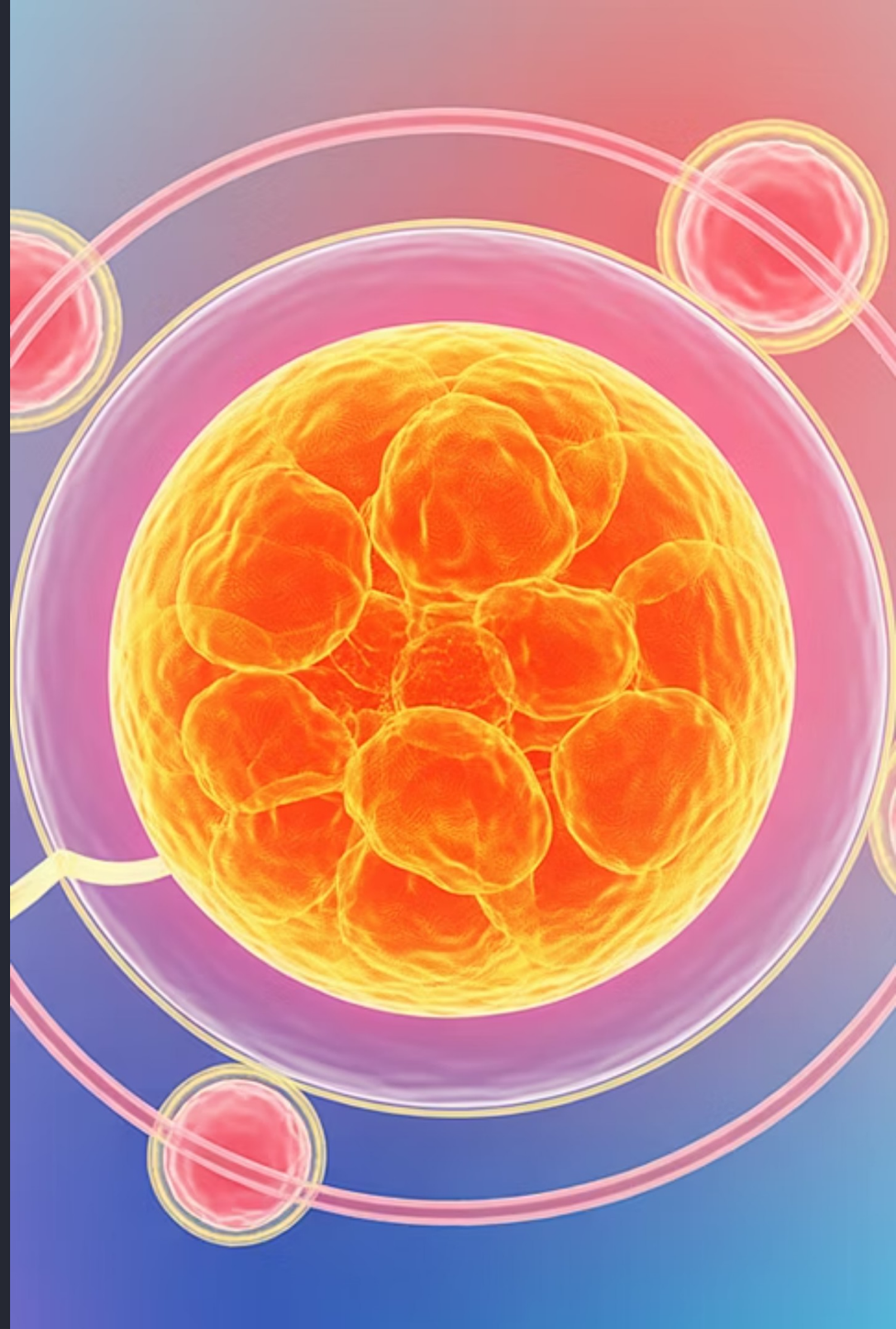
Oocyte Maturation: Preparing for Fertilization and Embryo Development

Oocyte maturation is defined as "reinitiation of the first meiotic division and NUCLEAR PROGRESSION to metaphase of Meiosis II, along with the accompanying cytoplasmic processes occurring within the oocyte that are essential for fertilization and support of early embryo development".

by Fertility Guidance Technologies



Breaking Down Oocyte Maturation

Nuclear Maturation

Oocyte nuclear maturation is pretty straightforward. This is the nuclear alterations that take place during the resumption of meiosis. The goal is to produce a HAPLOID chromosome complement from the previous DIPLOID state.

