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Preimplantation genetic diagnosis: polar body and embryo biopsy

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The introduction of IVF techniques into reproductive medicine in the late 1970s not only changed the approach to infertility treatment, but also permitted the in-depth study of human gametes and early preimplantation embryos. This has progressed considerably with the development of genetic analysis techniques which can be performed on a single cell for the assessment of its chromosomal or genetic constitution. Therefore, the analysis of one blastomere removed from a day 3 embryo was proposed, with the aim of assessing the genetic constitution of the originating embryo (Handyside *et al.*, 1990). This method is especially indicated for couples known to be at risk of transmitting genetic disorders or aneuploidy to their offspring.

There are two basic approaches to obtaining nuclear material for the genetic analysis of in-vitro generated embryos. One is the aspiration of the first and second polar bodies from normally fertilized oocytes. However, this method is informative only for disorders of maternal origin. The other method entails the removal of one or two blastomeres from embryos at the early cleavage stages. This method can also diagnose defects arising from the father and does not appear to affect the embryo's potential to implant, despite the reduction in the overall embryo mass.

The techniques currently used for genetic screening are: (i) polymerase chain reaction (PCR) for single gene defects. This test enables the amplification of short sequences of DNA in a few hours, in such a quantity that makes possible the detection of even single base mutations; and (ii) multicolour fluorescent in-situ hybridization (FISH) for aneuploidy. The labelling of chromosome-specific probes with different fluorochromes permits the simultaneous enumeration of several chromosomes in interphase nuclei.

Polar body biopsy

The oocyte's first meiotic division results in the extruded polar body containing the counterpart of the chromosomes present in the oocyte. Its removal is accomplished through manipulation of the oocyte ~4 h after oocyte retrieval, when the polar body is completely detached from the oolemma (Figure 9.1) (Verlinsky *et al.*, 1990). The oocyte is held by the holding pipette with the polar body at the 11–12 o'clock position. A slit is drilled mechanically in the zona pellucida and the polar body is aspirated with a thin, polished glass needle. The oocyte is then inseminated using the intracytoplasmic sperm injection (ICSI) technique, by introducing the injection needle through the breach already opened in the zona. The whole procedure does not adversely affect either fertilization or cleavage rates, as the polar body is not involved in these processes.

In order to improve the accuracy and reliability of the results obtained from genetic analysis, the second polar body should also be investigated. The use of this strategy permits chromosomal abnormalities associated with the second meiotic division to be identified and, in the case of single-gene mutations, the occurrence of crossing-over between homologous chromosomes can be verified. The second polar body is removed by introducing the aspiration needle through the slit previously made in order to remove the first polar body. This step is delicate as connections frequently still exist between the oolemma and this by-product of the second meiotic division. As an alternative, both polar bodies can be removed simultaneously (Figure 9.2), thus reducing the time and possible stress to which the oocyte is exposed during the micromanipulation procedures, and limiting the biopsies and genetic analysis only to normally fertilized oocytes. However, some concerns still remain regarding the frequent degeneration observed in the first polar body at the time of pronuclear observation.

In conclusion, the polar body biopsy technique is advantageous because it maintains the embryo's integrity, as only meiotic by-products are used to assess the condition of the oocyte. Nevertheless, it is hampered by the impossibility of diagnosing paternally-derived defects, and those originating after fertilization or first cleavage events.

Embryo biopsy

Embryo biopsy is the removal of one or more cells from the preimplantation embryo. The effects of biopsy upon embryo viability and development have been studied at different stages in the mouse. The incidence of embryo damage as the result of the procedure itself was very low, and the highest live birth rates were obtained when the biopsy was performed at the 8-cell stage, compared with earlier stages, possibly due to the reduction of the removed embryonic mass (i.e. 1/8 instead of 1/4).

In humans, the removal of one or two blastomeres is generally carried out 62–64 h after insemination. At this time, compaction starts to take place and cellular damage ensues if the biopsy is attempted when cell–cell interactions and junc-

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tions have begun to assemble. As an option, embryos can be incubated transiently in a medium deficient in divalent cations. However, the disruption of cell adhesions and tight junctions at this stage could alter the subsequent allocation of cells to the trophectoderm and inner cell mass.

