

## **In Vitro Maturation of Oocytes**

### ***Response to the Human Fertilisation and Embrology Authority Working Group on New Developments in Reproductive Technology***

Prepared by Policy & Practice Sub Committee of the BFS.

Members: Richard Fleming, Umesh Archaya and Richard Kennedy.

Reliable in vitro maturation (IVM) of human oocytes is an intellectual, scientific and clinical challenge with a number of potential applications. The introduction of IVM in routine clinical in vitro fertilisation (IVF) programmes, is for the present a distant goal, since established methods, using mature oocytes, achieve live birth rates in excess of 20% per treatment cycle<sup>1</sup>. However, the potential of this technology to restore fertility in women anticipating sterility secondary to cancer treatment is an important and exciting prospect.

#### **Background**

In the natural human menstrual cycle many follicles are recruited for growth. After some 14 days only one or two of these follicles usually reach maturation, ~20mm diameter, and are ovulated. The remainder may undergo atresia and their growth arrests. Human oocytes obtained for IVM are aspirated from antral follicles around 2-12mm diameter early in the follicular phase of the menstrual cycle. At the time of retrieval, follicular and oocytes growth is incomplete, and some follicles may already have initiated the process of atresia. Oocytes are matured in the 48 hours subsequent to retrieval, a shortened period of time as compared to the natural cycle, where nuclear maturation follows the luteinisation signal and cytoplasmic maturation is a progressive phenomenon<sup>2</sup>. This truncated growth phase is associated with maturational and developmental abnormalities in the oocyte<sup>3,4,5</sup> evidenced by poor fertilisation and embryo cleavage rates<sup>6,7</sup>.

Maturation in vitro of immature oocytes has been achieved in small mammals, even from primordial follicles, using a number of methods. It is important to distinguish between oocytes from antral follicles and those from earlier stages of maturity. Reliable results have only been obtained using oocytes derived from antral follicles in their final stages of growth. There is evidence that removing human oocytes from their normal follicular environment prior to luteinisation yields oocytes with compromised ability to be fertilised, as exemplified by the failure to elicit normal calcium ( $\text{Ca}^{++}$ ) signalling in response to sperm – egg fusion<sup>3</sup>. Consequently, intracytoplasmic sperm injection (ICSI) has almost universally been employed to achieve fertilisation in human IVM programmes.

Implantation rates following use of this technology are low, possibly consequent upon high rates of aneuploid cells in the embryo<sup>8</sup>. However, the preliminary results on the limited numbers of babies born so far (little more than 20 reported) have been normal<sup>9</sup>.

Research in this area of human biology is difficult to carry out, but a number of centres worldwide are working in this field.

#### Potential of the technology

The principal justifications for exploring the use of IVM in a clinical setting include:

- The simplification of ovarian stimulation protocols
- A reduction in cost of treatment
- A reduced risk of ovarian hyperstimulation syndrome (OHSS)It should provide information required to advance research in the maturation of oocytes from cryopreserved ovarian tissue.

At the present time there are no data confirming these benefits.

#### Clinical setting

There are currently 4 principal scenarios in which IVM technology could be considered for further study at the present time. Given the pace of development in this field, other indications may emerge, so this should not be considered an exclusive list.

#### 1. IVF (ICSI) in women with Polycystic Ovary Syndrome (PCOS) after mild stimulation.

These patients have a tendency to a vigorous response to ovarian stimulation and are at significant risk of OHSS. A milder stimulation protocol as envisaged in IVM would be expected to reduce this risk. Blastocyst development and pregnancy rates are lower in these patients and they have an increased risk of pregnancy loss. It remains to be seen whether IVM techniques influence these outcome measures.

#### 2. IVF (ICSI) in normal women after mild stimulation.

For the reasons as outlined above milder stimulation could be safer for such women but no reliable data as yet exists on outcomes. Potentially milder stimulation regimes perhaps combined with GnRH antagonists could be considered. Recent publications<sup>4,5</sup>, have suggested that normal offspring can result from this form of treatment but further work is required.

#### 3. Salvage of immature oocytes for IVF (ICSI) after standard stimulation.

There is a definite (low) incidence of cases where women, responding to standard stimulation protocols, yield a significant percentage of oocytes at the

germinal vesicle (GV) stage which will not fertilise using conventional IVF techniques. Refinement of IVM protocols might allow such oocytes to be “rescued” and lead to an improvement in fertilisation, embryo cleavage and clinical pregnancy rates. A recent paper from the Brussels group<sup>6</sup> which used ICSI to effect fertilisation after IVM in such cases, showed a high incidence (21%) of non-cleavage of embryos. A further 27% failed to develop beyond the first division. In addition, the remaining embryos showed high rates of aneuploidy, although control evidence was lacking. At the present time it is clear that it would be unwise to use these embryos outside a research setting, and that further research in to refining IVM protocols is required.