Cytoplasmic Maturation

Oocyte cytoplasmic maturity means 3 things:

1. Production and presence of specific factors
2. Relocation of cytoplasmic organelles
3. Post transcriptional modification of mRNAs that have accumulated during oogenesis

Somatic Cell Regulation in Oocyte Maturation

Active Role of Oocyte

The oocyte plays an active role in its own development. Oocyte granulosa cell bi-directional communication via gap junctions is essential.

Granulosa Cell Function

The oocyte is responsible for the proliferation, development, and function of the GC (granulosa cells).

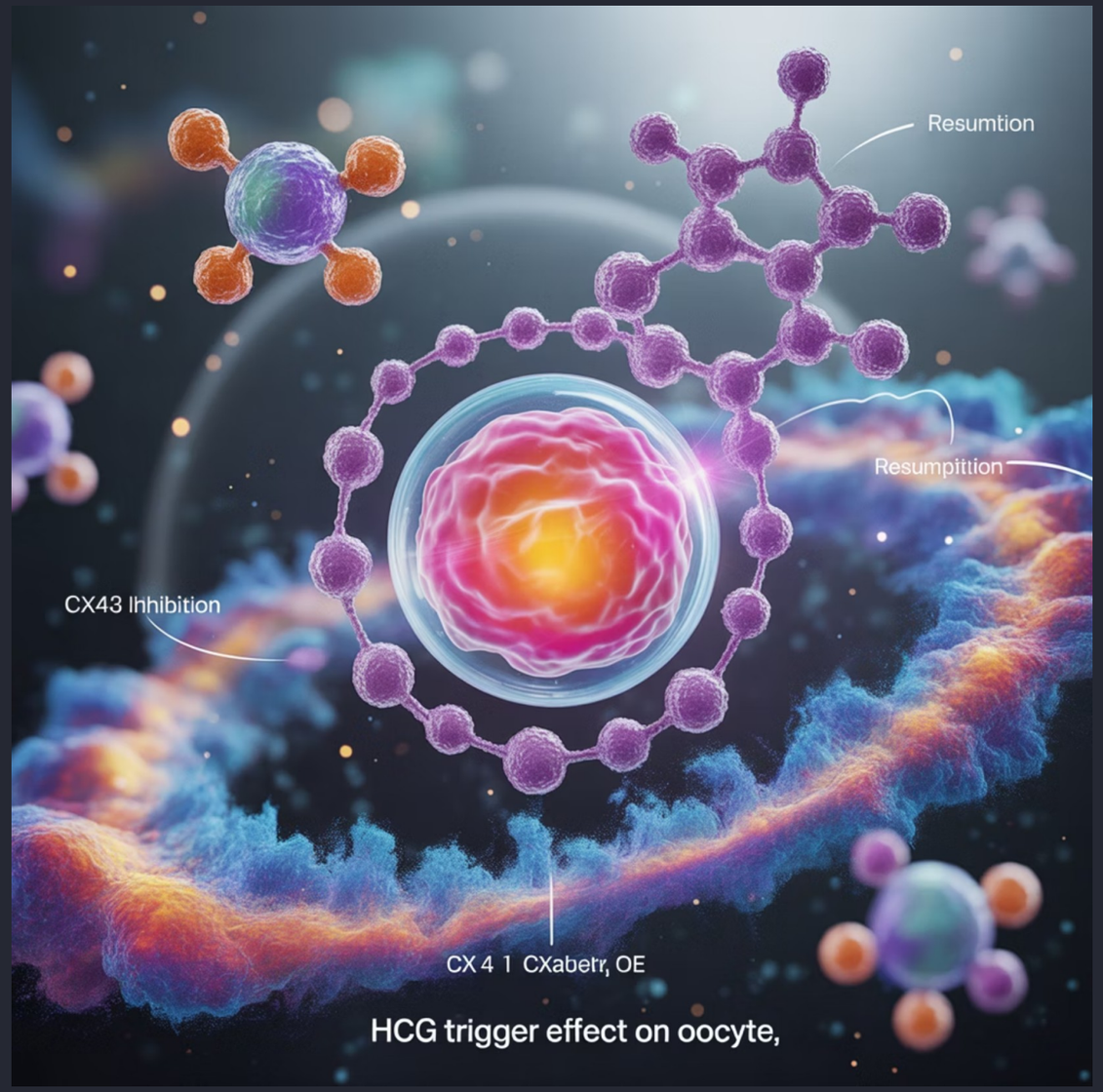
Gap Junction Proteins

The messenger molecules that play a role in follicle maturation. These are called connexins. Cx37, Cx43.

