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Current perspectives on *in vitro* maturation and its effects on oocyte genetic and epigenetic profiles

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In vitro maturation (IVM), the maturation in culture of immature oocytes, has been used in clinic for more than 20 years. Although IVM has the specific advantages of low cost and minor side effects over controlled ovarian stimulation, the prevalence of IVM is less than 1% of routine *in vitro* fertilization and embryo transfer techniques in many reproductive centers. In this review, we searched the MEDLINE database for all full texts and/or abstract articles published in English with content related to oocyte IVM mainly between 2000 and 2016. Many different aspects of the IVM method may influence oocyte potential, including priming, gonadotrophin, growth factors, and culture times. The culture conditions of IVM result in alterations in the oocyte or cumulus cell transcriptome that are not observed under *in vivo* culture conditions. Additionally, epigenetic modifications, such as DNA methylation or acetylation, are also different between *in vitro* and *in vivo* cultured oocytes. In sum, current IVM technique is still not popular and requires more systematic and intensive research to improve its effects and applications. This review will help point our problems, supply evidence or clues for future improving IVM technique, thus assist patients for fertility treatment or preservation as an additional option.

***in vitro* maturation, oocyte, fertility, genetics, epigenetics**

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INTRODUCTION

Definition and application

Oocyte *in vitro* maturation (IVM), is defined as the *in vitro* maturation of immature cumulus-oocyte complexes collected from antral follicles (Smitz and Cortvrindt, 1999). IVM has been used in clinic for over 20 years, and since the first IVM birth in 1991 (Cha et al., 1991), more than 5,000 IVM infants have been born worldwide (Chian et al., 2013; Yang and Chian, 2017).

Significance of IVM

Retrieval of immature oocytes from unstimulated ovaries, followed by IVM was originally proposed in order to avoid side effects of gonadotropin administration. Controlled ovarian hyperstimulation (COH) is widely used to produce multiple mature oocytes to improve the treatment success rate in routine *in vitro* fertilization (IVF) and embryo transfer. COH may, however, cause ovarian hyperstimulation

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syndrome (OHSS), which is a serious and common complication, although the rate of OHSS has come down after introduction of GnRH agonist trigger in antagonist stimulation. Therefore, for patients at high risk for ovarian hyperstimulation syndrome, IVM of immature oocytes obtained in an unstimulated cycle is a safer, less expensive, and simpler procedure than conventional COH (Chian et al., 2004). IVM has the potential to become more popular in the assisted reproduction field once the success rate of IVM is on par with traditional *in vitro* fertilization.

On the other hand, The IVM technology can be extended to the female fertility preservation, which is a frontier in the field of reproductive health. Fertility preservation is the only option for patients with cancer hoping to conserve their fertility (Qiao and Li, 2014). Cryopreservation of ovarian tissue before treatment or radio-chemotherapy of estrogen-sensitive tumors, followed by *in situ* or ectopic implantation, can restore ovarian and reproductive function; however, ovarian tissue cryopreservation and transplantation leads to the risk of reinfusion of malignant cells especially on cancer of blood system (Dolmans et al., 2010; Donnez et al., 2006; Rosendahl et al., 2010). For patients not suitable for ovary transplants, follicle culture and IVM may be the most suitable fertility preservation option available. In addition, fertility preservation is attractive for healthy couples who wish to postpone childbearing. They would also very likely benefit from IVM. Accordingly, IVM technology has broad application prospects.

Problems of IVM

IVM is, however, far from being a widely accepted procedure in many reproductive centers and is not so popular as standard IVF. IVM technology is still considered to be experimental (Yang and Chian, 2017). In 2013, the Practice Committees of the American Society for Reproductive Medicine and Society for Assisted Reproductive Technology declared that IVM should be performed only as an experimental procedure in carefully selected patients (Pfeifer et al., 2013).

