IN VITRO OOCYTE MATURATION

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M.SC SEMESTER III CC 02

Oocyte maturation is a lengthy process, during which the oocyte attains the competence to be fertilized and undergo embryogenesis. Most oocytes in the ovary are not growing and are small and immature. At regular intervals, a number of them start to grow and mature; but, of this cohort, only one will be ovulated, while the rest will die by atresia. On average, a healthy fertile woman will only ovulate approximately 400 of these unique cells. It is also thought that oocytes rest in the ovary, which are correspondence to irreplaceable, are vulnerable to environmental and toxic insults over time mature oocytes.

Concepts of oocyte maturation

Human oocytes usually become arrested in prophase I of meiosis during fetal life. At birth, the oocytes remain in the dictyate phase and each ovary has more than 500,000 healthy non-growing or primordial follicles. Throughout the reproductive life of the woman, cohorts of oocytes are removed from this non-growing pool and commence growth. The earliest follicular growth phase is determined mainly by an increase in oocyte size and forms a few layers of granulosa cells around the oocyte. Once oocytes build their cytoplasm up, follicular growth becomes concentrated on the granulosa cell proliferation and differentiation. This differentiation drives antral cavity formation in follicles. In the antral phase, which is initiated in response to FSH secreted by the anterior pituitary, fluid accumulates between granulosa cells. Acentral cavity is formed, with the mural granulosa cells located at the periphery. The oocyte remains surrounded by closely associated granulosa cells, referred to as cumulus cells, forming the compact cumulus–oocyte complex (COC). At this developmental stage, the follicles become gonadotrophindependent for further development. Under the influence of FSH, the follicles develop from the early antral stage to preovulatory stage. At late follicular phase (middle of menstrual cycle), the pre-ovulatory surge of luteinizing hormone (LH) induces germinal vesicle breakdown (GVBD) and chromosomes progress from metaphase I to telophase I. The completion of the first meiotic division is characterized by the extrusion of the first polar body and formation of the secondary oocytes, both of which contain a diploid chromosome complement. The second meiotic division is initiated rapidly after completion of the first meiotic division and the oocytes reach

the metaphase II stage prior to ovulation. Oocyte maturation is defined as the reinitiation and completion of the first meiotic division from the germinal vesicle stage to metaphase II, with accompanying cytoplasmic maturation necessary for fertilization and early embryonic development. Oocyte maturation is often conceptually divided into nuclear and cytoplasmic maturation. Nuclear maturation is a term that refers to the resumption of meiosis and progression to metaphase II. Cytoplasmic maturation is a term that refers to preparation of oocyte cytoplasm for fertilization and embryonic development. However, these two processes are not completely separated processes. Nuclear maturation is controlled by cytoplasmic maturation. In addition, increasing evidence indicates that modifications and changes have occurred on the surface of oocyte membrane during maturation. Here reference is made to membrane maturation, in order to distinguish the process from cytoplasmic maturation.

In-vitro maturation of immature oocytes Mammalian oocytes acquire a series of competences during follicular development (oocyte growth and maturation) that play critical roles at fertilization and subsequent early embryonic development. Early studies have shown that nuclear maturation can occur spontaneously following culture in vitro of animal and human immature oocytes. However, the developmental competence after fertilization of these oocytes is questionable. Oocyte maturation in vitro is profoundly affected by culture conditions. The percentage of oocytes that can develop to the blastocyst stage is generally considered a suitable indication of developmental competence. However, recent data from animal studies suggested that ‘blastocyst formation’ is a limited predictor of development. The successful production of morphologically normal blastocyst stage embryos has not proved reliable in indicating whether a successful pregnancy will be established. It is difficult for clinical practice to use these important markers at the present time. Further research is required to develop reliable markers for assessing oocyte and embryo viability.