The hCG Trigger and Meiosis Resumption

In IVF cycles, it is the administration of the hCG trigger (LH), that causes the Cx43 molecule to be inhibited, disrupting cumulus oocyte communication and bringing on the resumption of Meiosis!!

The biological definition of oocyte maturation is the reinitiating of the first meiotic division to metaphase-II (M-II) stage, accompanied by cytoplasmic maturation, to successfully prepare the oocyte for fertilization and early embryonic development until the zygotic and embryonic genome activation.



The LH Surge and Oocyte Maturation

LH Surge Trigger

The LH surge triggers oocyte maturation from germinal vesicle (GV) stage to M-II.

In Vitro Maturation

Human immature oocytes can be matured spontaneously to M-II stage in vitro when they were removed from the antral follicles and cultured in the proper culture media.

History of In Vitro Maturation (IVM)

1930s

In vitro maturation (IVM) of human immature oocytes is a technique that was initially attempted and performed in the 1930s. At the same time, human in vitro fertilization (IVF) started by using in vitro matured oocytes.

1

1960s

2

The landmark work of IVM using human immature oocytes was carried out in the 1960s, and the technique of in vitro fertilization (IVF) for human was established with in vitro matured oocytes.

Later Decades

IVM of human immature oocytes was developed as a clinical procedure a few decades after the first live birth from in vitro matured oocytes, which is more than a decade after the procedure of in vivo matured oocyte following IVF.

3

Current Status of IVM Technology

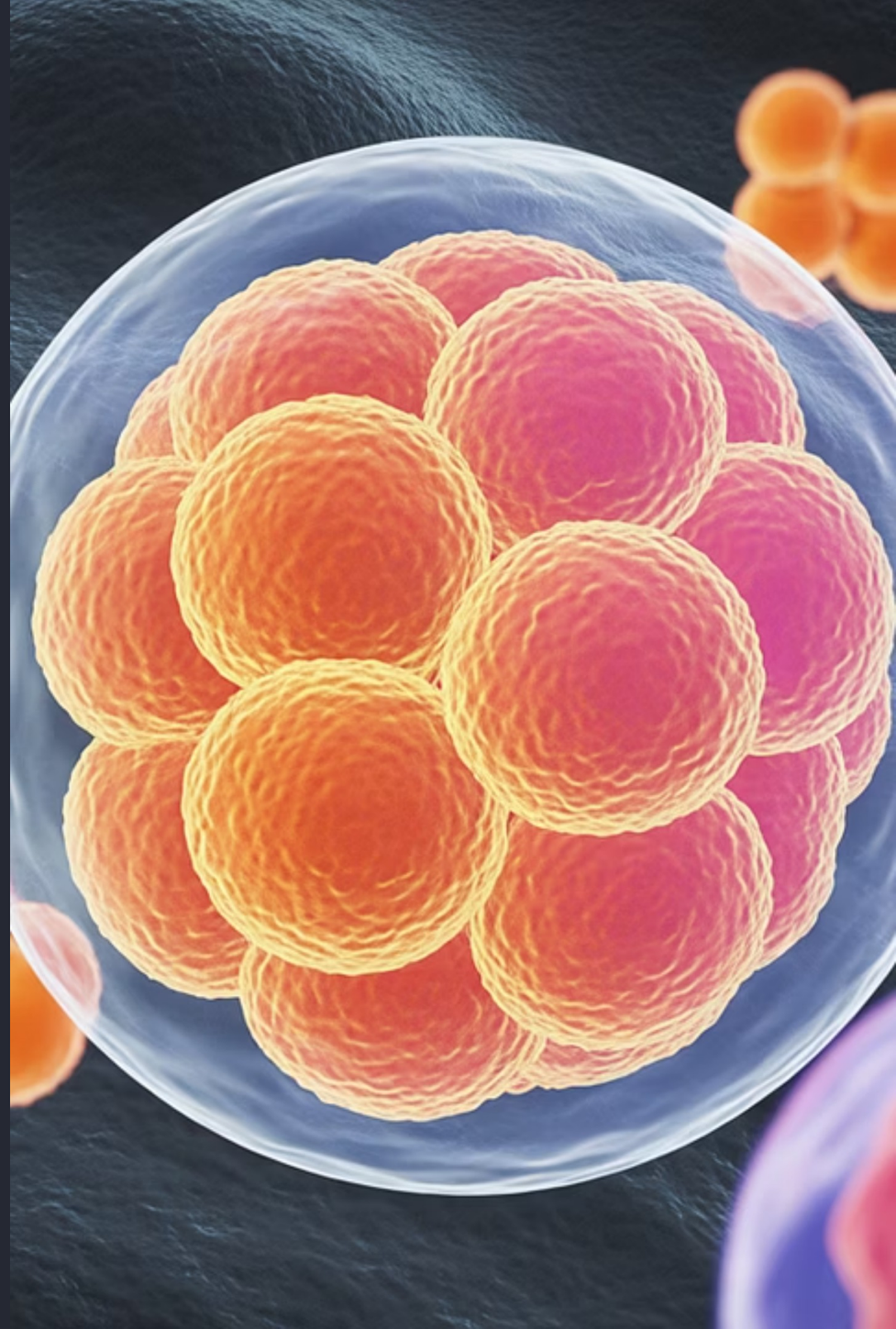
To date, assisted reproductive technologies (ARTs) have helped millions of women overcome infertility globally. Although thousands of healthy babies were born from IVM procedures, as shown in the reports by Trounson et al. and Chian et al., their efficiencies are quite different depending on the source of immature oocytes obtained.

