

Assisted hatching

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One of the factors that can cause embryo implantation to fail is the failure of zona rupture. Cohen *et al.* (1990) suggested that zona hardening and subsequent failure of hatching could be frequent after IVF. This hypothesis was suggested by previous observations of the in-vitro hatching process following zona drilling of mouse and human embryos. Hatching occurred earlier and embryos implanted more often after micromanipulation (Malter and Cohen, 1989). It was suggested that many in-vitro fertilized embryos could be deficient in lysins necessary for hatching. So, if this hypothesis was correct, the implantation rate of embryos should be increased by micromanipulation to open the zona pellucida.

Before beginning a clinical trial, several experiments were performed showing that the development of mouse embryos was not altered by such an invasive procedure, if the hole was large enough.

At present, there are three methods that can be used to improve the hatching of day 3 embryos selected for transfer: (i) the mechanical technique, i.e. partial zona dissection using glass microneedles; (ii) chemical assisted hatching, using acidic Tyrode's solution; and (iii) laser-assisted hatching.

Mechanical technique: partial zona dissection (Figures 8.1–8.4)

The zona pellucida is pierced with a very thin glass microneedle through both sides, the needle tip position being controlled in the perivitelline space by eye. Then the suction of the holding pipette is stopped and the holding pipette is rubbed against the trapped area of the zona until this area has been completely abraded.

Acidic Tyrode's assisted hatching (Figures 8.5–8.6).

The fine micropipette containing acidic Tyrode's solution is brought very close to the zona pellucida and the acidic solution expelled gently over a small area (~20–30 µm) until the zona is dissolved through to the inside. After the procedure, the embryos are thoroughly washed in fresh medium and cultured until the time of transfer.

Laser assisted hatching

The laser has been shown to be efficient and safe in mice and humans to carry out zona pellucida drilling for assisted hatching as well as for polar body or blastomere biopsy. Recently, infrared diode-laser light focused through a microscope objective has enabled rapid non-touch microdrilling. Embryos are maintained in their culture medium. Special care should be taken to open the internal side of the zona. No washing of the embryos is needed after laser drilling (Germond *et al.*, 1996). This procedure appears to be quick, precise and is chemical free.

Indications

There are questions concerning the efficiency of, and indications for, assisted hatching, and whether patients should be selected or not: A few early randomized prospective studies had demonstrated an increased implantation rate after assisted hatching but this was not confirmed by some later studies. One must also take into account the possible adverse effects of the technique on certain embryos, e.g. those with a thin zona, and the fact that the manipulation deprives the embryo of its protective coat. Monozygotic twinning has been described as associated with mechanical assisted hatching.

So it has been suggested that patients whose embryos had a poor prognosis for implantation should be selected. The criteria used were: the patient's age, basal concentrations of follicle stimulating hormone (FSH), previous unexplained implantation failures, and abnormal zona morphology. In most studies it was shown that, under these conditions, the assisted hatching procedure could significantly increase implantation rates without increasing miscarriage. The benefit for older women is still unclear and some recent investigations (Lanzendorf *et al.*, 1998) do not confirm it. Frozen-thawed embryos may also be considered for assisted hatching, since Check *et al.* (1996) found an improvement of the implantation rate. In summary, assisted hatching increases the implantation rate in some selected populations but further studies are still needed to confirm all its indications.

References

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- Lanzendorf, S.E., Nehchiri, F., Mayer, J.F. *et al.* (1998) A prospective, randomized, double-blind study for the evaluation of assisted hatching in patients with advanced maternal age. *Hum. Reprod.*, **13**, 409–413.
- Malter, H. and Cohen, J. (1989) Blastocyst formation and hatching *in vitro* following zona drilling of mouse and human embryos. *Gamete. Res.*, **24**, 67–80.

Figure Legends

Figures 8.1–8.4

The mechanical method of assisted hatching is shown in detail. The opening is clearly visible in Figure 8.4 (courtesy of M.Bergère and J.Selva, Paris, France).

Figure 8.5

Assisted hatching using acid Tyrode's solution, which is blown from the glass needle in close proximity to the embryo. The zona pellucida starts dissolving within a few seconds (courtesy of M.Bergère and J.Selva, Paris, France).

Figure 8.6

Assisted hatching using acid Tyrode's solution; the opening of the zona pellucida using acid Tyrode's solution is shown between the arrows. The opening should be $<25\text{--}30\ \mu\text{m}$ diameter (courtesy of S.Kahraman, Ankara, Turkey).

