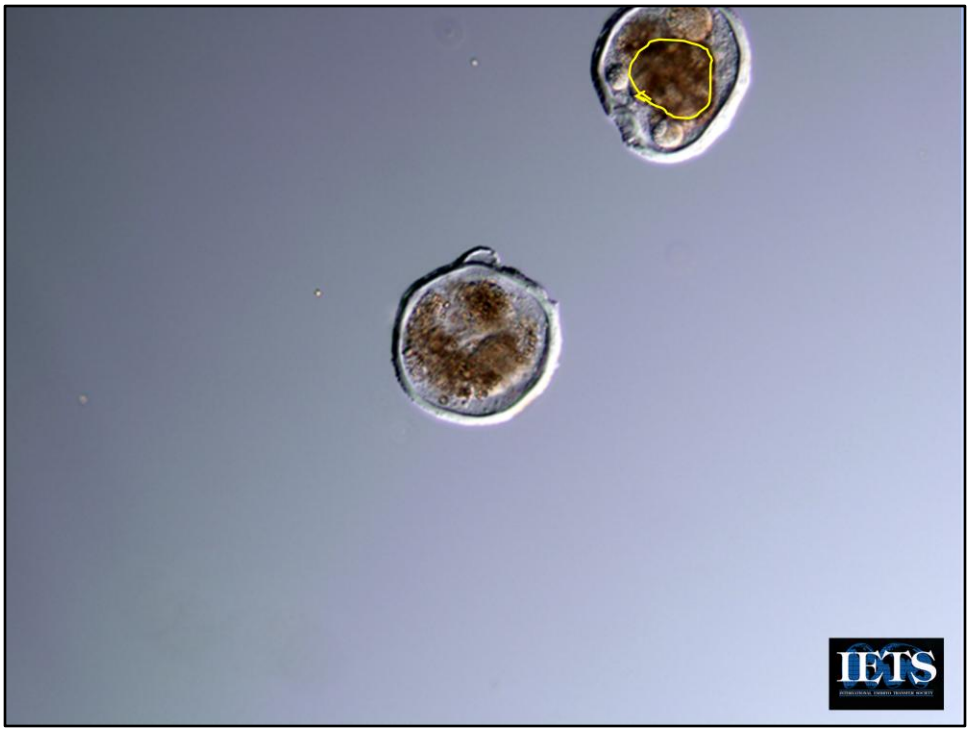


400x BF. Stage 8, Grade 1. This is a hatched blastocyst in almost perfect condition. Normally, the ratio of blastocoele to inner cell mass would be greater than in this embryo.

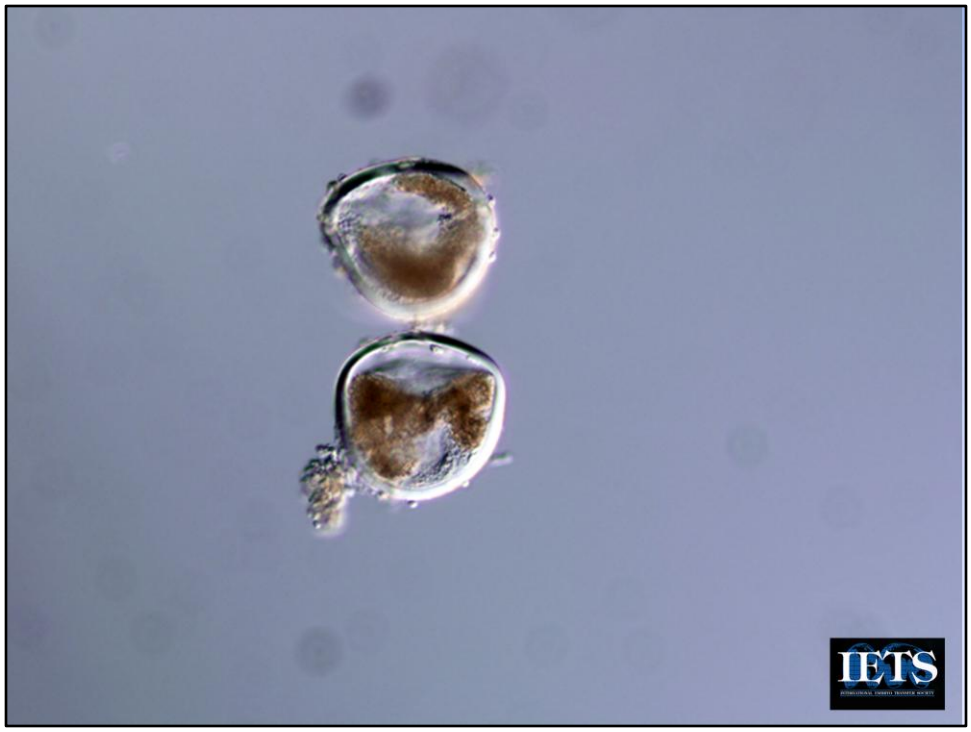
Unusual Ova/Embryos



100x DIC. The ovum in the center of the slide is a UFO with a small strip of zona beginning to peel away from itself at 12 o'clock. The embryo at the top is Stage 3, Grade 2. Its zona is oblong and has weakened, if not torn, at 7 o'clock. It has a healthy mass of fused blastomeres (circle animation – see next slide) with a few extruded dead cells surrounding it. It could be argued that this embryo should be classified as a Grade 3.