The biopsy procedure requires an opening in the zona pellucida of ~20 μm diameter, which is performed chemically, mechanically or using contact laser. The location of the embryo is selected in order to have a nucleated blastomere at the three 'clock position (Figure 9.3). The blastomere is then slowly aspirated (Figure 9.4) and gently released into the medium (Figure 9.5). Extreme care should be taken to avoid cell membrane rupture and damage to the surrounding blastomeres. The biopsied embryo is then thoroughly washed and incubated until transfer, whereas the blastomere undergoes genetic analysis.

Data derived from the implementation of embryo biopsy for preimplantation genetic diagnosis in the human demonstrate that one out of four embryos transferred is capable of implantation. Therefore, the removal of one cell from a day 3 embryo, despite being an invasive technique, does not negatively affect its competence to develop. The extension of the culture period prior to embryo replacement, has made possible the daily monitoring of the embryo growth and comparison with the cleavage rate observed in non-biopsied embryos. Interestingly, the number of cells observed 24 h after the biopsy is lower than expected, implying that either the reduction in cellular mass or the trauma associated with the procedure itself may have an effect on the rate of embryo growth. Nevertheless, compaction, blastocyst formation and hatching take place normally, giving rise to viable, normally-growing concepti.

The chromosomal analysis of embryos on day 3 of development demonstrates a relationship between aneuploidy, maternal age and morphology (Munné *et al.*, 1995). The frequency of chromosomal abnormalities varies depending on the cellular stage and increases with retardation in cell division, with the lowest rate in embryos presenting 7–8 cells at the observation performed 62 h post-insemination. Moreover, analysis of the chromosomal defects shows a relationship between the type of abnormality and the embryo's mitotic rate: monosomy and trisomy prevail in embryos growing within a normal time frame, whereas other abnormalities such as mosaicism, haploidy and polyploidy are more frequent in slow-cleaving embryos (Gianaroli *et al.*, 1997). As a result, the morphological criteria for the selection of embryos to be transferred are now supported by a genetic foundation. In conclusion, the removal of one or two blastomeres from day 3 embryos permits the analysis of the genetic constitution resulting from both the paternal and maternal contributions. In addition, concerns regarding the reduction of embryonic mass have not been substantiated.

Some technical hazards are still inherent to preimplantation genetic diagnosis which requires more research and refinements of the present methods to ensure accuracy and reproducibility of the diagnostic procedures. The ultimate goal is to provide the embryologist with the best tools to recognize those embryos that are capable of full, healthy development. An understanding of the mechanisms and origin of genetic defects, and their possible relationship with the embryo's morphological appearance represents a fundamental progression towards the comprehension of the first stages of human life.

References

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Figure Legends

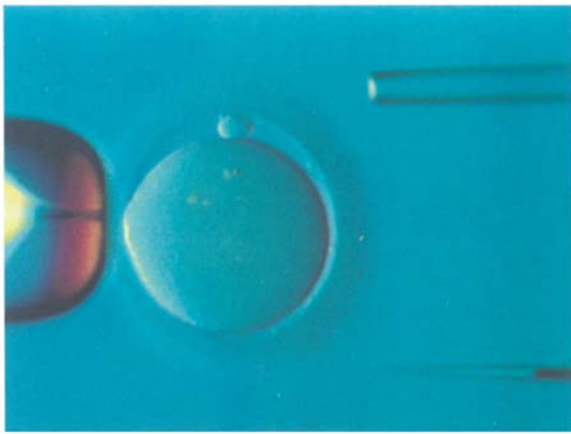
Figure 9.1
First polar body biopsy performed on a metaphase II oocyte, at ×40 magnification. (**9.1.1**) The oocyte is positioned by the holding pipette with the polar body at the 12 o'clock position; the needles for piercing the zona pellucida and aspirating the polar body are aligned. (**9.1.2–9.1.3**) The zona pellucida is perforated by the needle and (**9.1.4**) a slit is made by rubbing it against the holding pipette. (**9.1.5–9.1.9**) The polar body is removed by inserting the aspiration needle and (**9.1.10**) releasing it from the oocyte (courtesy of Y.Verlinsky, Chicago, US).

Figure 9.2
First and second polar body biopsy performed on a normally fertilized oocyte. (**9.2.1**) The zygote is anchored by the holding pipette with the polar bodies at the 11–12 o'clock position; the needles for piercing the zona pellucida and aspirating the polar body are aligned. (**9.2.2**) At ×40 magnification, the zona pellucida is perforated by the needle and a slit is made by rubbing it against the holding pipette. (**9.2.3–9.2.6**) The polar bodies are removed by inserting the aspiration needle and releasing them from the zygote (courtesy of Y.Verlinsky, Chicago, US).