In 2021, the Practice Committees of American Society for Reproductive Medicine (ASRM) for ARTs suggested that IVM technology should no longer be considered experimental and that IVM has the potential for wide clinical application.

IVM is not applicable to every patient and only those with a high antral follicle count (AFC) are considered good candidates.

Source of Human Immature Oocytes

In mammals, including humans, transcription ceases during the final stages of oocyte growth and only resumes when the zygotic and embryonic genomes are activated after fertilization. The oocytes can only use the stored mRNA to synthesize new proteins to support subsequent early embryonic development. Thus, the proper storage of maternal mRNA during oocyte growth is the key point for oocyte maturation through meiosis to generate mature and haploid oocytes.



Oocyte Size and Growth

120 µm

Average Mature Oocyte

It is common belief that once the antrum of follicles is formed, the inside of the contained oocyte is fully grown and the size of the fully grown human oocyte is approximately 120 µm in diameter.

60-171 µm

Size Range

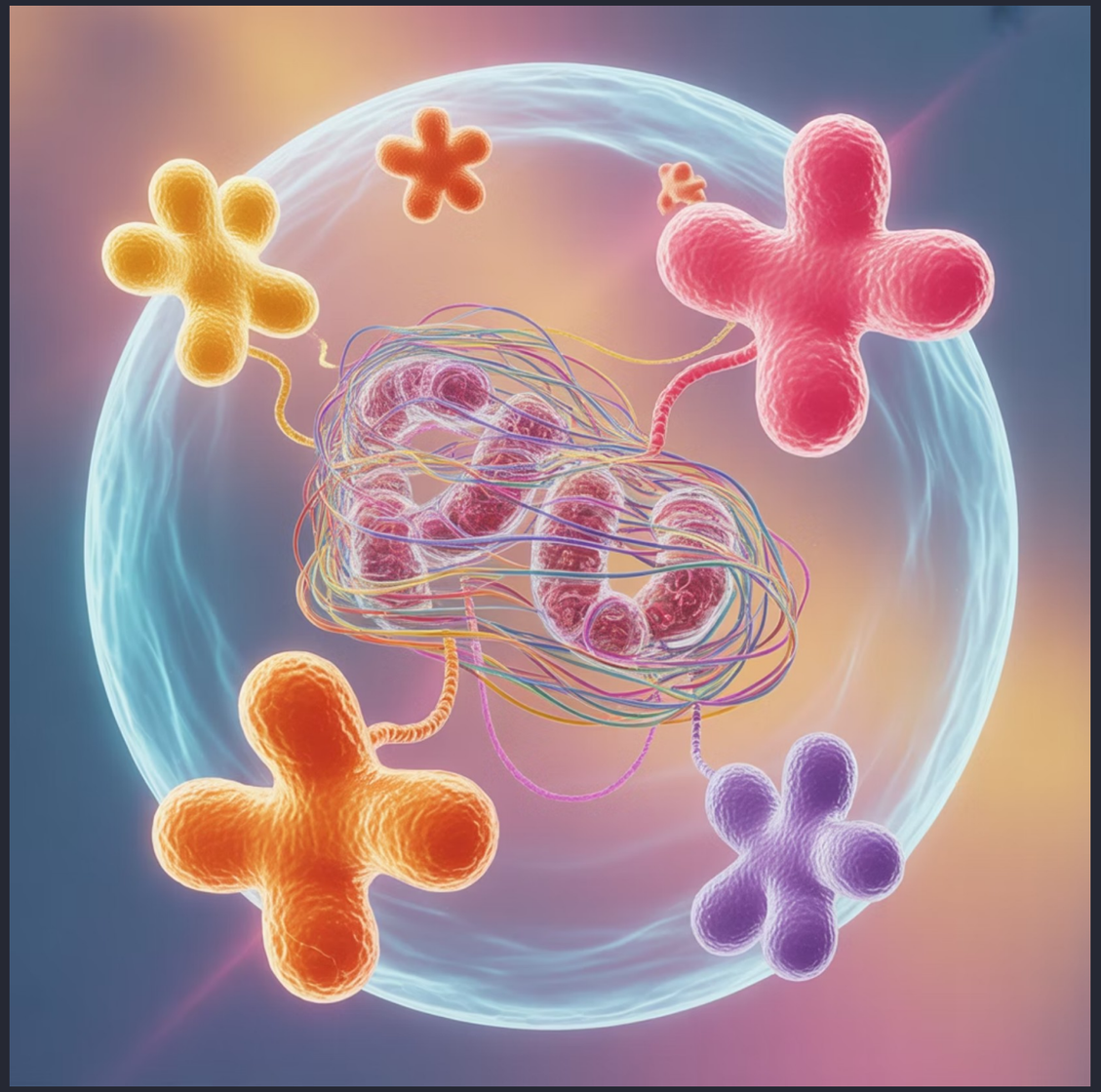
It has been reported that the diameter of human immature oocytes ranges from 60 to 171 µm with a mean of 115 µm and an interquartile range from 107 to 124 µm, when collected from ovarian tissues for fertility cryopreservation derived from women between 14 and 41 years of age.