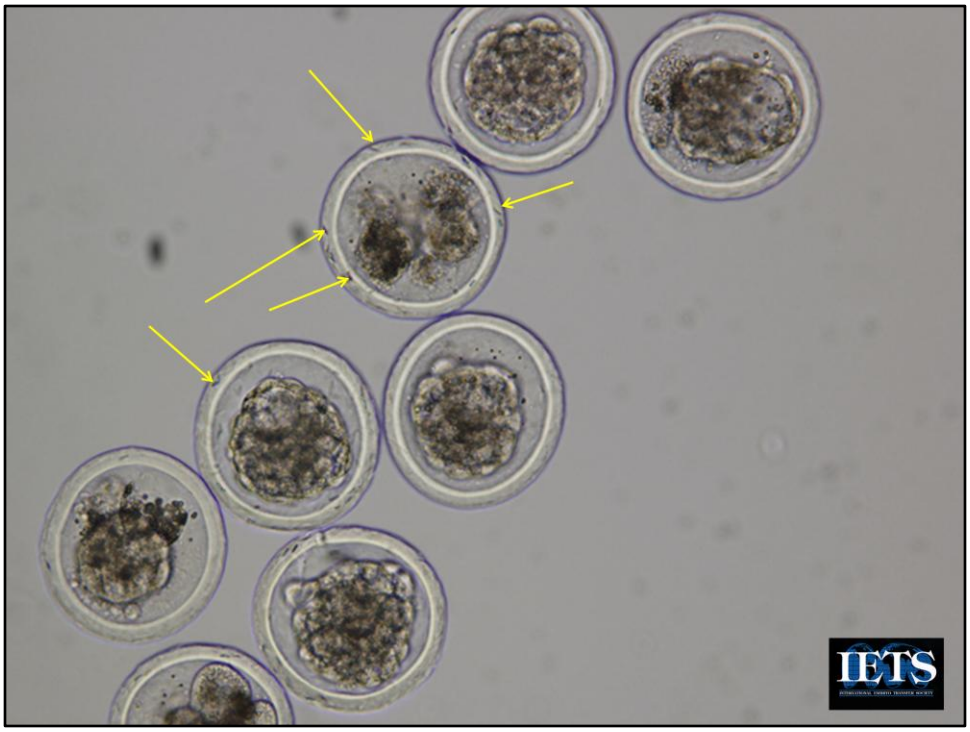


100x DIC. The ovum in the center of the slide is a UFO with a piece of zona beginning to peel away from itself at 12 o'clock. The embryo at the top is Stage 3, Grade 2. Its zona is oblong and has weakened, if not torn, at 7 o'clock. It has a healthy mass of fused blastomeres (yellow animation) with a few extruded dead cells surrounding it. It could be argued that this embryo should be classified as a Grade 3.

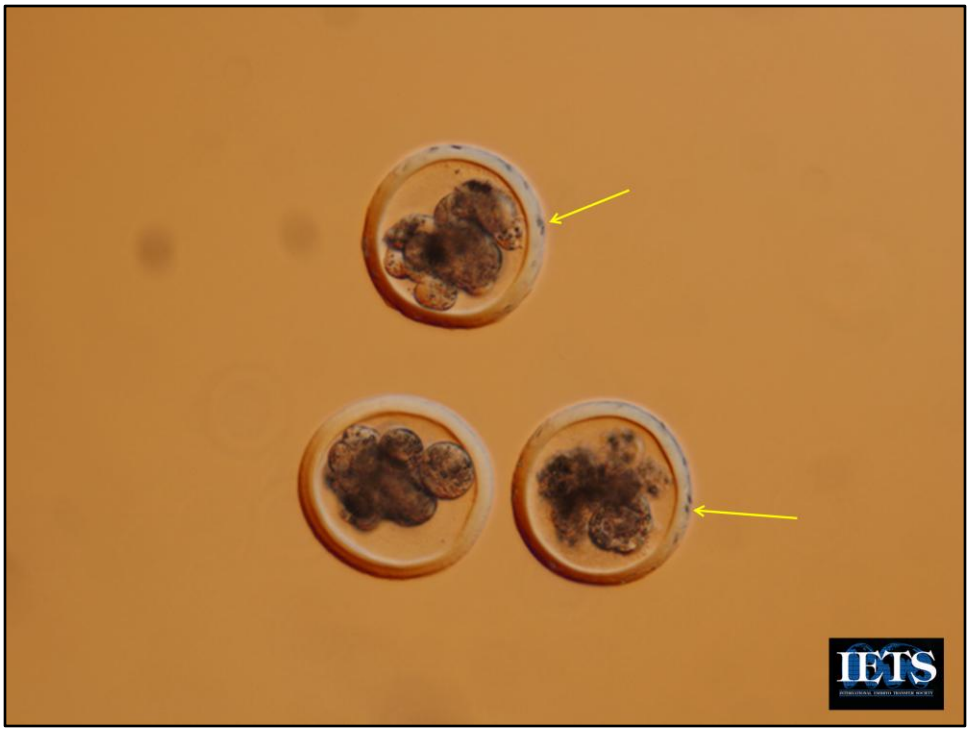


100x DIC. These two UFOs have misshapen zonas, and have uterine endometrial cells adhering to their zonas. If these were healthy morula and proper washing and trypsinizing failed to remove the cellular debris, they would not qualify for export.