A weakness in the procedure is the low maturity of oocytes, as opposed to mature oocytes obtained *in vivo*, and blastocyst formation rate, implantation rate, pregnancy rate, and live birth rate of IVM oocytes is significantly lower, although fertilization rates are similar (Nogueira et al., 2012; Ben-Ami et al., 2011; Mikkelsen, 2005; Zheng et al., 2012a). The rate of abnormal embryonic development and embryo arrest is also significantly higher for IVM than for *in vivo* maturation sources (Ben-Ami et al., 2011; Piquette, 2006; Zheng et al., 2012a). Overall, the application of IVM is constrained largely because of the limited level of oocyte developmental competence *in vitro*, which is influenced by a number of factors. For example, the quality and developmental capacity of oocytes can vary and depend on the intrinsic properties of the follicles (e.g., size and level of atresia). Indeed, acquisition of oocyte developmental potency requires mutual interactions between oocyte and surrounding somatic cells (granulosa or cumulus cells and theca cells), that is the important follicle microenvironment, and which would be interrupted once oocytes are retrieved from follicles. The *in vitro* nutrient environment is therefore particularly crucial and inadequate culture conditions are generally recognized as the major reason for poor development potential of oocytes (Zheng, 2007). These challenges underscore the need to identify the optimal culture methods.

Oocyte maturation includes both nuclear and cytoplasmic programs. Nuclear events refer to oocytes with the competence to complete meiosis (i.e., extrude the second polar body during fertilization), whereas cytoplasmic maturation enables the oocyte to be successfully fertilized and support later embryo development. Full developmental competence is guaranteed only when these two programs are closely integrated. The completion of cytoplasmic maturation, which lags behind meiotic maturation, is difficult to assess microscopically and depends on a complex cascade of events. Any dysfunction of organelles or biochemical processes, mitochondrial malfunctions, defects in maternal mRNA storage, insufficient synthesis of proteins or imprinting defects etc., may result in lower oocyte developmental competency and consequently lead to detrimental effects on embryo quality and development (Coticchio et al., 2012; Gilchrist and Thompson, 2007; Yerushalmi et al., 2011).

We reviewed papers about IVM published mainly from 2000 to 2016 (Figure 1). Our objective of this review is to summarize the present culture methods of human or mainly large mammalian oocytes, explore possible epigenetics effects of IVM, that is understanding current IVM various aspects and problems, thus lay the foundation for further improving the IVM technique.

METHODS AND ADDITIVE SUBSTANCES IN IVM

Priming or not before oocyte retrieval

Preparations before oocytes retrieval for IVM technique include stimulation cycle and natural cycle. Follicle stimulating hormone (FSH) priming generates immature germinal vesicle (GV) stage oocytes that are capable of undergoing meiosis *in vitro*. Therefore, it is widely accepted as encompassing FSH priming and it was adopted in human IVM since 1998 (Wynn et al., 1998). Priming with FSH typically involves daily administration of 150 IU for 3 days, starting from day 3 of the cycle, followed by a coasting period of 2–5 days in preparation for oocyte recovery (Choavaratana et al., 2015; Mikkelsen and Lindenberg, 2001). A prospective,

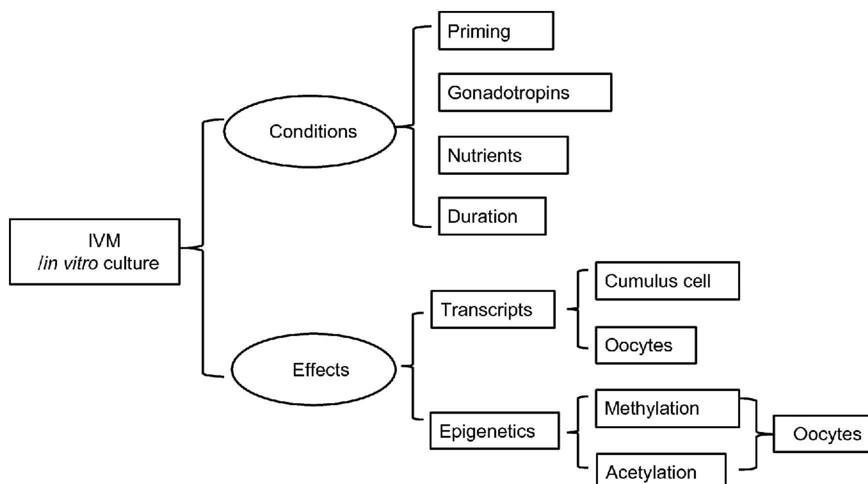

Figure 1 Flowchart of the review on IVM/*in vitro* culture conditions and effects.

randomized controlled study showed that, following priming with 150 IU recombinant FSH per day for 3 days, no differences in the developmental competence of oocytes were observed between coasting for 2 days or for 3 days (Mikkelsen et al., 2003). Studies have shown that using FSH pretreatment can improve the recovery efficiency of immature oocytes, the matured oocytes rate, the implantation rate of the cleaved embryos and pregnancy rate (Chian and Cao, 2014; Mikkelsen and Lindenberg, 2001).