This indicates that the diameter of human immature oocytes is a highly determining factor in the nuclear maturation of oocytes during IVM. Interestingly, it has been previously reported that human immature oocytes still grow during IVM when the immature oocytes are collected from the unstimulated ovaries with PCOS and the stimulated ovaries for ICSI cycles, indicating that different sources of human immature oocytes can have different growth profiles in vitro.

Mitochondria and mRNA in Oocytes

Recently, it also has been reported that a mitochondria-associated membrane-less compartment controls mitochondrial distribution and regulates maternal mRNA storage, translation, and decay to ensure fertility in mammals, indicating that maternal mRNAs and RNA-binding proteins are mainly deposited around mitochondria in the oocytes.

Although many mRNAs are stored during oocyte growth and translationally activated when the oocytes resume meiosis or after fertilization, it still is largely unknown that whether mitochondrial distribution is changed, and new protein synthesized during the process of oocyte meiotic maturation.



Clinical Definition of IVM

Biological Definition

The biological definition of IVM refers to the reinitiation of the first meiotic division to metaphase-II (M-II) stage, accompanied by cytoplasmic maturation.

Clinical Definition

The clinical definition of IVM technology should be defined as IVM of any immature oocytes, regardless of oocytes with GV stage or metaphase-I (M-I) stage to M-II, because of its involvement in the IVM procedure for immature oocytes (GV and M-I).

