Zygote

cambridge.org/zyg

In vitro maturation of oocytes in light of ovarian mitochondrial improvement: effectiveness and safety

Nikos Petrogiannis[1], Kalliopi Chatzovoulou[1], Maria Filippa[1], Grigoris Grimbizis[2], Efstratios Kolibianakis[2] and Katerina Chatzimeletiou[2]

[1]ART Unit, Naval Hospital of Athens, Athens, Greece and [2]Unit for Human Reproduction, 1st Department of Obstetrics and Gynaecology, Aristotle University Medical School, Papageorgiou General Hospital, Thessaloniki, Greece

Review Article

Cite this article: Petrogiannis N *et al*. (2024). *In vitro* maturation of oocytes in light of ovarian mitochondrial improvement: effectiveness and safety. *Zygote*. page 1 of 7. doi: 10.1017/S0967199424000182

Received: 11 January 2024
Revised: 14 April 2024
Accepted: 8 May 2024

Keywords:
clinical pregnancy; *In vitro* maturation (IVM); live birth rate; mitochondria; oocyte; safety

Corresponding author:
Kalliopi Chatzovoulou;
Email: kchatzovoulou@icloud.com

Summary

In vitro maturation of oocytes (IVM) represents an assisted reproductive technique that involves the minimal or absence of ovarian stimulation and is beneficial to specific groups of patients. These may include women with polycystic ovarian syndrome and/or patients who need a fertility preservation option before undergoing gonadotoxic treatment. However, when IVM is applied in cases where it is not recommended, it can be considered as an add-on technique, as described by the ESHRE Guideline Group on Female Fertility Preservation. Interestingly, IVM has not been proven yet to be as effective as conventional IVF in the laboratory, in terms of clinical pregnancy and live birth rates, while concerns have been raised for its long-term safety. As a result, both safety and efficacy of IVM remain still questionable and additional data are needed to draw conclusions.

Introduction

Since the birth of the first baby conceived by *in vitro* fertilization (IVF, R. Edwards, 1978), a series of innovative events throughout the years have taken place in the field of Human Assisted Reproduction Technologies (ARTs), attempting to improve clinical outcomes. In particular, an alternative method to conventional IVF has been introduced by reproductive scientists, through which immature oocytes could mature *in vitro*, mimicking in vivo conditions, a technique known as *in vitro* maturation of oocytes (IVM). This method was first described in mammals, specifically using rabbit oocytes (Pincus and Enzmann, 1935), as a way to improve the efficiency of animal breeding in agriculturally important species. Moving towards the application of IVM in humans, the first successful fertilization of human *in vitro* matured oocytes was described in the late 1940s (Rock and Menkin, 1944; Menkin and Rock, 1948), while the remarkable work of Edwards defined the kinetics of oocyte nuclear maturation, as well as the ideal culture conditions for a successful IVM protocol (Edwards, 1962, 1965; Edwards *et al.*, 1969). Following the significant work made to optimize the technique, the first IVM birth was reported in 1991 by Cha and colleagues (Cha *et al.*, 1991), paving the way for a novel ART technique translated into clinical practice.

Application of IVM and clinical indications

IVM is based on the collection of immature cumulus-oocyte complexes (COCs) from antral follicles that are subsequently cultured *in vitro* until they reach the metaphase II (MII) stage (Edwards, 1965; Mikkelsen *et al.*, 1999; De Vos *et al.*, 2021). Patients undergoing IVM receive no or minimal ovarian stimulation, instead of a conventional controlled ovarian stimulation IVF (COS-IVF) protocol. Once maturation in the laboratory is completed, IVM oocytes are normally fertilized and treated exactly as the oocytes retrieved after conventional IVF (Thompson and Gilchrist, 2013).

