Practical aspects of “Laser Assisted Blastocyst Collapsing“ prior to vitrification using Octax lasers

Blastocyst collapsing is a common procedure to remove the fluid inside the blastocoel in order to increase the survival rate after cryopreservation procedures. The easiest and safest way to collapse the blastocyst is to use a laser system such as Octax. It is important to understand how and when the blastocyst should be collapsed. You will find a suggested protocol below.

Which embryos to treat?

Collapse any embryo that shows stretching of the zona. That is, if the cavity is sufficiently expanded to cause the zona to stretch and thin, the embryo can be collapsed. If the embryo has a small cavity, that has not caused any stretching of the zona, collapsing is unnecessary.

When to collapse?

The procedure is applied just prior to vitrification. In general, embryos will need a few minutes to collapse (up to 10 mins max) after being hit by the laser, 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 microscope stage (see below). Return the embryos to the culture incubator. Now, set up the vitrification solutions, straws and other items needed to perform vitrification. This usually takes 5-10 minutes, during which time the majority of laser treated embryos will have collapsed nicely.

How to use the laser for collapsing?

Identify two spindle-shaped 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).

Using a well serviced Octax laser system, a pulse length of 1.3 – 1.5 msec should be sufficient for collapsing. **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.
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 five minutes back in the incubator. Occasionally, an embryo will not collapse even after time back in the incubator, but these will collapse in either the equilibration solution or the vitrification solution of your vitrification media kit.

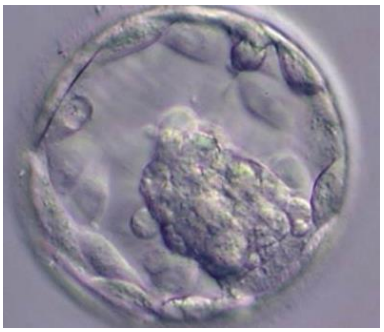

Figure 1.
Identify the joint between two trophectoderm cells to be targeted by the laser.

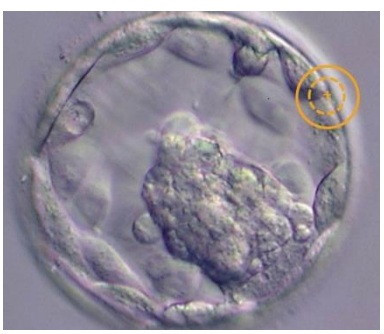

Figure 2.
Align the laser over the selected spot and fire once only, on the pulse settings recommended above.