100x DIC. All of these ova/embryos have misshapen zonas except the the 4 cell degenerated embryo in the middle. The ova to its immediate left and lower right are tear shaped UFOs. The embryo on the far left classifies as a Stage 6, Grade 3. The zona has a teat-like protrusion at 2 o'clock and has a large rip or tear. The embryo proper is relatively small for its age (day 7.5 collection), but has a blastocoele larger than its inner cell mass. The embryo on the far lower right is a Stage 5, Grade 2 due to the imperfection in the zona at 4 o'clock. All of these ova/embryos came from the same collection.



100x BF. The small dark slashes embedded in the zona pellucida are zonary sperm. They have been trapped in the zona due to the zona block resulting from the first sperm penetrating the zona and fusing with the VM. These embryos are not necessarily unusual, but it's not often that this many zonary sperm are observed in a set of embryos.



100x DIC. The yellow background in this photo allows for more contrast so the zonary sperm (dark dashes in the zona) are easier to see. The embryo on the lower left of the slide does not have any zonary sperm in the focused plane.

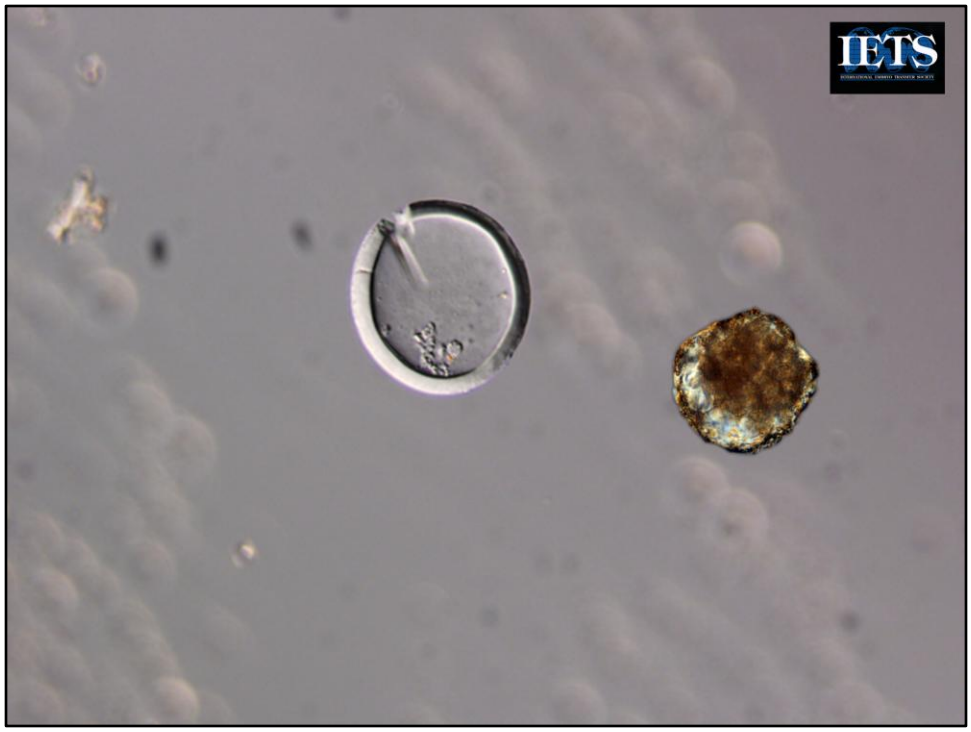
40x photo taken with stereomicroscope. The elongated piece of tissue is a retained mass of cumulous cells. It has 4 ova/embryos embedded inside the mass (see next slide with yellow arrows). Careful manual dissection with two 20 gauge needles handheld will free the ova from the mass. The embryos are as likely to be healthy and viable as the ones outside the cumulous mass. The viable embryos can be transferred fresh or frozen with good results. All ova outside the mass are good quality viable embryos collected from the same flush.

40x Stereomicroscope. Same as previous slide with arrows.