As far as IVM protocols are concerned, there are four major protocols that are practiced in the laboratory. To start with, the standard IVM protocol represents the original one that was initially developed by Edwards (Edwards, 1965). The latter includes the collection of immature/GV-stage COCs, which undergo *in vitro* maturation in a single step until they reach the MII stage and are subsequently inseminated. It is of great importance that the cumulus cell-oocyte communication structure remains intact during *in vitro* culture from the GV to the metaphase II stage. Of note, follicle-stimulating hormone (FSH) might have been administered to patients, or not, prior to oocyte pick-up. Second, the so-called biphasic IVM protocol, which was the evolution of the standard IVM, involves a two-step procedure. The biphasic IVM protocol represents a variation of the standard IVM protocol, whose main difference relies on the additional pre-IVM step. More specifically, once collected, GV-stage COCs are cultured in a pre-IVM medium for approximately 24 h, where meiosis is inhibited at the GV stage, due to the

presence of meiotic inhibitors in the culture medium. In the next step, oocytes finally mature from the GV to MII stage, through meiosis-inducing factors, such as epidermal growth factor (EGF-p). The meiotic induction step lasts 30–48 h in humans (Gilchrist, 2011; Richani and Gilchrist, 2022). Interestingly, both oocyte meiotic arrest and resumption are regulated by the C-type natriuretic peptide/ cyclic guanosine monophosphate (CNP/ cGMP) signalling pathway, upstream of the intra-oocyte cyclic adenosine monophosphate (cAMP) (Gilchrist *et al.*, 2016). Based on the above, the main principles of the biphasic IVM culture system include 1) the maintenance of the oocyte in a meiotically arrested- GV stage (*in vitro*), 2) the stable and not impaired communication between oocyte and cumulus cells, 3) the acquisition of the oocyte developmental competence during the pre-IVM step, and lastly 4) the resumption of meiosis under conditions mimicking the endogenous post-LH surge effect. Concerning the FSH priming during the implementation of the biphasic protocol, it remains optional. Finally, the pre-IVM procedure, also known as "capacitation-IVM" (CAPA-IVM) was tested for its safety and efficacy through pre-clinical trials, while its use in clinical practice is associated with healthy live birth rates, comparable to conventional IVF protocols (Gilchrist *et al.*, 2024). Furthermore, the hCG-Primed IVM protocol represents an alternative protocol, where patients are triggered with human chorionic gonadotropin (hCG), in order to increase maturation success, while FSH priming still remains optional. The so-called "Truncated" IVM protocol, thus results in the presence of both immature (GV, MI) and mature (MII) stage oocytes, which are inseminated at different time points in the laboratory (Son *et al.*, 2008). In other words, oocytes are treated differently in the laboratory, with the MII oocytes necessitating fertilization on the same day of the oocyte retrieval, while the maturing and/or immature oocytes require IVM culture before the fertilization step, which might be a source of an additional laboratory burden. The hCG-primed IVM protocol thus excludes the use of any pre-IVM culture system. Finally, the "Rescue-IVM " or Conventional IVF protocol, includes the *in vitro* maturation of immature oocytes (GV and/or MI stage), collected after a conventional IVF cycle, where FSH is normally being administrated and ovulation triggering is mostly followed after hCG priming. The oocytes collected by such protocols are commonly regarded as non-usable oocytes for medical practice and are normally discarded in the corresponding cycles. The "rescue-IVM" oocytes are usually denuded of their cumulus cells, after the oocyte retrieval and prior to intracytoplasmic sperm injection (ICSI) and as a result, these oocytes are invariably cultured *in vitro* in a denuded state, from the GV to the MII stage. Overall, due to their suboptimal quality and the presence of meiotic defects, the success of Rescue-IVM procedures remains questionable (De Vos *et al.*, 2016).