Whether or not using hCG priming is debated. A team of IVM specialists from Canada propose that IVM also include retrieval of oocytes from small and intermediate sized follicles even after hCG or GnRH triggering (Dahan et al., 2016). Reinblatt et al. believed that combination Gns and hCG can increase the number of oocytes retrieved and maturation rate (Reinblatt et al., 2011). Previous findings in our laboratory are that hCG priming can increase the rate of COCs maturation, but the fertilization rate and cleavage rate were not significantly different from non-priming ones (Zheng et al., 2012b). In addition, we summarized the natural cycles IVM data of our center in recent 10 years, comparing the hCG priming group with the control group. There is no difference in the clinical pregnancy rate (37.66% vs. 35.64%, P=0.064) or live birth rate (22.94% vs. 16.48 % P=0.21), between the two groups (Zheng et al., 2012a). A systematic review of Reavey et al. in 2016 believed that there is no clear evidence of hCG effects on the IVM clinical pregnancy rate, live births rate or miscarriage rate (Reavey et al., 2016).

However, recently, Coticchio G believed that use of short FSH priming and/or hCG triggering in IVM cycles have generated much confusion about the definition and clinical outcome of IVM (Coticchio et al., 2016a). Michel De Vos redefined the "IVM" as the existing definition of IVM and included priming with FSH or FSH analogues for follicle growth, but excluded cycles primed with gonadotrophins that are intended to trigger oocyte maturation *in vivo*, such as hCG or GnRH agonists (De Vos et al., 2016). Indeed, these trigger protocols would generate a mixed population of immature (GV), maturing and matured (MII) COCs, which are matured *in vitro* for varying intervals. Therefore, we did not review papers involving that triggered by hCG or GnRH agonists.

Gonadotrophins added in culture medium

The driving forces of follicle development and oocyte maturation are gonadotrophins. FSH is generally recognized as a hormone that improves the health status of follicle cells (Hillier et al., 1995). The addition of FSH to the IVM culture system has long been shown to improve oocyte developmental competence (Farsi et al., 2013). In previous studies, luteinizing hormone (LH) was shown to enhance maturation of immature oocytes due to its natural role as a promoter of ovulation and oocyte maturation *in vivo* (Zuelke and Brackett, 1990). However, recent evidence indicated that addition of LH to the IVM system might be irrelevant for oocyte maturation *in vitro* since human cumulus cells (CCs) express very few LH receptors, especially those of small-medium sized follicles that are targeted in IVM cycles (Maman et al., 2012).

Growth factors or other nutrients

IVM effects are not only influenced by FSH and LH/hCG but also by the combinations and concentrations of local growth factors. Several studies indicate that growth factors are beneficial for oocyte maturation *in vitro* because of the paracrine pathway derived from granulosa cells (Gilchrist et al., 2008; Matzuk et al., 2002; Shimada et al., 2006). The following is the relatively more used factors in culture. Be-

sides these, other growth factors and cytokines, such as bone morphogenetic factor 15 (BMP15) (Sudiman et al., 2014), Cumulin (Mottershead et al., 2015), steroids (especially E2) (Beker et al., 2002; Beker-van Woudenberg et al., 2004; Kim et al., 2005; Tkachenko et al., 2015), and EGF-like peptides (Nyholt de Prada et al., 2009; Richani et al., 2014) were also reported.

EGF

Epidermal growth factor (EGF) stimulates cell growth, proliferation, and differentiation by binding to its receptor EGFR. EGF is reported to stimulate mitosis and induces the resumption of meiosis in oocytes in a range of mammals (Guler et al., 2000; Lindbloom et al., 2008). Recently, Richani et al. reviewed the role of EGF pathway in the complex signalling network which finely regulates oocyte maturation (Richani and Gilchrist, 2017). In an IVM culture system of rhesus macaque cumulus-oocyte complexes (COCs), tyrphostin AG-1478 (a selective EGFR inhibitor) reduced the maturation rate of oocytes and also the percentage of embryos that developed to the blastocyst stage, indicating that the EGFR pathway is important in this setting (Nyholt de Prada et al., 2009). Using porcine oocytes, Uhm et al. showed that EGF could functionally replace the role of FSH on meiotic resumption and could increase oocyte nuclear maturation rate in serum- and hormone-free IVM medium (Uhm et al., 2010). Additionally, these authors found that supplementation of EGF in combination with FSH significantly improved nuclear and cytoplasmic maturation of oocytes, suggesting that EGF has synergistic effects with FSH on cytoplasmic maturation.