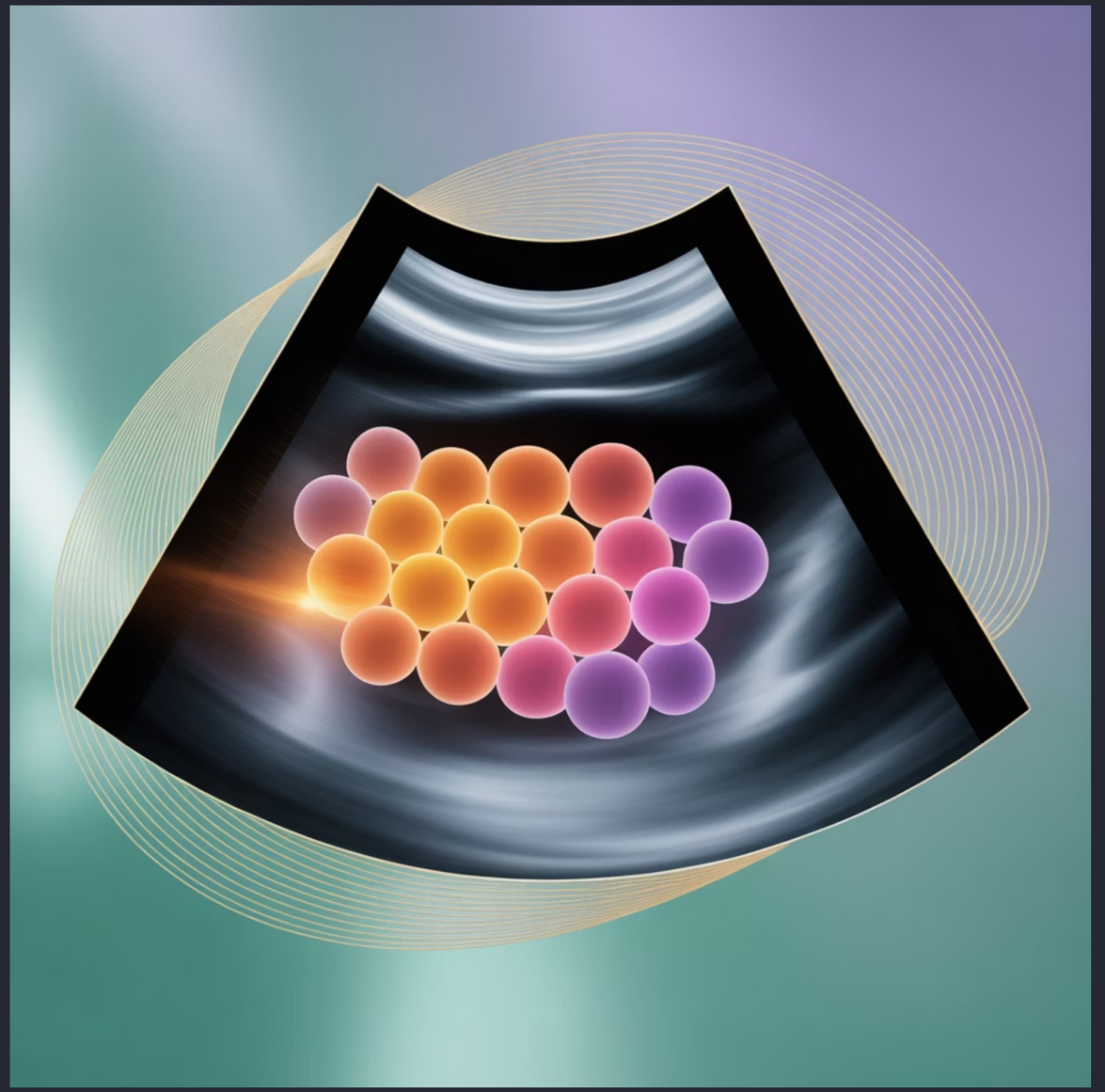
Terminology Note

Maybe, it is interesting to mention here that commonly the mature oocytes is referred as "egg" regardless of the fact if it was matured in vivo or invitro.

Benefits of IVM for PCOS Patients

IVM technology can be a useful technique for infertile women and fertility preservation. IVM is particularly effective for women with polycystic ovaries (PCO) or polycystic ovary syndrome (PCOS) – related infertility, since there are more antral follicles within the ovaries of this group of women.

This group of women is sensitive to ovarian stimulation with the use gonadotropins and has an increased risk of ovarian hyperstimulation syndrome (OHSS) compared to women who have normal ovaries.



Immature oocytes may be retrieved from women after priming with or without the use of FSH, hCG or a

Immature Oocytes from Non-Primed Ovaries

As mentioned above, the initial attempts of IVM for patient own immature oocytes were from women with PCO and PCOS. The immature oocytes were retrieved from the ovaries of follicular and luteal phases, and the immature oocytes were aspirated from the size of follicles at 2–10 mm in diameter. Apparently, those immature oocytes were all at GV stage and the IVM rate of those obtained immature oocytes was relatively low, reporting that the efficiency of IVM technology for infertility treatment was low.

Follicle Size and Menstrual Cycle

Early studies reported that the size-dependent ability for meiotic competence depends not only on the size of the follicles but also the stage of the menstrual cycle. It means that although the immature oocytes can be retrieved from the follicles of luteal phase in the ovaries, the quality of oocytes and maturation rates may be different which compared to the immature oocytes were obtained from the follicles of follicular phase.

Factors Affecting Quality

Therefore, the size and the phase of of follicles may be important factors for the quality of immature oocytes in term of in vitro maturation potential because the maturation rates are directly related to the size and the phase of of follicles.

