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EDITORIAL

MICROMANIPULATION AND HUMAN REPRODUCTION

Scientists are employing micromanipulation of gametes and embryos in invertebrates for nearly 100 years now. Advent of human in-vitro fertilisation (IVF) and about two decades of research with micromanipulation in mice and other animals have now enabled fruitful employment of these sophisticated techniques for assisting human fertilisation and for studying human blastomeres. Micromanipulation is also being profitably used in animal husbandary. In human reproduction micromanipulation can be employed for :-

(A) Microassisted fertilisation
(B) Preimplantation genetic diagnosis
(C) Therapeutically to control oocyte function or to administer gene therapy and
(D) Possibly for embroyonic banking.

(A) MICROASSISTED FERTILISATION

I. Indications

Zona pellucida is considered to be the main block to sperm penetration of the egg in mammals. When the ability of the sperm to penetrate the zona is believed to be markedly reduced (sperm, number, motility and morphology too poor to attempt IVF) or proved to be absent (failure of fertilisation at IVF) micromanipulation techniques can be employed to assist fertilisation by bypassing the zona. Such microassistance is indicated in -

(1) Severe oligospermia - when progressively motile sperm is less than 0.5 m/ml possibility of fertilisation by standard IVF procedures is extremely reduced

(2) Motility of sperm markedly decreased or absent.
(3) Multiple sperm problems - when there are 3 or more defects in the sperm the possibility of fertilisation at IVF is less than 8%
(4) Failure of fertili-sation at IVF - unless the semen sample is too poor to attempt IVF, failure of fertilisation must be demonstrated at least once at IVF before resorting to micromanipulation.

II. Procedure

(a) Oocytes

After hyperstimulation of ovaries followed by HCG administration the oocytes are aspirated under transvaginal sonography. They are stripped of cumulus corona complex by exposure to 0.1% bovine hyaluronidase in Earle's medium for about 5 min. and gentle aspirations through very fine Pasteur pipettes. They are now examined and those in metaphase II are used. The oocytes are placed in culture medium containing 0.1 to 0.2 M sucrose to shrink the ooplasm and enlarge the perivitelline space. Sucrose is washed off immediately after the procedure.

(b) Sperm

Sperm are prepared by appropriate standard procedure employed for IVF. In mammals. sperm must undergo acrosome reaction before they can fuse with the egg plasma membrane. Since it is not possible to isolate acrosome reacted sperm, many workers advocate that capacitated sperm be held in the capacitated state by substituting calcium in the culture medium by stroncium (2.4 mM $SrCl_2$). Subsequent transfer of the sperm into culture medium containing calcium, prior to micromanipulation, synchronises acrosome reaction increasing the possibility of sperm capable of fusing with the oolema being selected for the procedure.

(c) Micromanipulation techniques

The oocyte is held by a mild suction with a holding pipette having 10-15 μm inner diameter and about 20 μm outer diameter.

(1) Zona cracking - This is done by two fine glass hooks controlled by a micromanipulator. Insemination is now done by placing the oocyte in culture medium containing sperm. The gap produced in the zona is large and this procedure is not used any longer.
(2) Partial Zona Dissection (PZD) - Zona is partially opened by mechanical force with a fine glass pipette and the oocyte placed in culture medium containing sperm. PZD carries a slightly greater risk of polyspermy than SUZI.
(3) Microinsemination sperm transfer (MIST) or Subzonal insemination (SUZI) - Access to the perivitelline space is obtained by zona drilling. This can also be done by using enzymes like trypsin or pronase. Mechanical perforation after softening the zona with chymotrypsin is another alternative. For insemination a few sperm are aspirated, tail first, in the injection pipette having 5-10 μm inner diameter,

10-15 μm outer diameter and 30^0-40^0 bevelled tip. Three or four sperm with just minimal volume of culture medium are deposited in the perivitelline space through the opening in the zona. SUZI needs a few but functional sperm.