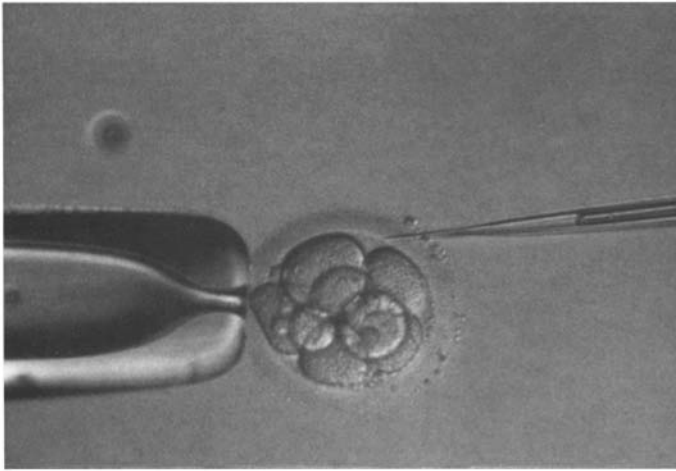


Figure 8.1

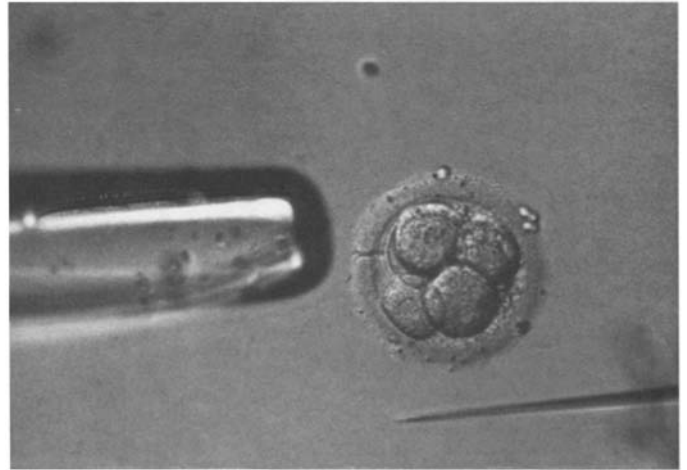


Figure 8.4

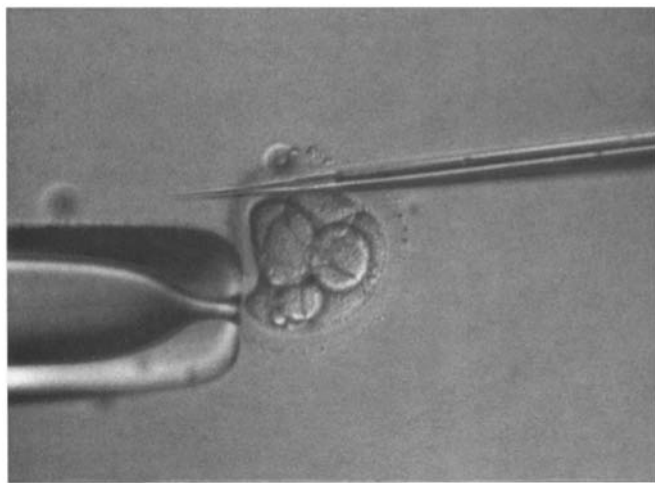


Figure 8.2

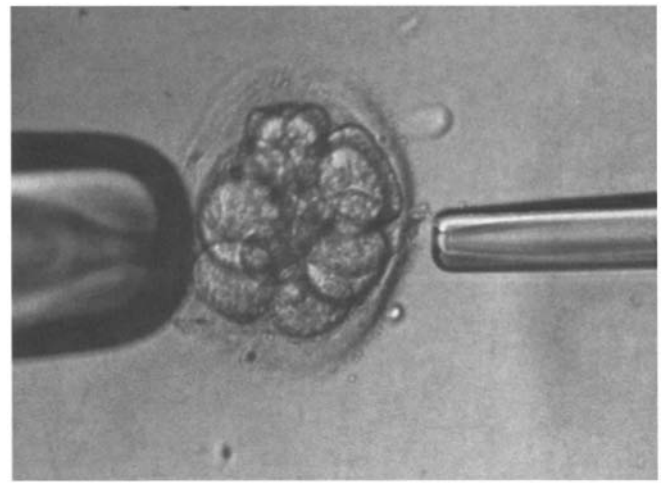


Figure 8.5

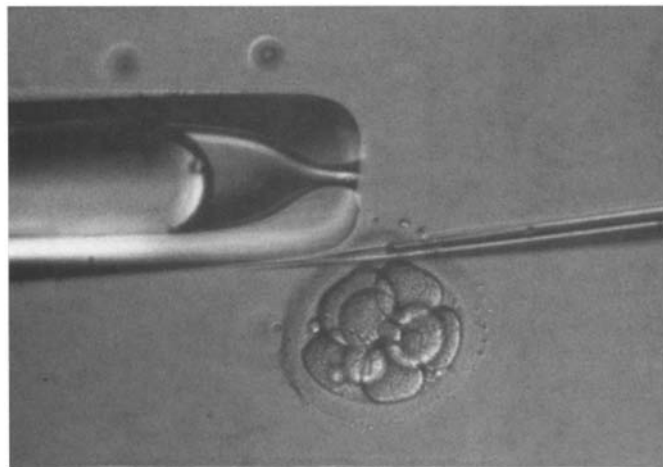


Figure 8.3

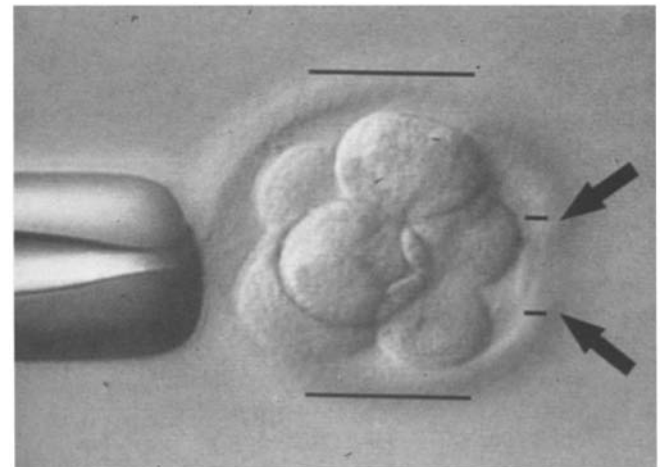


Figure 8.6