In a study of marmoset oocytes, Tkachenko et al. evaluated the effects of 10 ng mL^{-1} EGF in combination with 1 or 10 IU mL^{-1} of gonadotrophins (FSH/hCG 1:1 ratio) during IVM. They found that EGF had different effects depending on gonadotrophin concentration in IVM medium. Addition of EGF increased degeneration and decreased first cleavage rate in the presence of low levels of gonadotrophins but, contrastingly, partially protected oocytes from the negative effects of high gonadotrophin levels (Tkachenko et al., 2010). Supplementation of the maturation medium with the EGF-like growth factors Areg and Ereg significantly improved the maturation rate of human GV oocytes *in vitro*, which in turn enabled the production of a larger number of human embryos (Ben-Ami et al., 2011). Moreover, the addition of exogenous Ereg during mouse IVM significantly increased blastocyst rates, and blastocyst quality was higher with the addition of Areg and Ereg than FSH or EGF, suggesting that Areg and Ereg might serve as better IVM additives than FSH or EGF (Richani et al., 2013).

EGF is reported to stimulate meiotic resumption in mouse oocytes by elevating intracellular calcium concentrations of CCs and decreasing natriuretic peptide receptor 2 (NPR2) guanylylcyclase activity and cGMP levels (Wang et al., 2013). Additionally, this study found that calcium-elevating reagents ionomycin and sphingosine-1-phosphate mimicked the effects of EGF on oocyte maturation and cGMP levels.

BDNF

Brain-derived neurotrophic factor (BDNF) is a protein encoded by the *BDNF* gene in humans. BDNF is a member of the neurotrophin family of growth factors and plays multiple roles in humans, including a regulatory role in ovary development and oocyte maturation. Expression of *BDNF* is found in granulosa cells and oocytes in humans and other mammalian species (De Sousa et al., 2004; Lee et al., 2007; Seifer et al., 2002; Seifer et al., 2006).

BDNF promotes the first polar body (PB1) extrusion of mice oocytes and the *in vitro* development of embryos (Kawamura et al., 2005). It is also reported to specifically promote cytoplasmic maturation of bovine oocytes without influencing nuclear maturation in an IVM model, and can improve the developmental competence and subsequent developmental potential of the bovine early embryo (Martins Da Silva et al., 2005).

Combination treatment with BDNF and metformin improves bovine oocyte maturation rate and early embryo development, although the individual addition of these factors has no effect on embryo development, suggesting that BDNF might play a collaborative role in IVM culture (Hong et al., 2009). However, one paper reported that in human oocytes, addition of BDNF to standard IVM culture had no detectable effect on maturation rate, but almost completely inhibited cleavage of activated oocytes. Treatment with blocking antibodies to BDNF resulted in an increased frequency of polar body emission with activated oocytes (Anderson et al., 2010).

IGF

Insulin-like growth factors (IGFs) are proteins with high sequence similarity to insulin. Insulin-like growth factor-1 (IGF-1), also known as somatomedin C, is a protein that in humans is encoded by the *IGF1* gene. IGF-1 is widely implicated in the regulation of growth hormone and occurrence of cancer, and also plays a role in embryonic and post-natal growth (Baker et al., 1993; Hankinson et al., 1998). In previous studies, IGF-1 was beneficial for oocyte maturation and preimplantation development via autocrine stimulation of cumulus and granulosa cells in a range of species including cattle and pig (Makarevich and Markkula, 2002; Xia et al., 1994). However, IGF-I has been reported to have no effect on murine oocyte maturation rate, although embryo development and blastocyst cell numbers were enhanced when follicles were cultured with IGF-I (Demeestere et al., 2004).