In terms of clinical application, IVM was basically designed as an alternative to standard ovarian stimulation protocols, in order to overcome the negative effects and risks associated with ovarian stimulation, such as ovarian hyperstimulation syndrome (OHSS) in high responders. In this regard, women with polycystic ovary syndrome (PCOS) represent the best candidates for IVM, as on the one hand, these women are expected to have a higher number of immature oocytes at oocyte pick-up, which is associated with better clinical outcomes when using IVM, while on the other hand, they risk being affected by OHSS (Cha *et al.*, 2000). More specifically, in women who are high responders and the final oocyte triggering is performed by GnRH agonist, IVM would be a useful approach. GnRH agonists have been successfully used to trigger final oocyte maturation in IVF cycles, occasionally leading to the collection of immature and/or reduced number of oocytes, as a preventive approach to OHSS (Casper, 2015; Gonen *et al.*, 1990). Indeed, IVM represents a possible alternative to ovarian hyperstimulation, while priming in IVM cycles using GnRH agonists seems to be equally effective as hCG priming, although GnRH priming seems to be best-suited for fertility preservation in hormone-sensitive cancers and urgent fertility preservation cases (Hachem *et al.*, 2018).

Furthermore, a good indication for IVM is also considered to be in cases of urgent fertility preservation, when conventional ovarian stimulation protocols cannot be applied and/ or are contraindicated. Such cases that cannot be treated with gonadotrophins include cancer patients who are scheduled to be exposed to gonadotoxic treatments and prepubertal girls (ESHRE Guideline Group on Female Fertility Preservation *et al.*, 2020). Lastly, IVM should ideally be applied in rare cases of patients with resistant ovary syndrome (ROS). ROS represents a rare endocrine disorder whose symptoms include hypergonadotropic anovulation and infertility, while patients experience primary or secondary amenorrhoea (Talbert *et al.*, 1984; Huhtaniemi and Alevizaki, 2006). On the other hand, IVM may not be suitable for a certain group of patients. In fact, the use of IVM in normo-ovulatory patients (patients with regular cycles) might end up with a lower oocyte yield, compared to a conventional oocyte stimulation protocol, meaning fewer usable embryos, thus resulting in lower clinical pregnancy rates (Gilchrist and Smitz, 2023). Accordingly, IVM is not indicated for poor responders with low ovarian reserve, as well as women of advanced reproductive age, since the success of IVM depends on the number of oocytes collected (the more the better), as already mentioned (Braga *et al.*, 2010). A higher number of oocytes collected after IVM might be able to compensate for the suboptimal clinical outcomes (De Vos *et al.*, 2021; Gilchrist and Smitz, 2023). Finally, a small group of patients presenting oocyte meiotic defects, who obtain no mature oocytes after conventional oocyte stimulation procedures, are not suitable for IVM either. In fact, current IVM protocols are not able to "correct" oocyte meiotic abnormalities and thus do not result in encouraging clinical outcomes (Hourvitz *et al.*, 2010; Galvão *et al.*, 2018).

Taking into consideration the cases of patients that are suitable for an IVM procedure, IVM itself is not considered an experimental/add-on technique. On the contrary, this is not the case for the group of patients where IVM is contraindicated and therefore shouldn't be applied, as recently declared by the ESHRE Add-ons working group (ESHRE Add-ons working group *et al.*, 2023). It is worth mentioning that the need for universal guidelines during the use of alternative IVF protocols, such as IVM, is urgent, as well as the expertise of reproductive scientists who need to discuss the clinical outcomes and protocol modifications, in the context of an inter-centre communication and exchange of knowledge and skills.

Effectiveness of IVM in clinical practice

Moving towards the efficacy of IVM on a clinical scale, reproductive scientists are questioning whether IVM increases success rates when applied to certain groups of patients (with an indication of PCOS, high responders and/or fertility preservation cases). Based on the recently published data, when comparing the outcomes of conventional IVF protocols to standard IVM in patients with a defined infertility cause (e.g. PCOS), IVM clinical pregnancy rates still remain lower (Vuong *et al.*, 2020; Gilchrist

https://doi.org/10.1017/S0967199424000182 Published online by Cambridge University Press

