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Collapsing Protocol

Human Blastocysts

Have all necessary materials, tools and equipment ready and handy before starting procedure.

COLLAPSING PROTOCOL

1. **Which embryos:** Collapse any embryo that has stretching of the zona. That is, if the cavity is sufficiently expanded to cause the zona to stretch and thin, the embryo needs to be collapsed. If the embryo has a small cavity, that has not caused any stretching of the zona (typically <50% of the volume of the embryo), collapsing is unnecessary.
2. **When to collapse:** This procedure is performed just prior to vitrification. In general, embryos will need a few minutes to collapse (up to 10 minutes maximum) after trophectoderm disruption, so collapsing is usually performed prior to setting up the solutions and straws for the procedure. Identify the embryos for collapsing and perform the collapsing procedure on a heated stage. Return the embryos to the culture incubator. Then set up the vitrification solutions, straws, labels and other items needed to perform vitrification. This usually takes 5–10 minutes, during which time the majority of embryos will have collapsed nicely.
3. **How to collapse using a laser:** Identify two trophectoderm cells that are located well away from the inner cell mass (ICM), and which can be visualized cleanly on the edge of the embryo (see Figure 1). Cells that are nicely stretched out are ideal. Position the laser such that it will fire at the junction between the two cells (see Figure 2). The laser should be set on its minimum setting, which is 50 mW for the Research Instruments device. Fire the laser at the targeted spot **once and only once** (this is very important). Typically, the embryo will not begin to collapse immediately, and there is no need to fire the laser again. In fact, firing the laser a second time can actually seal up the hole made by the first shot.

It is not necessary to breach the zona for successful collapsing. If the zona is thick at the chosen spot, it will likely not be completely breached. If the zona has thinned considerably, the hole may cut through the entire zona. Usually, embryos with the complete breach will collapse more quickly, but even without the breach, the vast majority will be nicely collapsed after 5 minutes back in the incubator. Occasionally, an embryo will not collapse even after time back in the incubator, but subsequently will collapse in either the equilibration solution (ES) or vitrification solution (VS).

Fig. 1

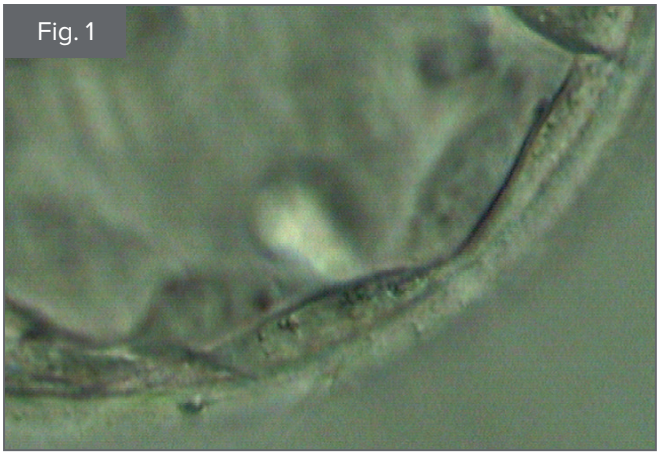

Figure 1. Identify the joint between two trophectoderm cells as the place to make a hole.

Fig. 2

Figure 2. Align the laser over the selected spot and fire once only, on the lowest setting available.

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4. **How to collapse using a glass needle:** Collapsing is easily achieved with a glass needle if a laser is not an option. However, this does involve the setting up of a dish of HEPES buffered medium with 10% serum into which the embryo(s) should be moved for the procedure. A glass holding pipette and an ICSI pipette are first mounted on the micromanipulators in much the same way as for an ICSI procedure. Again, an area between two trophectoderm cells and well clear of the ICM is identified for making a hole. The embryo is held firmly with the holding pipette and the ICSI needle is pushed gently into the junction between the cells (see Figure 3). It is not necessary to use the ICSI pipette to aspirate the fluid because the needle itself is sufficient to disrupt the blastocoel. As the needle passes into the cavity of the blastocyst, it creates a small hole which will allow fluid to escape. The tight junctions between the cells can repair the hole, so it is important to move the needle in and out several times to establish a good breach between the cells.