GDF9

Oocyte-secreted factors, particularly BMP15 and growth differentiation factor-9 (GDF9), enhance oocyte developmental competence and provide evidence that COC microenvironment is an important determinant of oocyte developmental programming (Gilchrist et al., 2008; Hussein et al., 2006). GDF9 is a member of the transforming growth factor-beta (TGFβ) superfamily and is one of just two known oocyte-specific paracrine growth factors. It has a critical role in granulosa cell and theca cell growth, as well as in differentiation and maturation of the oocyte. The most notable effects of GDF9 on oocyte competence are observed in the presence of EGF and FSH. Accordingly, Yeo et al. showed that mouse oocytes matured with exogenous GDF9, in the presence of FSH and EGF, had higher rates of development and a higher percentage of hatching blastocysts and blastocyst total and inner cell mass cell numbers; however, in the absence of EGF and FSH, addition of exogenous GDF9 during IVM also improved embryo development as determined by an increase in hatched blastocysts (Yeo et al., 2008). In addition, the presence of 200 ng mL^{-1} GDF9 *in vitro* culture medium could significantly increase bovine oocyte maturation rates, the cleavage rate, and blastocyst formation rates of bovine cloned embryos (Su et al., 2014).

Vitamins

Vitamins are reported to affect glucose metabolism in embryos in a range of species, including mouse and sheep (Gardner et al., 1994; Lane and Gardner, 1998). Inositol, a water-soluble vitamin, reportedly promotes the rate of blastocyst expansion in embryo cultures (Fahy and Kane, 1992). Moreover, addition of vitamins to minimal essential medium is beneficial for the maturation and subsequent development of caprine oocytes (Bormann et al., 2003).

Amino acids

Essential and/or non-essential amino acids play critical roles in diverse processes such as protein synthesis, and as osmolytes, intracellular buffers, heavy metal chelators, and energy sources, acting as physiological regulators(Bavister, 1995; Edwards et al., 1998). Essential and/or non-essential amino acids are common supplements in culture medium used for mammalian *in vitro* embryo development and oocyte IVM. Accumulated evidence shows that the addition of amino acids to the IVM system supports oocyte maturation in many species (Ka et al., 1997; Rose-Hellekant et al., 1998). Indeed, supplementation of vitamins and amino acids to an ovine IVM system significantly increases the blastocyst development rate (Kafilzadeh et al., 2012).

cAMP regulators

Cyclic adenosine monophosphate (cAMP) is a second messenger that plays an important role in many biological processes. cAMP is produced from ATP by a G-protein-dependent enzyme in the oocyte and is used for intracellular signal transduction via the cAMP-dependent pathway. High levels of intra-oocyte cAMP maintain the oocyte in meiotic arrest by suppressing maturation-promoting factor activity mediated by stimulation of cAMP-dependent protein kinase A.

Few studies have analyzed the effects of cAMP on human oocyte maturation in culture and the majority of studies have focused on animal models. It has been shown that a decrease in oocyte cAMP levels might contribute to spontaneous mouse oocyte maturation *in vitro* (Downs et al., 1989). In addition, the elevated levels of cAMP triggered by glucose treatment associate with a lower rate of GV breakdown (GVBD) in mouse oocytes (Downs, 1995). Also, elevation of cAMP or activation of protein kinase C was shown to inhibit porcine oocyte meiotic maturation, which can be likely attributed to down-regulation of mitogen-activated protein kinase activation (Fan et al., 2002; Laforest et al., 2005; Yeo et al., 2008).

Accumulating evidence showed that exposure of pig COCs to dibutyrylcAMP (dBcAMP) during the first 24 hours of culture enhances their developmental competence (Bagg et al., 2006; Somfai et al., 2003). Also, it has been recently demonstrated that supplementation IVM cultures with dBcAMP increases the normal fertilization rate and lowers the rate of adherent COCs in pig oocytes, while maintaining the blastocyst rate (Appeltant et al., 2015). The addition of a cAMP modulator, forskolin, to the IVM culture medium significantly improves the developmental competence of vitrified-warmed bovine GV oocytes (Ezoe et al., 2015). Moreover, the developmental competence of mouse oocytes is positively influenced by the addition of the cAMP modulators 3-isobutyl-1-methylxanthin and forskolin, as well as FSH, during IVM (Zeng et al., 2014).