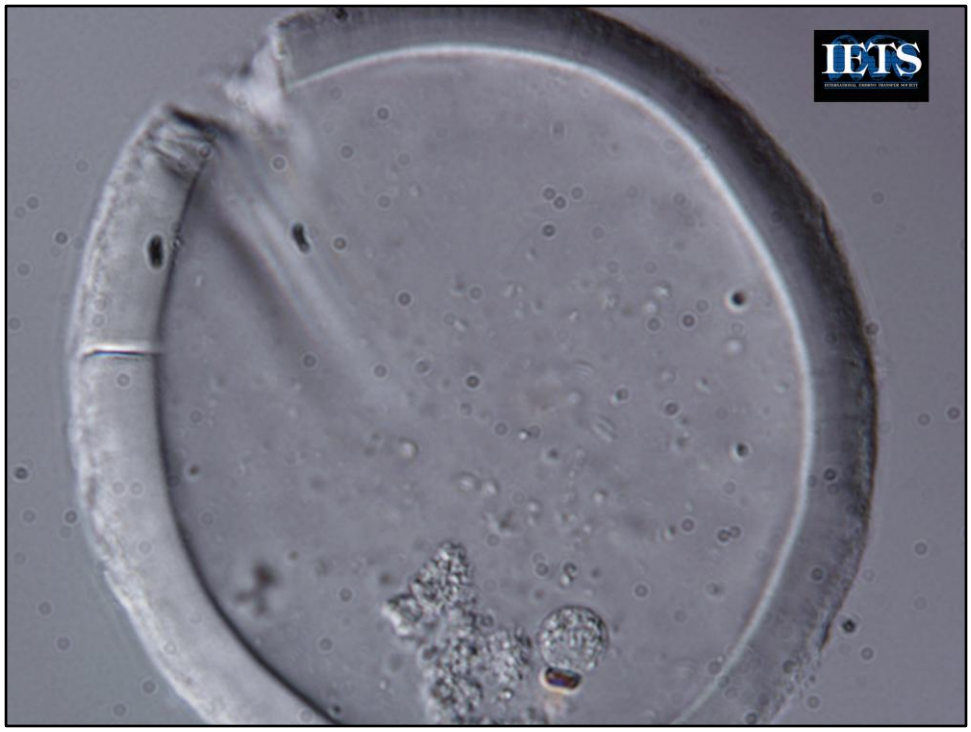
40x BF. This is a single UFO (see blue arrow next slide) embedded inside a cumulous matrix. It's interesting that these embedded ova can traverse the oviduct and be transported into the uterus.

40x BF. Same as previous slide with arrow.

100x DIC. This empty zona has been severely cracked or torn at about 11 o'clock. There is a small amount of ooplasm remaining inside the zona at about 6 o'clock. Sometimes, the tear can be very subtle and can lead a practitioner into believing the ovum was only a zona without a cytoplasm. It is not uncommon to see empty zonas after an embryo collection procedure (flush). It is thought to be a result of physical sheering of hydrostatic forces created when filling and manipulating the uterus.

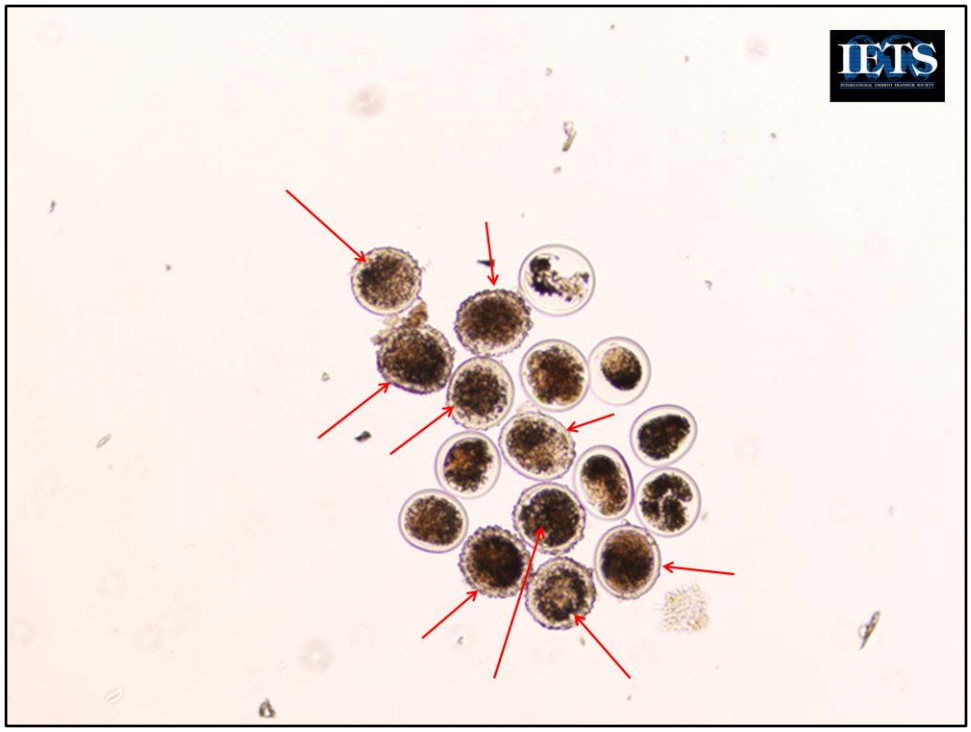


100x DIC. Same slide as previous, but with Stage 5, Grade 1 embryo proper. The zona was torn during embryo collection, but the embryo proper was discovered by the search technician. This embryo can be transferred fresh with good results. It can be frozen, but is not exportable.