and Smitz, 2023). Interestingly, several studies suggest that oocytes collected after an IVM procedure; once they reach the MII stage and therefore are inseminated, result in lower fertilization rates, as well as lower quality of embryos, contrasted to conventional IVF outcomes (Braga *et al.*, 2010). In fact, it is hypothesized that the significantly low clinical outcomes might be the result of a dysfunctional maturation process, an asynchronous nuclear-cytoplasmic maturation, owing to the *in vitro* culture conditions (De Vos *et al.*, 1999; Bao *et al.*, 2000). Concerning the implantation capacity, a significant improvement in the success rates of IVM embryo transfers has been observed when choosing the freeze-all strategy and deferred transfer per cycle (De Vos *et al.*, 2011; Chang *et al.*, 2014; Vuong *et al.*, 2021). In particular, endometrial development seems to be compromised and insufficiently prepared for a fresh embryo transfer during an IVM cycle, compared to the natural and/or stimulated ones (De Vos *et al.*, 2011; Walls *et al.*, 2015; Ortega-Hrepich *et al.*, 2019). Finally, IVM pregnancy rates are still controversial between centres, as observational studies demonstrate a live birth rate of 15,9% per retrieval (Child *et al.*, 2001; Buckett *et al.*, 2004), while others report pregnancy rates at approximately 22% (Söderström-Anttila *et al.*, 2005). Based on the official report of the ESHRE Add-ons working group, ongoing pregnancy rates following IVM range from 36.8 to 31.9% in women aged from 20 to 39 years, while clinical pregnancies in women over 40 years are rarely detected (ESHRE Add-ons working group *et al.*, 2023). Of note, a positive correlation between IVM live birth rates and the number of oocytes collected at the time of egg retrieval has been observed, with a minimum of five oocytes needed to achieve a pregnancy (Al-Sunaidi *et al.*, 2007; Fadini *et al.*, 2011; Yang *et al.*, 2012). Interestingly, a very recent article by Mostinckx and colleagues reported comparable reproductive outcomes between patients undergoing a conventional ovarian stimulation protocol and patients included in an IVM cycle, with serum anti-Müllerian hormone levels ≥10 ng/ml. Data from a large cohort of patients showed that ongoing pregnancy rates where not different in predicted hyper-responders undergoing ART after IVM compared with conventional IVF cycles (Mostinckx *et al.*, 2024)

Safety issues and aspects of IVM

As expected, modified and/or newly performed ART protocols raise safety and ethical concerns about their potential adverse effects and the long-term safety of children conceived with these techniques, such as IVM. To overcome safety issues, scientists are investigating the impact of ART interventions performed on a clinical scale in human populations by using animal models, which represent an alternative approach to both understand the complexity of such reproductive treatments, as well as to collect useful data. Interestingly, the main advantage of studying ART treatments in animal models, compared to human clinical studies, is that animals selected for the studies normally do not present fertility complications, which could introduce a confounding factor of infertility in the population performing ART. Moreover, animal populations are characterized by a higher genetic homogeneity compared to human populations, which might also play an important role in detecting the variability in ART treatment effects.

As far as IVM is concerned, animal studies are primarily based on the bovine model, while clinical and laboratory protocols are slightly modified compared to human methodologies, during the hormonal priming and the *in vitro* maturation steps (Krisher, 2022). Overall, animal studies investigating the impact of IVM are focusing on several clinical outcomes, such as birth weight, length of gestation, cardiovascular (e.g. blood pressure) and metabolic (fasting glucose, insulin) parameters, behavioural traits and finally lifespan. First, results from a meta-analysis in bovine models showed a significant increase in the birthweight of the IVM group, when compared to the *in vivo* controls, while also a longer gestational length was found in the IVM group versus the controls (Beilby *et al.*, 2023). Studies focusing on the mouse model reported a significant increase in the systolic blood pressure in female mice conceived with IVM (Le *et al.*, 2019), where metabolic outcomes from bovine studies (serum glucose and insulin levels after birth) were not found to be significantly different in the IVM group (Jacobsen *et al.*, 2000; Sangild *et al.*, 2000; Bertolini *et al.*, 2002). Finally, no differences were reported in newborn behavioural traits, such as standing and suckling time, as well as respiratory distress, between IVM and *in vivo* conceived calves (Bertolini *et al.*, 2002), although the need for a breathing stimulus at birth was found to be significantly increased in calves conceived with IVM when compared to *in vivo* conceived animals (van Wagtendonk-de Leeuw *et al.*, 2000). Lifespan data were not available for animals conceived with IVM (Beilby *et al.*, 2023).