C-type natriuretic peptide

C-type natriuretic peptide (CNP) and its receptor NPR2 play important roles in maintaining oocytes meiotic arrest (Zeng et al., 2014). As the essential component of medium of mice oocytes *in vitro* culture, also containing FSH and GDF9, the first polar body extruding rate and blastocyst formation rate were both significantly improved (Romero et al., 2016). 200 nmol L^{-1} CNP pre-treatment before IVM could increase quality of bovine oocytes and blastocyst rate (Zhang et al., 2016). After using CNP to delay meiotic resumption, the developmental competence of goat oocytes was enhanced. The oocyte maturation rate, the cleavage rate, blastocyst rate and total cell number of blastocysts were all significantly increased (Zhang et al., 2015). 10 and 50 nmol L^{-1} CNP pretreatment significantly improved fertilization and blastocyst rate of mouse oocytes (Wei et al., 2015). Prematura-

tion culture in the presence of CNP followed by IVM using FSH and AREG can increase the human oocytes meiotic maturation rate than routine standard IVM (Sánchez et al., 2017).

Culture time

Identifying the best culture time of IVM for maximum oocytes potential is crucial and not easy. It can discriminate metaphase-II (MII) stage oocytes with extrusion of the first PB from GVBD stage oocytes after removing the CCs. However, cytoplasmic maturation not only lags behind nuclear maturation, but also is not easily observed. That is, the cytoplasm may not mature even if the nucleus did. Therefore, in many situations nuclear maturation is accompanied by an immature cytoplasm during short time culture. It has been reported that a proportion of oocytes had already developed to MII stage within 24 h culture (Ge et al., 2008); thus it is recommended that human oocyte maturity be assessed after 1 day in culture. Some studies reported a culture time of 24–36 h (Mikkelsen and Lindenberg, 2001; Söderström-Anttila et al., 2005), and a majority of IVM studies showed that complete mature time requires 48 h (Benkhalifa et al., 2009; Son et al., 2008; Wei et al., 2008). Altogether, there is different culture time that may also be associated with different priming condition for oocytes retrieval. Most reported the culture time were during 24 and 48 h, while only two reported last for up to 52 h (Das et al., 2014; Jurema and Nogueira, 2006; Shavit et al., 2014). Conversely, prolonged culture periods may lead to oocyte aging and a decline in quality. One study of animal comparing 24 and 48 h *in vitro* culturing of bovine oocytes showed that a 48 h period led to aging-like alterations in bovine oocytes, and suggested that epigenetic mechanisms are critically involved in oocyte aging at this time. Additionally, cleavage rates and blastocyst yields were significantly lower in oocytes after 48 h *in vitro* culture than after 24 h (Heinzmann et al., 2015). In conclusion, the precise maturation time for high quality oocytes needs to be examined further and might be individualized depending not only on the oocytes, but also on the patient's endocrine condition or the culture system used.

Our work

Previously, to increase the efficacy of IVM, we chose three factors: EGF, IGF-1 and BDNF, added to the human oocytes IVM medium and found that they individually can significantly improve the oocytes maturation rate, but not the fertilization or balstocyst rate. Thereafter, the combination of EGF, BDNF and IGF-1 can effectively increase human denuded GV or MI oocytes maturation rate and quality *in vitro*, and significantly improve the oocyte quality in terms of morphology and normal spindle levels. Also, the developmental competence of fertilized oocytes to 8-cell and blastocyst stages was improved by the addition of growth factors (Yu et al., 2012). However, when similar methods were used for culture of immature oocytes retrieved from PCOS patients by transvaginal immature follicles aspiration, we did not observe the significant increase of oocytes maturation rate by these factors (P=0.053, unpublished data).

DIFFERENCES IN THE EFFICACY OF *IN VITRO* AND *IN VIVO* MATURATION

Some efforts have been made to elucidate the differences in efficacy of *in vitro* and *in vivo* maturation (Nogueira et al., 2012; Yerushalmi et al., 2011). Compared with *in vivo* matured oocytes, normally fertilized IVM oocytes of PCOS patients were significantly larger at the sperm injection and second polar body extrusion stages (Walls et al., 2016). Large mitochondria-vesicles complexes partially replaced mitochondria-smooth endoplasmic reticulum aggregates in IVM oocytes by using transmission electron microscopy for morphometric criteria of evaluation (Coticchio et al., 2016b). *In vitro* matured mouse oocytes are more susceptible than *in vivo* matured ones to mock ICSI induced mitochondrial distribution pattern change (Uppangala et al., 2015). The following section describes the influence of *in vitro* culture on genetic and epigenetic aspects of oocyte maturation including those triggered by hCG or GnRH.