Figure 9.3
Blastomere biopsy from an 8-cell embryo, performed at 62 h post-insemination, magnification ×40. The embryo is maintained by the holding pipette with a nucleated blastomere at the 3 o'clock position. (**9.3.1**) The zona drilling needle is loaded with acid Tyrode's solution and placed close to the zona pellucida. (**9.3.2**) The acidic solution is expelled until a breach of ~20 μm is opened. (**9.3.3**) The needle is then withdrawn after aspirating the excess of acidic solution spread around the embryo during the procedure (courtesy of L.Gianaroli, Bologna, Italy).

Figure 9.4
The biopsy needle is introduced into the perivitelline space through the hole opened in the zona. (**9.4.1–9.4.2**) Very slow suction permits the aspiration of the blastomere. During the procedure, the cell membrane reacts to mechanical stress with elasticity. Extreme care is necessary to avoid the rupture of the membrane and the consequent loss of the nuclear material (courtesy of L.Gianaroli, Bologna, Italy).

Figure 9.5
The removed blastomere is released into the medium; it recovers its original shape within a few seconds. The nucleus is clearly evident.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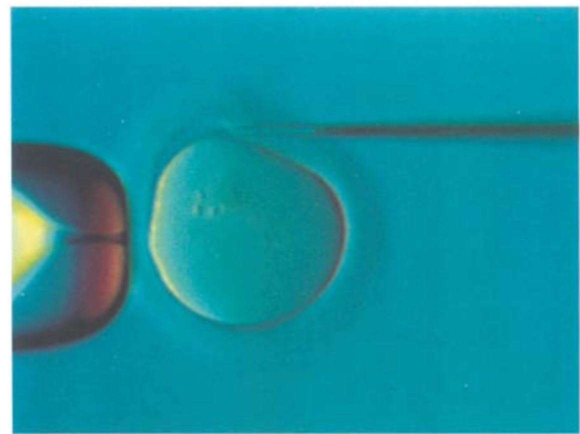
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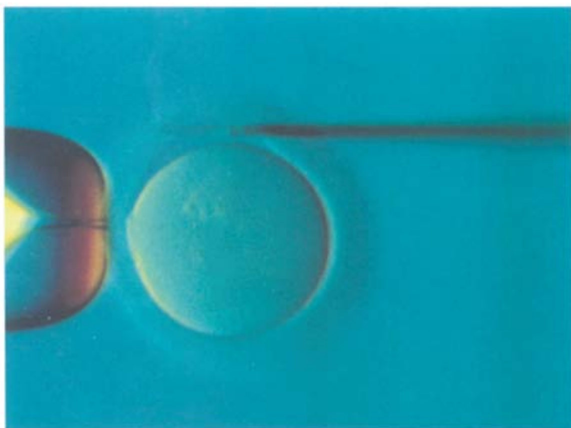
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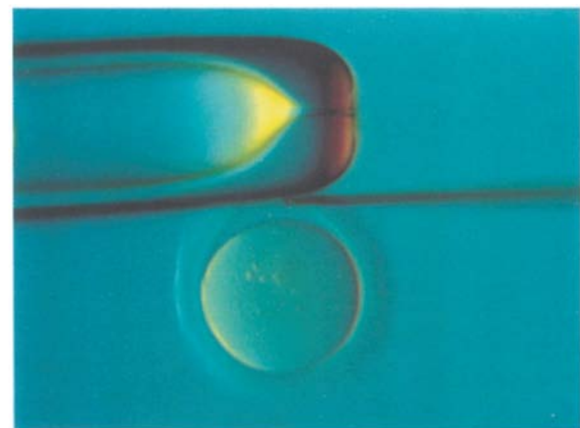
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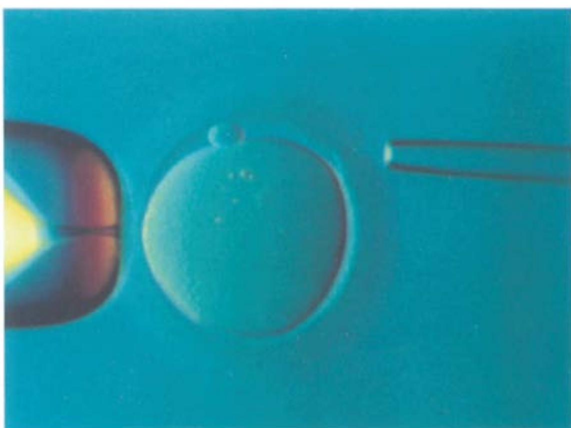
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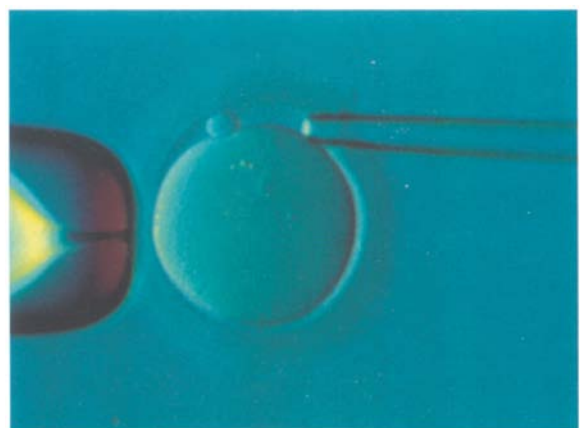
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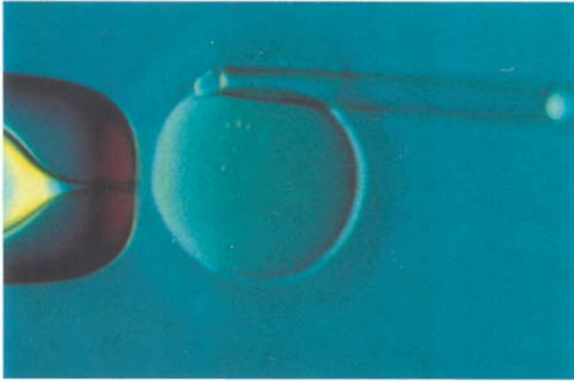
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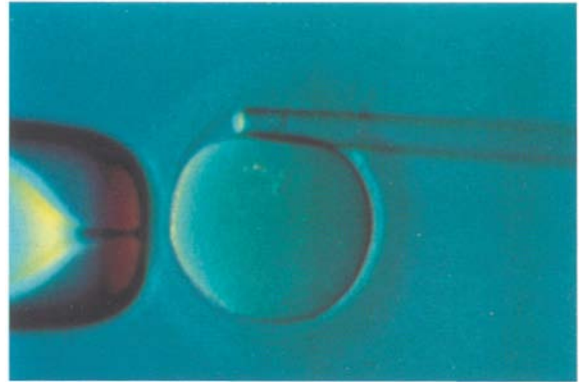
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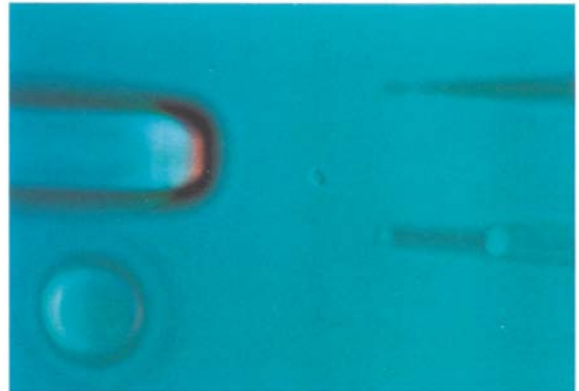
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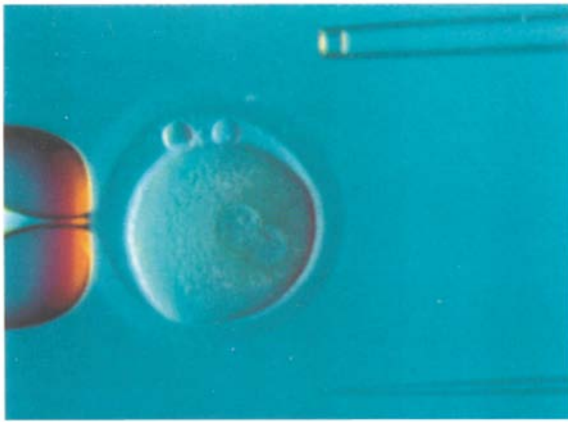