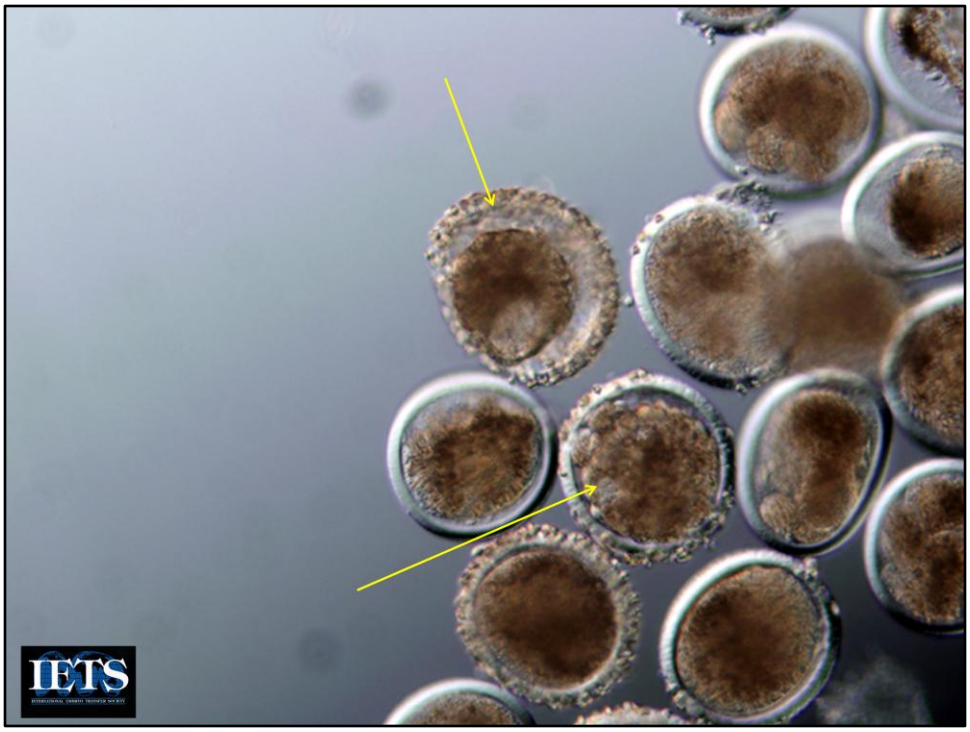


400x DIC. Same slide but higher magnification. If an empty zona is found that is thin it could be a sign that the embryo has hatched, or that a Stage 7 embryo has been sheered and force hatched. In either case, the embryo search technician should look for the embryo proper, which could be transferred or frozen for domestic use.

400x DIC. This is an empty zona with a severe tear from 2 to 4 o'clock that extends into the center of the ova. Close inspection reveals the porous nature of the zona (arrow). The zona has striations that allow cumulous cell filaments to penetrate and communicate with the ooplast.

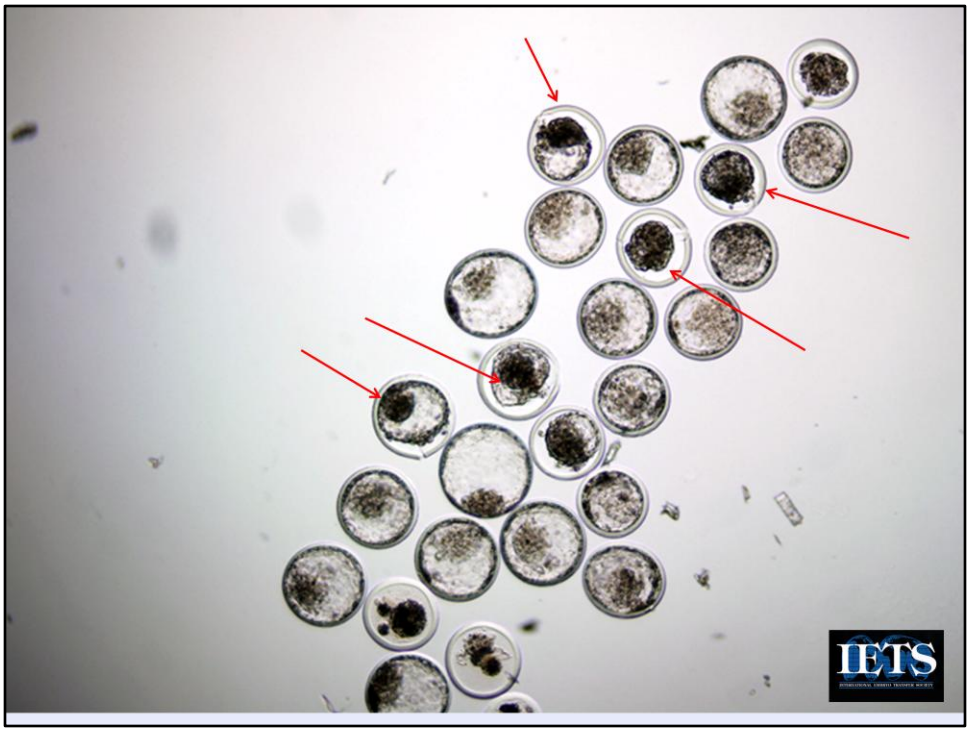


40x BF. Some of the ova/embryos in this collection have neutrophils invading the surface of their zona (red arrows). The neutrophils look like small nodules adhering to the outer surface of the zona. The other ova/embryos appear to be unaffected. Neutrophils are a sign of bacterial infection presumably of the oviduct or uterus. Embryos being phagocytized by neutrophils can be transferred successfully. An attempt should be made to physically remove the white blood cells (WBCs) by using some form of micromanipulation with a micropipettor, or Vortexed. They would not be suitable for export.