Effects of *in vitro* culture on cumulus cell gene expression

In a study comparing the transcription profiles of CCs derived from COCs matured *in vitro* or *in vivo*, 64 genes were found to be differentially expressed between the two groups. Key genes associated with cumulus expansion (*TNFAIP6*) and regulation of oocyte maturation (*INHBA* and *FST*) were down-regulated in *in vitro*-derived CCs, whereas stress response genes (*HSPA5* and *HSP90AB1*) were upregulated (Tesfaye et al., 2009).

Microarray technology was used to compare the expression profiles of CCs from COCs at GV, MI, and MII stages following IVM or *in vivo* maturation. The molecular signature of CCs from IVM was found to be different from that of CCs matured *in vivo*. Specifically, the molecular signature of CCs from COCs matured *in vitro* with OCs at MII stage was characterized by over-expression of the GV and MI stage signatures, whereas genes belonging to the CCs from *in vivo*-matured COCs were down-regulated or undetectable. Furthermore, the expression of genes involved in cumulus expansion (*TNFAIP6*, *PTGS2*, and *PTX3*) as well as those related to oocyte maturation, including several EGF-like growth factors (EREG, AREG and BTC), were lower in CCs from *in vitro*-matured oocytes than in equivalent cells from

in vivo-matured oocytes. Additionally, cell cycle-related genes, such as cyclins and CDKs, were upregulated in CCs from IVM oocytes, implying that CCs matured *in vitro* are not yet fully mature (Ouandaogo et al., 2012; Ouandaogo et al., 2011).

Effect of *in vitro* culture on oocyte transcripts

Although no data were presented for later fecundity, Ibáñez et al. found that mouse oocytes matured *in vitro* had a fewer number of cytoplasmic microtubule organizing centers (MTOCs) than *in vivo* counterparts, and also a larger spindle and polar body size, irrespective of the IVM conditions (Ibáñez et al., 2005). In another study, two-cell-stage embryos derived from *in vitro*-matured bovine oocytes had a dramatically lower amount of many maternal mRNAs than *in vivo*-matured oocytes, which partially accounts for the reduction in developmental potential of these IVM oocytes (Lequarre et al., 2004; Zheng, 2007).

Profiling of human genome arrays generated from *in vitro*- or *in vivo*-matured oocytes showed that greater than 2,000 genes were upregulated more than 2-fold and 162 genes were upregulated 10-fold or more in oocytes matured *in vitro* than in oocytes matured *in vivo*. Most of these genes are related to transcription or other cellular events (Jones et al., 2008).

In a similar study using cDNA arrays to compare the transcriptomes of *in vitro*- and *in vivo*-matured monkey oocytes, a small set of just 59 mRNAs were differentially expressed between the two conditions. mRNAs were associated with cellular homeostasis, cell-cell interaction, and mRNA stability and translation function. In particular, the level of expression of two maternally imprinted genes, *PLAGL1* and *MEST*, was significantly higher in *in vitro*-matured oocytes than in their *in vivo* counterparts, implying abnormal epigenetic programming (Lee et al., 2008).

Effects of IVM on epigenetics of oocytes

Epigenetic reprogramming during gametogenesis and early embryonic development refers to scheduled global chromatin modifications without alteration of the DNA sequence, which leads to the re-establishment of gene imprinting patterns. Genomic imprinting is a specialized epigenetic mechanism that marks genes during gametogenesis to allow their parent-of-origin specific expression. DNA methylation at CpG islands and histone acetylation are two major epigenetic marks that alter the functional state of chromatin through activation or repression of gene expression.

Oocyte maturation is a critical period of oocyte development and differentiation during which the oocyte genome is epigenetically reprogrammed. Oocyte growth and maturation appear to be vulnerable to environmental factors. Whether human oocytes matured *in vitro* are intrinsically compromised or whether culture conditions induce epigenetic alterations to deregulate gene expression is contentious. Here, we mainly focus on reports in large animals and humans.