#### 4. IVM of immature cryopreserved oocytes for fertility preservation.

This circumstance might include women about to undergo cancer treatment.

### **Recommendations**

The concept of IVM technology complementing current IVF methodology remains attractive. The techniques are at present at a developmental stage but their potential may have far reaching consequences for the way in which clinical IVF is carried out. Basic research into the biological mechanisms of human oocyte maturation and development is to be encouraged and the British Fertility Society would encourage the HFEA to permit further development in this exciting area.

### **The Society Recommends**

- IVM is defined as the culture of oocytes for more than 24h following retrieval and before exposure to sperm.
- Treatment licenses should be provided for clinics who submit appropriate clinical laboratory protocols, patient information sheets (indicating that it is a new treatment with unproven success rates) and consent forms.
- Outcome data from IVM cycles should be collected and reported in a manner similar that proposed for pre-implantation genetic diagnosis (PGD).
- The HFEA should be strongly encouraged to support clinical and laboratory research in this field.

### **References**

1. The patient's Guide to IVF Clinics. *Human Fertilisation and Embryology Authority* (1999)
2. Cheung A., Swann K., Carroll J. The ability to generate normal Ca<sup>2+</sup> transients in response to spermatozoa develops during the final stages of oocytes growth and maturation. *Human Reproduction* **15** 1389-95 (2000)

3. Herbert M., Gillespie J.I., and Murdoch A.P. development of calcium signalling mechanisms during maturation of human oocytes. *Molecular Human Reproduction* **3** 965-73 (1997)
4. Expression of genes encoding antioxidant enzymes in human and mouse oocytes during the final stages of maturation. El Mouatassim S., Guerin P., Menezo Y. *Molecular Human Reproduction* **5** 720-25 (1999)
5. Regulation of human and mouse oocyte maturation *in vitro* with 6-methylaminopurine. Anderliesz C., Fong C-Y., Bongso A., Trounson A. *Human Reproduction* **15** 379-88 (2000)
6. In-vitro maturation of human oocytes from regularly menstruating women may be successful without follicle stimulating hormone priming. Mikkelsen AL, Smith SD, Lindenberg S. *Human Reproduction* **14** 1847-51 (1999)
7. Impact of oestradiol and inhibin A concentrations on pregnancy rate in in-vitro oocyte maturation. Mikkelsen AL, Smith S, Lindenberg S. *Human Reproduction* **15** 1685-90 (2000)
8. Nuclear status and cytogenetics of embryos derived from in-vitro matured oocytes. Nogueira D., Staessen C., Van de Velde H., Van Steirteghem A. *Fertility & Sterility* **74** 295-8.
9. Cha KY, Han SY, Chung HM, Choi DH, Lim JM, Lee WS, Ko JJ, Yoon TK. Pregnancies and deliveries after in vitro maturation culture followed by in vitro fertilisation and embryo transfer without stimulation in women with polycystic ovary syndrome. *Fertility & Sterility* **73** 978-83 (2000)

### **Other relevant publications**

#### Mouse model

Sztejn JM, O'Brien MJ, Farley JS, Mobrauten LE, Eppig J. Rescue of oocytes from antral follicles of cryopreserved mouse ovaries: competence to undergo maturation, embryogenesis and development. *Human Reproduction* **15** 567-71 (2000)

#### Human

Cobo AC, Requena A, Neuspiller F, Aragones M, Mercader A, Navarro J, Simon C, Remohi J, Pellicer A. Maturation in vitro of human oocytes from unstimulated cycles: selection of the optimal day for ovum retrieval based on follicular size. *Human Reproduction* **14** 1864-68 (1999)

Beckers NGM, Pieters MHEC, Ramos L, Zeilmaker GH, Fauser BCJM, Braat DDM. Retrieval, maturation and fertilisation of immature oocytes obtained from unstimulated patients with polycystic ovary syndrome. *Journal of Assisted Reproduction & Genetics* **2** 81-86 (1999)

Alak BM, Coskun S, Friedman CI, Kennard EA, Kim MH, Seifer DB. Activin A stimulates meiotic maturation of human oocytes and modulates granulosa cell steroidogenesis in vitro. *Fertility & Sterility* **70** 1126-30 (1998)