On the other hand, in humans, currently available data do not support a globally negative impact of the use of IVM in clinical practice. To start with, very recent reports have evaluated the quality and ploidy status of embryos generated by an IVM procedure. These studies demonstrate that the ability of *in vitro* matured oocytes to be fertilized and form good quality embryos, as well as the production of euploid blastocysts, was similar to *in vivo* matured oocytes, whereas pregnancy and perinatal outcomes of these embryos were similar (Li *et al.*, 2021, Li *et al.*, 2024). Furthermore, concerns have also been expressed about the epigenetic abnormalities and imprinting errors in embryos resulting from an IVM procedure, as oocyte meiosis occurs *in vitro*. In fact, possible epigenetic modifications, such as methylation, as well as dysfunctional gene expression of imprinting genes, might occur, although published data do not report imprinting gene disorders in embryos/foetuses after IVM, suggesting that IVM does not compromise the epigenetic landscape and genomic imprinting establishment (Kuhtz *et al.*, 2014; Pliushch *et al.*, 2015; Saenz-de-Juano *et al.*, 2019). However, while current data seem reassuring, increased methylation levels of the KvDMR1 locus were observed in arrested immature oocytes of unstimulated PCOS patients, compared to oocytes resulting from stimulated cycles, suggesting that stimulation may exert an impact over imprinting establishment (Khoueiry *et al.*, 2008; Market-Velker *et al.*, 2010), making the subject a highly controversial one. Concerning neonatal health, as well as the development of children born after IVM, in the majority of cases, normal perinatal outcomes have been observed, when compared to babies born after a conventional IVF. In fact, factors such as miscarriage rate, preterm birth, birth weight, congenital anomalies, mental development and other pregnancy complications, seem to not be different from the ones described for IVF babies (Mostinckx *et al.*, 2019; Belva *et al.*, 2020; Strowitzki *et al.*, 2021; Vuong *et al.*, 2022). While some reports do describe perinatal abnormalities (Cha *et al.*, 2005; Söderström-Anttila *et al.*, 2006; Buckett *et al.*, 2007), clinical outcomes cannot be accurately assessed, as only a small number of babies are born after an IVM procedure, highlighting the importance of data availability. Indeed, the follow-up of children conceived from IVM is limited to ≤2 years, although current results from published studies have not identified differences between children born after IVM compared

https://doi.org/10.1017/S0967199424000182 Published online by Cambridge University Press

with those born after a conventional IVF cycle (Strowitzki *et al.*, 2021; Vuong *et al.*, 2022; 2023). Consequently, longitudinal data from prospective studies with longer term follow-up are needed, in order to draw solid conclusions about the safety of IVM (Vuong *et al.*, 2023).

IVM and mitochondrial function

While safety issues and complications after an IVM procedure have not yet been fully elucidated, concerns about critical components of oocyte competence are still a matter of discussion and need to be further investigated. For instance, mitochondria play a pivotal role during oocyte maturation and growth, as these procedures require a large amount of energy in the form of ATP. As a result, the correct functioning of mitochondria is crucial, otherwise these important steps will be compromised.