Effects on DNA methylation

A study comparing the capability of pig oocytes matured either *in vivo* or *in vitro* to carry out epigenetic processes found that the monospermic fertilization rate was significantly higher in *in vivo*-matured oocytes than in IVM oocytes. IVM oocytes also had a reduced epigenetic competence and a reduced ability to transform the chromatin of penetrated sperm into male pronucleus (PN). Analysis of global DNA methylation in the late PN stage showed that male PN in IVM zygotes had a reduced active demethylation and histone H4 hyperacetylation epigenetic competence (Gioia et al., 2005). In a second study using bovine oocytes as a model to determine putative epigenetic mutations at three imprinted gene loci (*H19/IGF2*, *PEG3*, and *SNRPN*) in different *in vitro* culture conditions, or *in vivo*, no significant alterations in individual CpGs or in the entire allele methylation error rate were found between the IVM and the *in vivo* group; however, different mRNA expression profiles were found between *in vivo*-matured oocytes and their *in vitro* counterparts (Heinzmann et al., 2011).

A recent literature review of the risks for imprinting defects in human oocyte IVM found that no definitive conclusions could be drawn because of the lack of well-designed studies (Anckaert et al., 2013). In a small-scale study of human IVM oocytes, one quarter (5/20) were found to have an altered methylation pattern of the H19 differentially methylated region, which is normally unmethylated in maternal alleles (Borghol et al., 2006). In a similar study, three maternally-methylated (*LIT1*, *SNRPN*, and *PEG3*) and one paternally-methylated (*GTL2*) imprinted genes were compared in 38 *in vivo* and 71 *in vitro* matured oocytes. The latter were retrieved from 2–9 mm follicles of PCOS subjects in minimally stimulated cycles without hCG priming. No significant increase in imprinting mutations at *LIT1*, *SNRPN*, *PEG3*, and *GTL2* was found in IVM oocytes using limiting dilution bisulfite sequencing (single cell methylation analysis) (Kuhtz et al., 2014). Finally, in a recent study examining the possible transmission of epigenetic defects to the next generation, the methylation level of a range of developmentally important genes and interspersed repeats was measured in 11 human IVM newborns and 19 controls conceived by conventional assisted reproduction. No significant impact of IVM on chorionic villus and cord blood DNA methylation was detected (Pliushch et al., 2015).

Effects on acetylation

In contrast to DNA methylation, little is known about the influence of IVM on oocyte histone modification, another important mechanism of epigenetic reprogramming. In a

study using mouse MII oocytes matured *in vivo* or *in vitro* and preimplantation embryos, Wang et al. examined the expression of two enzymes controlling histone acetylation, histone acetyltransferase (GCN5), and histone deacetylase 1 (HDAC1), as well as their common target, acetyl-histone H3. They found that IVM down-regulated protein expression of GCN5 and mRNA expression of HDAC1, whereas the levels of acetyl-histone H3 were not significantly changed. Thus, global histone acetylation levels in IVM oocytes and embryos remain comparable.

Recently, a study comparing post-translational acetylation of histone H4 at lysine 8 (*AcH4K8*), 12 (*AcH4K12*) and 16 (*AcH4K16*) of equine oocytes matured *in vitro* and *in vivo* (naturally cycling) found that while no differences were observed in the deacetylated levels of K8 and K12 between the two conditions, K16 (*AcH4K16*) was abnormally deacetylated following IVM. The authors concluded that IVM conditions might adversely affect epigenetic reprogramming, although the functional meaning of residue specific acetylation requires further investigation (Franciosi et al., 2012).

Other aspects

Safian et al. analyzed meiotic spindle and zona pellucida birefringence of IVM oocytes in PCOS patients. In the IVM oocytes, the percentage of highly birefringent zona pellucida was significantly higher than that *in vivo*-matured ones. However, both groups have the similar fertilization rates and meiotic spindle detection outcomes (Safian et al., 2017). Del Collado et al. demonstrated that the occurrence of functional disruption in lipid metabolism and stress pathways, altered mitochondrial activity and energy metabolism during IVM of cattle cumulus-oocyte complexes (Del Collado et al., 2017),

CONCLUSION

At present, although the technique has been improved. IVM is still not as good as *in vivo* matured. Progress in the IVM technique seemed to have reached a bottleneck period. The mechanisms controlling oocyte nuclear and cytoplasmic maturation are still far from clear. Translation of animal data to human IVM seldomly occurred. What makes a good human IVM system remains a mystery and much improvement is still required. This review may point out questions, supply evidence or clues for future improving IVM technique. Only with better understanding of the mechanism controlling oocyte maturation and internal molecular cascades of subsequent embryo development, can the IVM efficiency be improved greatly. This in turn would increase the application of IVM not only for treating human infertility, but also for fertility preservation as an additional option.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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