5. **The collapsing process:** When the embryo collapses, it will first be seen to pull away from the zona on the side opposite to where the hole was made (see Figure 4). It will then continue to collapse in on itself and towards the hole as fluid leaves the cavity. Since the behavior of the embryos is predictable, we can use this pattern of collapsing to make sure that partially hatched embryos collapse back into the zona. If an embryo is hatching, there is a temptation to want to make the hole in the part that is coming out of the zona. However, making the hole in the side opposite to where the embryo is hatching ensures that the embryo collapses back into the zona.

6. **Assisted hatching:** The small hole or nick in the zona that is made during the collapsing process does not substitute for assisted hatching (AH). If AH is desired, it should be performed in the normal way, after the embryo is warmed. It is best done immediately after warming, when the embryo is still in a collapsed state, in an area of the zona well away from any cells. It is not necessary to find and enlarge the hole made during collapsing.

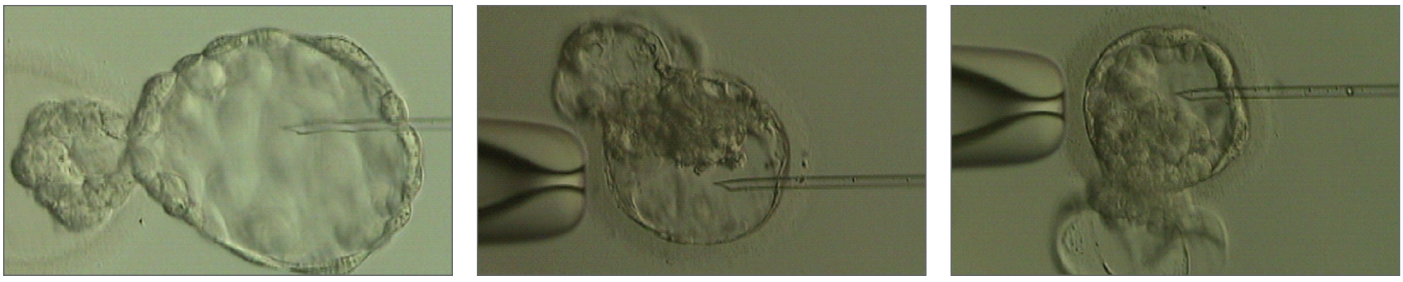

Figure 3. Collapsing blastocysts with an ICSI needle. It is necessary to move the needle back and forth several times after insertion to establish the hole between the trophectoderm cells.

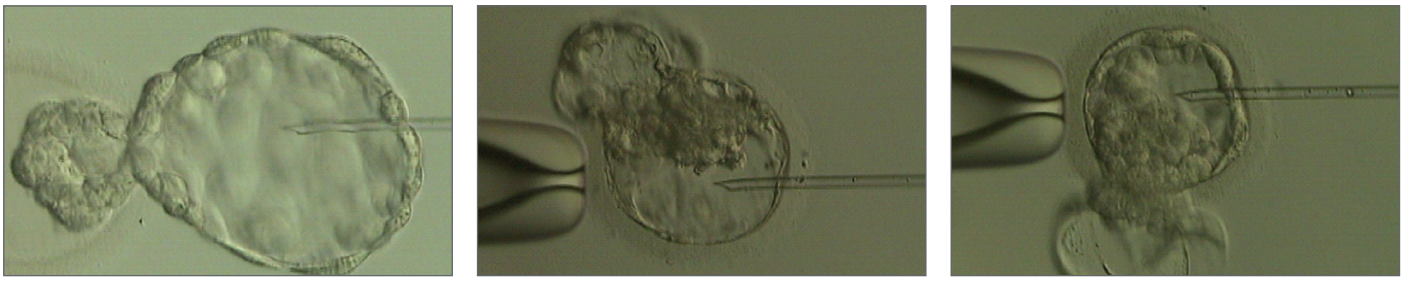

Figure 4. The embryo collapses towards the hole which is on the lower right at about the 4 o'clock position. Complete collapsing can take several minutes.

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