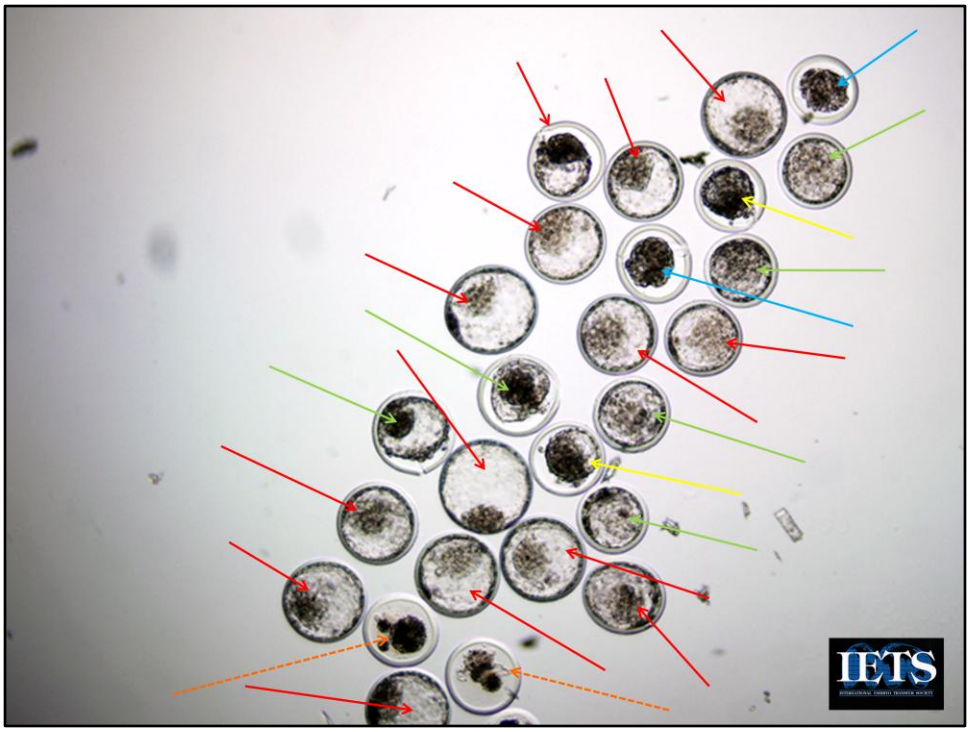


100x DIC. Most of these ova/embryos are either UFOs or degenerates. The embryos with arrows are transferrable. The uppermost embryo (arrow) is a Stage 5, Grade 2 (down graded from a 1 due to the neutrophils and an oblong zona). The other embryo is a Stage 4, Grade 2. Several of these ova/embryos are being phagocytized by neutrophils.