Clinical Outcomes with Non-Stimulated IVM

It may be true that the clinical pregnancy and live-birth rates were inferior with IVM technology due to the low maturity rate of immature oocytes in vitro when the immature oocytes were retrieved from non-stimulated ovaries, especially from women with PCOS compared to the standard ovarian stimulation IVF cycles.

However, it is not a suitable comparison using the different sources of mature and immature oocytes even though the oocytes were retrieved from the same infertile women with PCOS. As mentioned above, the source of mature and immature oocytes is the key for the successful treatment.

> Apart from the size and phase of follicles and stimulated or non-stimulated cycles, the quality of immature oocytes is also directly related to the age of the woman.

The process of pregnancy to live birth is complex, and multiple factors can affect pregnancy success in infertile women. When using IVM technology to treat infertile women, the age must be considered as a key factor affecting the outcome.

Oocyte Quality Comparison

Age and Oocyte Quality

In Vivo Matured Oocytes

The quality of in vivo matured oocytes reduces after a certain age (>35 years) and is an important factor influencing a successful ART treatment.

IVM Treatment

The same theory applies to the outcome of IVM treatment, and a key obstacle to successful infertility treatment using IVM technology is the process of obtaining high quality of immature oocytes.

Proper Application

IVM is just a technology and if used properly with high quality immature oocytes, the clinical outcomes can be the same as in vivo matured from ovarian stimulated cycles.

Immature Oocytes from Primed Cycles

In vivo meiotic oocyte resumption is initiated by the pre-ovulatory LH surge. The LH surge triggers oocyte maturation from GV to M-II stage. For infertility treatment using IVF technology, women are usually given hCG to induce the completion of follicular oocyte meiosis in natural or stimulation cycles. The final stage of oocyte meiosis can also be induced by the administration of luteinizing hormone-releasing hormone (LHRH) agonist after follicular stimulation for IVF treatment.

> Therefore, without the LH surge, most of the oocytes retrieved would be at an immature GV stage, from the large size of preovulatory follicles.

The IVM technology also involves priming with FSH and/or hCG before immature oocyte retrieval. As mentioned previously, successful pregnancy rate with IVM technology correlates with the number of immature oocytes retrieved.

FSH Priming for IVM

Efficient Recovery

FSH priming promotes efficient recovery of immature oocytes and maturation rate. Although a low-dose FSH priming from the luteal phase improves the efficiency of immature oocyte recovery, the rates of maturation and fertilization are not different between women with regular menstrual cycles and women with irregular cycles of PCOS.

Improved Potential

Similarly, it has been reported that priming with r-FSH during follicular phase before harvesting of immature oocytes from women with PCOS improves the maturational potential of oocytes and the implantation rate of the cleaved embryos.

Enhanced Development

It seems that the use of FSH priming at the beginning of follicular or luteal phases enhances more follicular development and promotes the maturational competence of retrieved immature oocytes.