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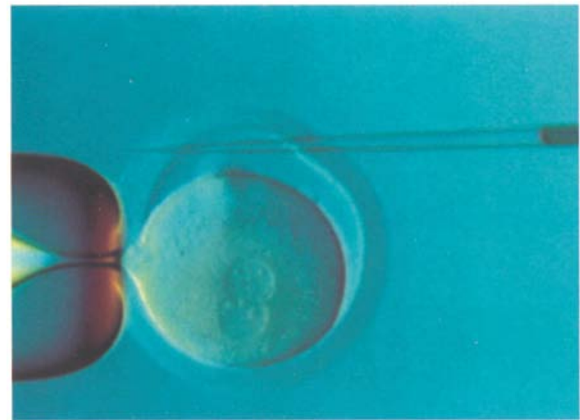

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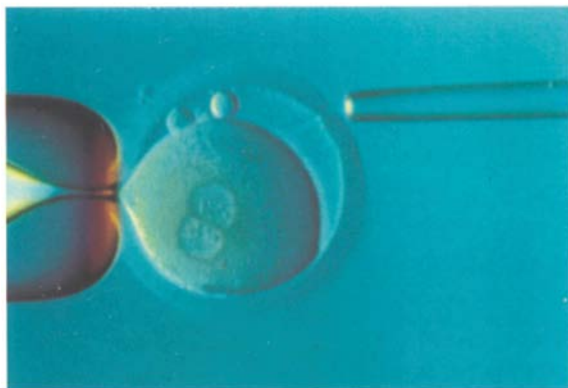

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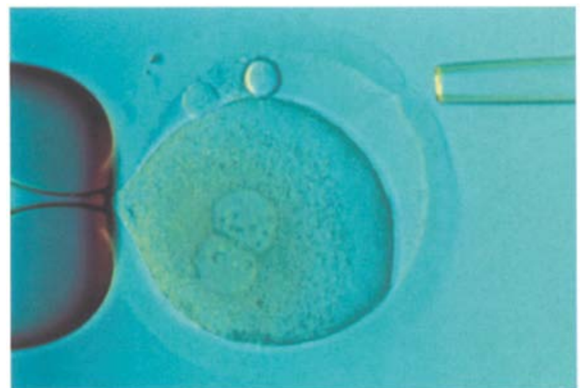

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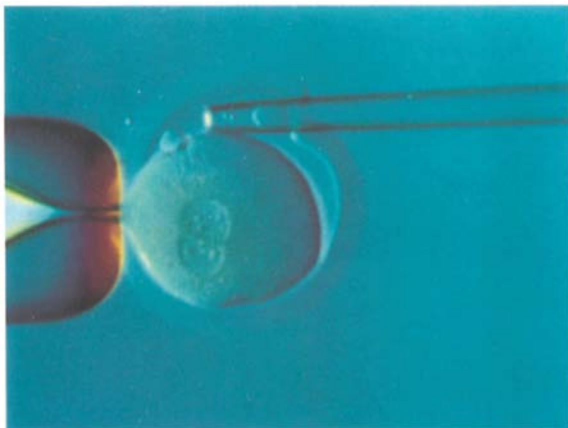

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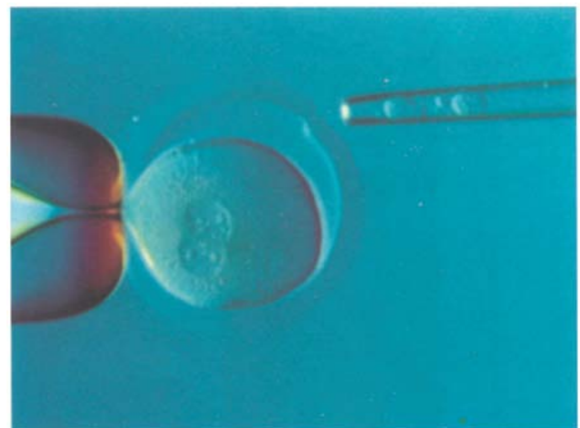