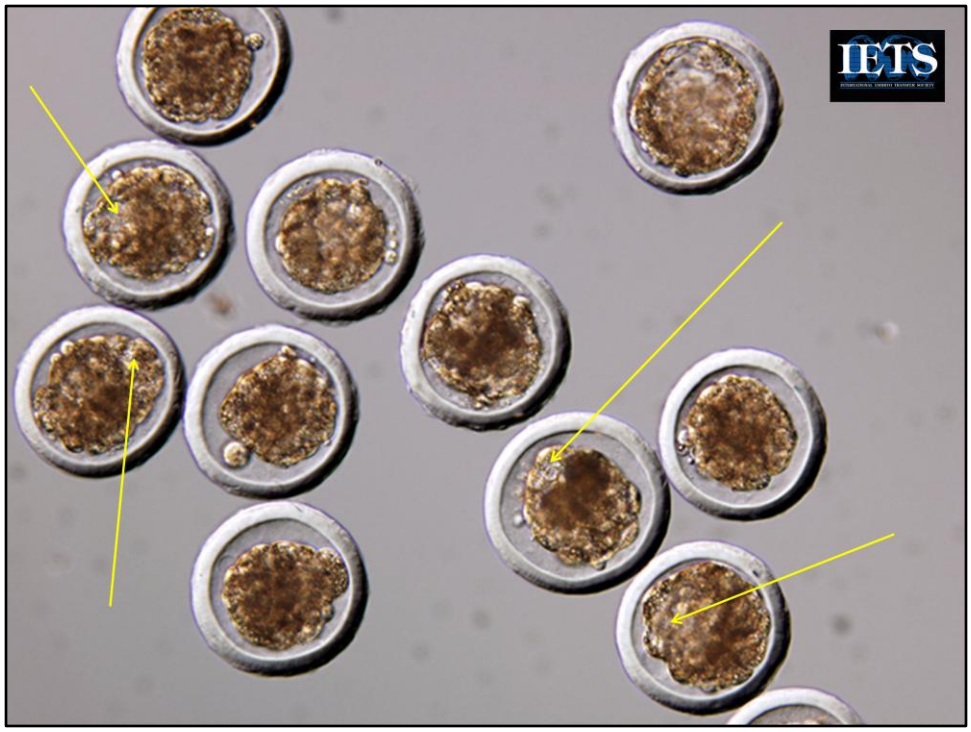
Collections of Embryos



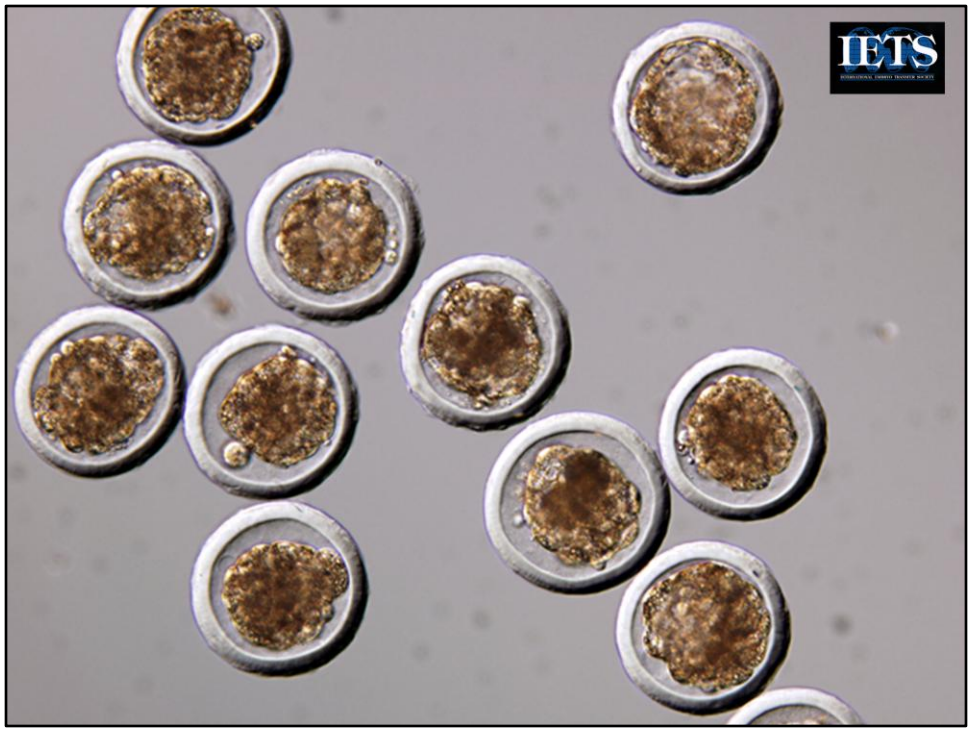
40x BF. The red arrows point to embryos with cracked zonas. There could be more embryos with cracks if they were rolled and inspected more closely. Many of these embryos classify as Stage 7 Grade 1.



40x BF. The red arrows point to Stage 7, Grade 1 embryos. The green arrows point to Stage 6, Grade 1 embryos. The yellow arrows represent Stage 5, Grade 1 embryos. The blue arrow points to Stage 4, Grade 1s. The orange dashed arrows point to fragmented UFOs. Any embryo with a cracked zona down grades from a Grade 1 to a Grade 2.



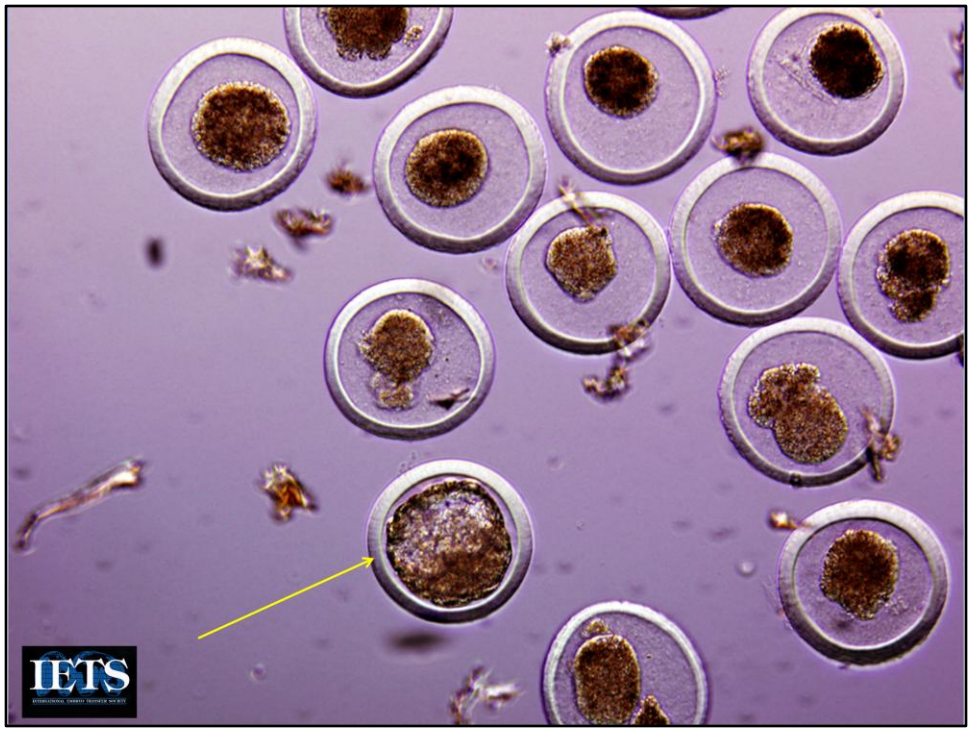
100x DIC. Most of these embryos are Stage 4, Grade 1. The embryo in the upper right corner is a Stage 5, Grade 1. Arguably the embryos with arrows pointing to them could be considered Stage 5 embryos. The arrows point directly to their early developing blastocoeles.



100x DIC. Same slide as previous without arrows.