(4) In microinjection of the sperm (MIMIC or ICSI) a very fine micropipette (outer diameter 6 μm) is passed through the zonal opening into the cytoplasm of the oocyte to deposit a single sperm therein. Motility of the sperm is not mandatory and even an immobile sperm can be used. Sometimes during SUZI the injection pipette accidentally enters the cytoplasm of the oocyte and the procedure is then converted into an unplanned ICSI.

Zona is avascular and hence the breach created in it does not heal but gets sealed in due course. The trauma inflicted on the zona does not damage the oocyte as proved by the fact that oocytes failing to get fertilised by SUZI can get fetilised if reinseminated by donor sperm.

(d) Embryo Transfer

The oocytes are inspected for fertilisation and subsequent clevage. Those clearing are transfered to the uterine cavity. Luteal phase is supported by progesterone.

(e) Results

Generally speaking 15% of oocytes subjected to SUZI get fertilised. Of the patients undergoing SUZI 30-40% achieve fertilisation. Needless to say that quality of the sperm especially total motile count affects fertilisation rate. Fertilisation rate being low a large percentage of patients having embryo replacement have only one embryo for replacing. This reduces the pregancy rate. Of the patients who have embryo replacement about 15% have resultant pregnancy. Once fertilisation and clevage occurs the chances for achieving pregnancy are nearly as good as after IVF. Thus, of the patients opting for SUZI less than 5% have an established pregnancy. This is not so discouraging when one considered that even IVF offers no hope to these patients.

ICSI carriers a higher fertilisation rate of about 40% of oocytes injected. But the percentage of patients achieving on going pregancy is not substantially different than that with SUZI, possibly because oocytes may be damaged during the procedure.

The procedure of micromanipulation causes parthenogenic activity in 0.3% of the eggs. The incidence of polyspermy ranges from 4 to 7%. This is not higher than that with IVF. PZD carries a slightly higher incidence of polyspermy. In any case, polyspermic eggs can be recognised and discarded. Although zona acts as the main block to polyspermy there appears to be some block at oolemma also.

Although, in micronsemination we are bypassing the sperm selection criteria at the level of zona, incidence of abnormal embryos and birth of abnormal babies is fortunately not increased.

(f) Conclusion

Micromanipulation techniques need expensive equipment and specialised training. Fertilisation rates are low. All the same, this is a practical alternative for couples when IVF has failed or when semen is too poor to attempt IVF. Proper counselling especially emphasizing the low success rate must be done before undertaking this treatment which is designed for situations otherwise untreatable.

(B) PREIMPLANTATION GENETIC DIAGNOSIS

Cells of early embryos are totipotent. Micromanipulation techniques enable us to remove one or two blastomeres for preimplantation diagnosis of genetic disorders or for sexing (in cases of sex-linked genetic problems) without disturbing the development of the blastomere into an individual. Single blastomere cell can be safely removed from a six cell to ten cell human embryo. Polymerase chain reaction and in-situ hybridization permit rapid genetic analysis on a single cell so obtained. Only those embryos which are free from gene defects are transfered to the uterus. Such preimplantation diagnosis prior to embryo transfer obviates the need for chorion villus biopsy or amniocentesis and eliminates termination of pregnancies for preventing birth of children affected by genetic disorders.

(C) THERAPEUTIC POSSIBILITIES

It may be possible to control or direct oocyte functions (eg. meiotic maturation, fertilisation etc.) by microinjection of intact proteins into the cytoplasm of oocytes. Electric field mediated transfer of substances can be an alternative to microinjection.

Cloned DNA can be injected into fertilised eggs for therapeutic purposes (Gene therapy).

(D) EMBRYONIC BANKING

It may even be possible to cryopreserve blastomere cells in the hope of permiting the future individual to utilise his own frozen totipotent cells for developing them into organs for replacing his damaged ones like liver, kidney, lumps, heart etc.

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