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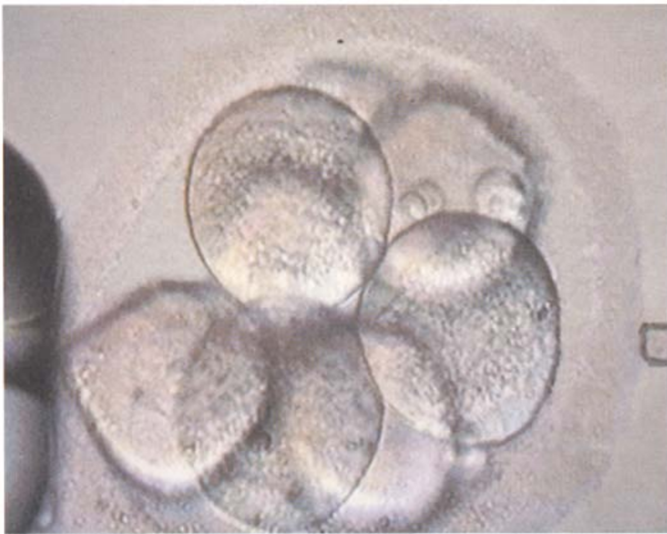
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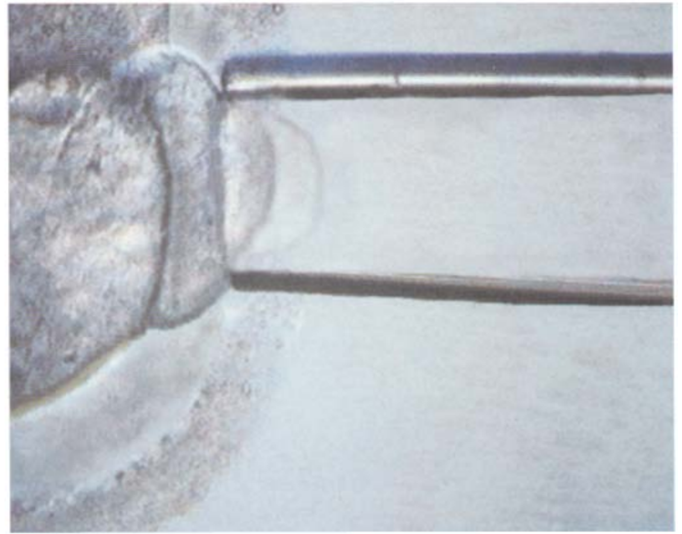
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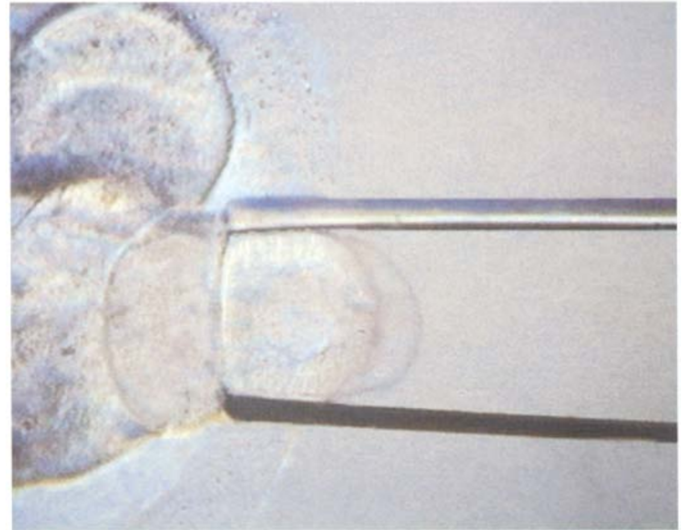
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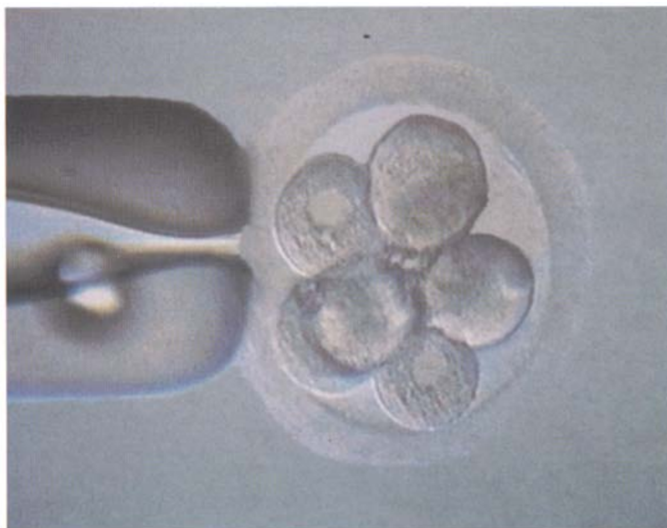
Figure 9.3.3

Figure 9.5