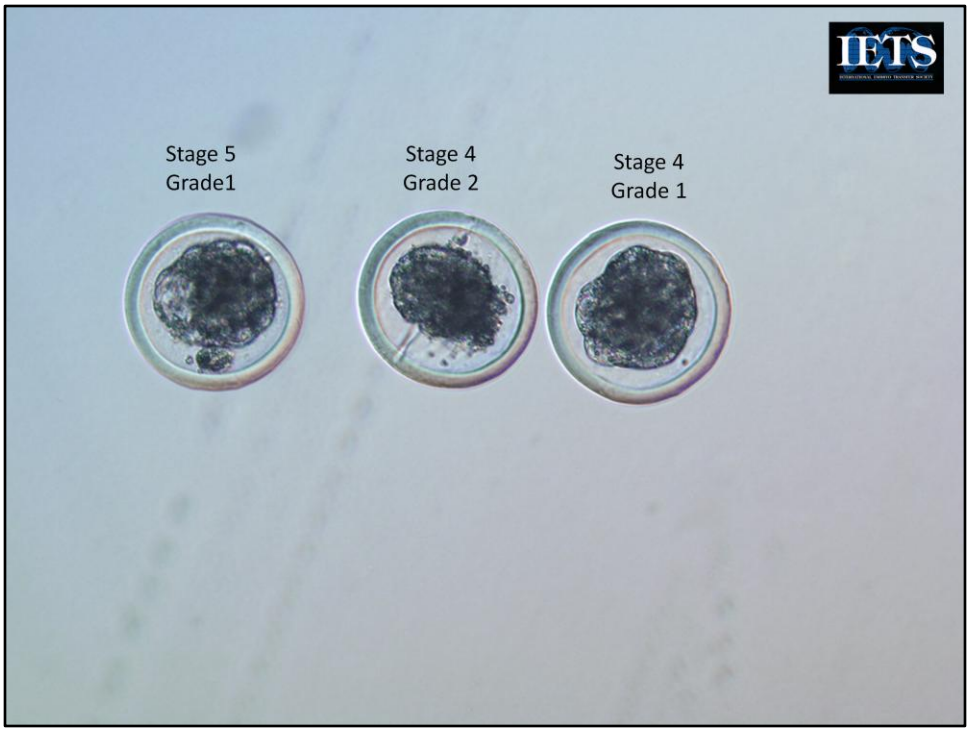
40x BF. #1 = Stage 6, Grade 1. #2 and #3 = Stage 7, Grade 1. #4 – Stage 6, Grade 1. The blastocoele appears to be smaller than the inner cell mass(ICM) in this view, but when rolled, the blastocoele is actually larger than the ICM. #5 = Stage 7, Grade 1. Notice the large diameter, plus the thinning of zona due to physical expansion of growth. #6 = Stage 7, Grade 1 (arguably a stage 6). #7 = Stage 6, Grade 1 (arguably a stage 5). #8 = Stage 7, Grade 1. #9 and #10 = Stage 5, Grade 1. #11 and #12 = Stage 7, Grade 1. #13 = Stage 5, Grade 1 (arguably a stage 6). #14 = Stage 4, Grade 2 (arguably a grade 1). 15 = Stage 5, Grade 1. #16 = Stage 7, Grade 1. #17 = Stage 4, Grade 3. The VM is very ill defined in this embryo. The VM has a "frayed" appearance from about 12 to 6 o'clock. There are most likely a few viable fused cells in the main mass, but many of the peripheral cells are degenerated. #18 = Stage 4, Grade 2. It has dead blastomeres at 6, 7, and 12 o'clock. #19 = Stage 5, Grade 1 (arguably a stage 6). #20 = Stage 6, Grade 1. #21 = Stage 7, Grade 1 (arguably stage 6). #22 = Stage 5, Grade 1. #23, 24, and 25 = Stage 4, Grade 1. #26 = Stage 4, Grade 2. The shape of the embryo proper is geometrically non-sperical. #27 = Stage 4, Grade 2 (arguably a stage 5) due to a cracked zona at 3 o'clock. #28 = Stage 7, Grade 1. #29 = Stage 6, Grade 1. This embryo is slightly oval shaped, but has no other flaws. #30 = Stage 7, Grade 1. #31 = Stage 6, Grade 1. #32 = Stage 7, Grade 1. #33 = Stage 6, Grade 1. #34 = Stage 4, Grade 1. #35 = Stage 7, Grade 1. #36 = Stage 7, Grade 1 (arguably stage 6). #37 = Stage 4, Grade 2 (cracked zona). This embryo could be a Stage 7 with a collapsed blastocoele cavity due to physical forces during the embryo collection procedure. The diameter of the zona is noticeably larger than that of embryo 34. The crack in the zona is at about 7 o'clock. #38 = Stage 7, Grade 1. #39 = Stage 5, Grade 1. #40 = Stage 6, Grade 1.

100x BF. #1 = Fertilized degenerated. #2 = Stage 4, Grade 2. There are extruded blastomeres from 12 to 5 o'clock. #3 and #4 are both fertilized degenerated due to the fact that they have no fused blastomeres like embryo #2. #3 could possibly have a few fused (live) cells in its interior, but not likely.



100x DIC. With the exception of the embryo with the arrow (Stage 6, Grade 1) all ova are UFOs.

100x DIC. This collection produced only 1 poor quality embryo (Stage 4, Quality 3). The others are either unfertilized or degenerated.



100x DIC. The middle embryo is downgraded from a Grade 1 to a Grade 2 due to the cracked zona at 7 o'clock.