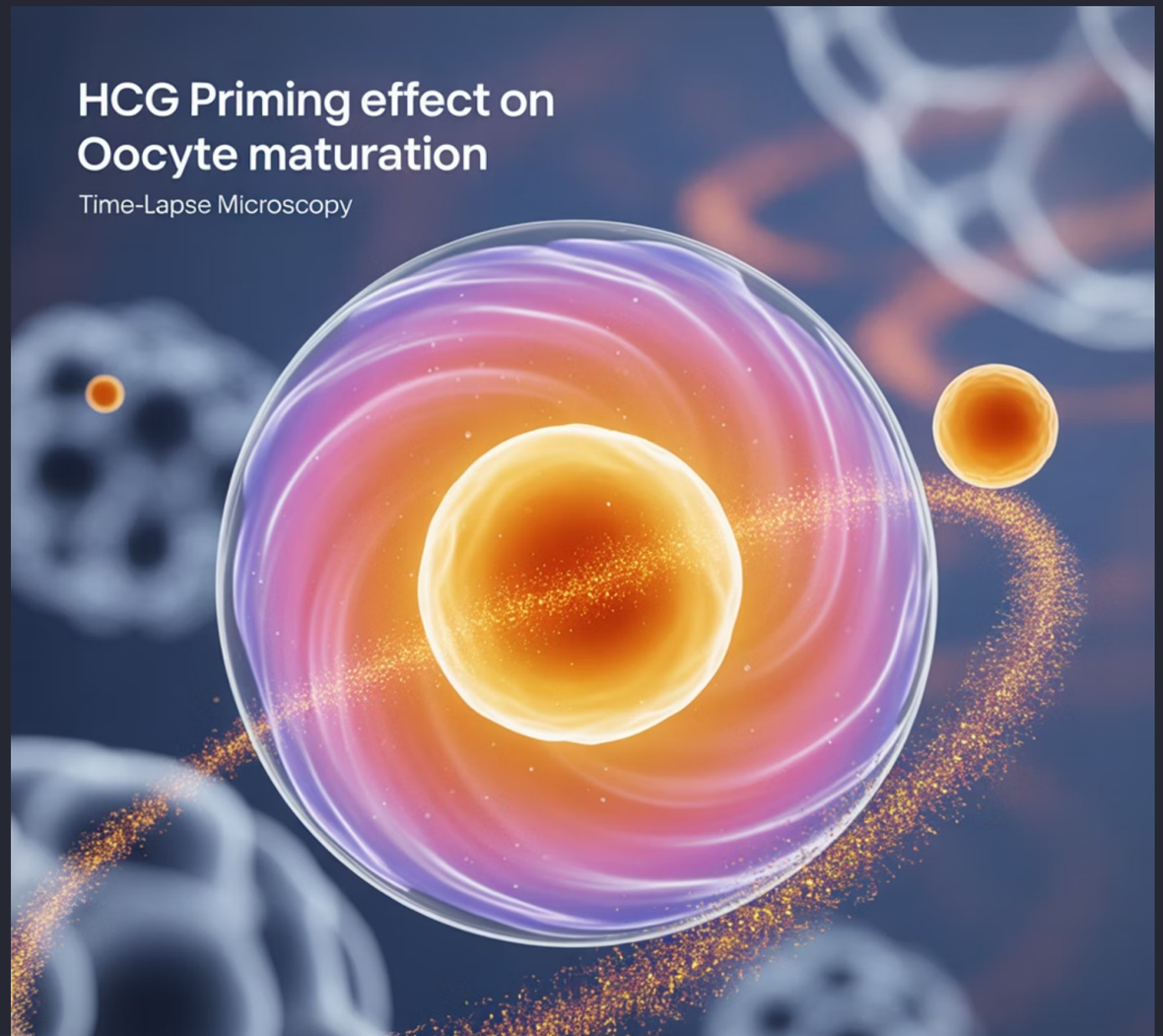
hCG Priming for IVM

It has been demonstrated that the time course of oocyte maturation in vitro is hastened, and the rate of oocyte maturation is increased by priming with hCG 36 h before retrieval of immature oocytes from women with PCOS.

It appears that the oocytes retrieved from follicles in response to hCG may promote the initiation of oocyte maturation in vivo, since there are LH/hCG receptors in the granulosa and cumulus cells from the antral follicles as small as 3.0 mm in diameter, suggesting a benefit to most follicles when using hCG priming before immature oocyte retrieval.

Indeed, although the final rates of oocyte maturation were not different between the immature oocytes priming with and without hCG, the time course of oocyte maturation was different.

Therefore, it is possible that the quality of oocytes and subsequent pregnancy rate may potentially improve by priming with hCG before immature oocyte retrieval.



Immature Oocytes from Ovarian Tissue

1

Surgical Materials

Immature oocytes may be obtained from surgical materials of ovaries at follicular and luteal phases, in which human IVM technology was developed. From those immature oocytes, the first IVM babies were born.

2

Menstrual Phase

It seems that the immature oocytes derived from different phases of menstrual cycle do not affect adversely oocyte maturation in vitro and subsequent fertilization and embryonic development.

3

Fertility Preservation

The immature oocytes from obstetric and gynecological surgical materials may provide the possibility for younger women for fertility preservation with immature oocyte collection.

IVM Media for Human Immature Oocytes

Human oocytes acquire a series of competencies during follicular development (oocyte growth and maturation) that play critical roles in fertilization and subsequent early embryonic development. Although high rates of IVM of immature oocytes may be obtained, the developmental competence of IVM oocyte is still suboptimal, as indicated by the relatively minimal development up to blastocyst stage and the poor implantation rates after transfer.

Oocyte IVM is profoundly affected by culture conditions. So far, numerous data have been accumulated from studies, but the current rationale for choosing a specific medium for IVM of human immature oocytes appears to stem largely from the adaptation of the methods developed for culturing other cell types.

All existing media for oocyte IVM are the base of complex culture media supplemented with different substances.