Indications and clinical applications

IVM was first introduced in patients with PCOS and patients who had severe OHSS in their previous IVF treatments but the indications were expanded in recent years and in almost all areas of infertility; IVM can be adapted as an option. Potential indications of IVM are;

-PCOS

-PCO-like ovaries

-Normo-ovulatory patients

-Previous failed IVF attempts

-History of OHSS

-Oocyte maturation problems

-Patients with testicular sperm extraction (microdissection-TESE)

-Emergency oocyte retrieval due to malignancies (estrogen-sensitive tumors)

-Oocyte retrieval from ovarian tissue before vitrification

-Poor responders

-IVM for rescuing IVF cycles

-Resistant Ovary syndrome

-Recurrent implantation failure

-Preimplantation genetic diagnosis (PGD)/preimplantation genetic screening (PGS)

Stimulation protocols and treatment modalities in in vitro maturation

The only difference of IVM from conventional IVF is the maturation of the oocytes under in vitro conditions. IVM is a laboratory term and obtaining immature oocytes is dependent on certain clinical protocols, monitorization, and timing of oocyte retrieval, which is why IVM per se is not a treatment protocol, it is the laboratory part of a stimulation protocol or a stimulation cycle. Types of stimulations are listed below:

- Unstimulated IVM cycles without hCG priming

- FSH priming IVM cycles (75 IU/day for 3 days. Start at day 3)

- hCG priming IVM cycles (10.000 IU-20.000 IU IM when the endometrium reaches 8 mm)

- FSH and hCG priming IVM cycles (the combination of above protocols)

- Cycle independent IVM in cancer patients (random start or letrozole use)

- IVM cycles converted from conventional IVF (rescue procedure)

- Aromatase inhibitor use for ovarian stimulation in IVM (letrozole 2.5 mg twice daily start at day 3 for five days)

- Estrogen-suppressed in IVM (estradiol valearate started on day 3 of the cycle)

Oocyte pick-up (retrieval)

Bovine studies have shown that the diameters of aspiration needles and vacuum aspiration pressure during immature oocyte pick-up (OPU) by the transvaginal route have significant impact on the morphology of COCs and this morphology is involved in the developmental capacity and competence of bovine oocytes

Laboratory procedures

Mammalian oocytes are dependent on the follicular environment for proper maturation. Oocytes and follicles have symbiosis-like interrelations because the follicle loses its competence when the oocyte ovulates from the follicle. In the meantime, oocyte development and meiotic resumption takes place in the follicular milieu after the LH peak. Removal of immature oocytes from the follicles blocks the completion of maturation processes and no well-developed culture environment will be sufficient to perfectly nourish and mature the oocytes derived from IVM. There have been more animal studies on IVM than human studies and information derived from large animal studies may illuminate the path of human oocyte IVM. The follicular conditions at the time of oocyte recovery and the oocyte chromatin distribution may have a great impact on the clinical outcomes in human IVM studies

Conclusion

- The course of IVM is progressing slowly and has a long way to go.

- IVM seems to remain an alternative option until standardized terminology and stimulation protocols are in place.

- The best IVM program may be FSH-hCG priming, yielding 100% GV oocytes with favorable clinical outcomes.

- Nuclear maturation and cytoplasmic maturation are not concordant and cytoplasmic maturation needs to be investigated extensively.

- Embryonic arrest seems more prevalent within the first three days but embryos beyond day 3 are more competent.

- Epigenetic changes in IVM are not significant and not more than changes in conventional IVF.

- IVM can be applied to all indications in which conventional IVF is applied.

- Enrichment of culture media for cytoplasmic maturity may increase the clinical outcomes in IVM cycles.

- For fertility preservation, IVM seems a remarkable option.

- PGS/PGD can be easily performed from the embryos of IVM cycles.

- Embryos derived from IVM oocytes are more susceptible to cryopreservation than GV oocytes.

The enrichment of culture media, standardization of the stimulation protocols and management of cytoplasmic maturity are strongly recommended for improved IVM cycles. Future fertility preservation and young age malignancies draw attention on IVM and as a conclusion, increasing the experience of IVM is recommended for all IVF laboratories, instead of neglecting it.