Mitochondria, also known as the power-house of the cell, are semi-autonomous organelles containing their own genetic information, called mitochondrial DNA (mtDNA). Their main functions include energy production for the cells, Ca^{2+} homeostasis, cell death regulation, iron metabolism and biosynthesis of several organic compounds (Spinelli and Haigis, 2018; Rossi *et al.*, 2019; Bock and Tait, 2020; Boyman *et al.*, 2020; Lill and Freibert, 2020). During oogenesis and follicular growth, the number of mitochondria in oocytes increases exponentially, rising from approximately 10,000 to 20,0000 organelles (Jansen and de Boer, 1998), while also the mtDNA copy number reaches up to 50,000 mtDNA copies in a mature oocyte (Reynier *et al.*, 2001). This highlights the importance of an adequate number and good quality of mitochondria required to sustain oogenesis and the early stages of embryogenesis. However, before oocyte maturity completion, the energy needed to support the process must be provided by the surrounding granulosa and cumulus cells, as mitochondria from immature oocytes remain in a naïve state (Dumollard *et al.*, 2008). As oocytes lose progressively their connections with the cumulus cells, they need to activate their own mitochondria to complete the final stages of maturation. Any deviation from this well-defined mechanism may result in diminished ovarian reserve. In fact, in women with primary ovarian insufficiency, which is also known as premature ovarian failure, oocytes were found to contain less mtDNA copies, compared to women with a normal ovarian profile, suggesting that low values of mtDNA copy number are associated with an abnormal mitochondrial biogenesis (May-Panloup *et al.*, 2005). Accordingly, mitochondrial distribution is also a crucial component during oocyte maturation, as it needs to be well-structured and dynamic (Takahashi *et al.*, 2016). In fact, mitochondria preferentially migrate towards the perinuclear region and represent 80% of the cytoplasmic volume. After the germinal vesicle breakdown stage and until oocytes reach the MII stage, mitochondria are equally distributed and occupy almost the whole of cytoplasmic volume (Trebichalská *et al.*, 2021).

However, during IVM, oocytes are exposed to different *in vitro* conditions, which may subsequently modify the well-defined pattern of mitochondrial function and localization. For instance, as mentioned before, upon maturation, mitochondria localize towards the perinuclear/central region of the oocyte, while their homogeneous distribution represents a sign of cytoplasmic maturity. On the contrary, the peripheral localization of mitochondria has been associated with meiotically incompetent oocytes (Sánchez *et al.*, 2015). Notably, it has been demonstrated that mitochondria from IVM oocytes tended to localize more abundantly in the peripheral region, instead of the inner cytoplasmic region, when compared to *in vivo* matured oocytes (Liu *et al.*, 2010). To continue, while both mitochondrial number and ultrastructure were not found to be different between IVM and *in vivo* matured oocytes (Coticchio *et al.*, 2016), data from mitochondrial DNA copy number assessment are scarce. Several studies based on the murine model have actually demonstrated that the mitochondrial DNA number of *in vitro* matured oocytes was significantly lower, compared to control oocytes, which was hypothesized to be a sign of a compromised mitochondrial biogenesis, as well as cytoplasmic immaturity (Ge *et al.*, 2012; Tao *et al.*, 2017). Furthermore, reports from mice showed that both mitochondrial membrane potential and the ATP amount were not found to be different between *in vitro* and *in vivo* oocytes (Ge *et al.*, 2012). However, single-cell transcriptomic data from both *in vitro* and *in vivo* human oocytes demonstrated a significant number of alterations in gene pathways associated with mitochondrial function in the IVM group (Zhao *et al.*, 2019), making this subject a highly controversial one.

Whether *in vitro* conditions exert an impact over mitochondrial patterns still remains an open question. Nevertheless, the degree of the possible alterations previously mentioned, might depend on the specific cultured conditions and protocols used during an IVM procedure that might influence both nuclear and cytoplasmic maturity of the oocyte. In fact, it remains unclear whether IVM media influence the mitochondrial integrity of oocytes, as there is a large variety of protocols used in clinical practice. For instance, studies on the bovine model demonstrated that during an IVM procedure, relatively high oxygen concentrations (20%) resulted in a better embryonic yield, when compared to lower oxygen concentrations (5–7%) (Pinyopummintr and Bavister, 1995; Whitty *et al.*, 2021). However, attention should be paid to the oxygen concentrations applied, as excessive oxygen levels may result in increased production of reactive oxygen species (ROS), which subsequently compromise mitochondrial function. Although the impact of different oxygen concentrations, as a component of culture conditions, is not clear yet, it is likely that culture media composition might affect the redox state of the cells and ROS production (Cobley, 2020).

To overcome these issues, several studies propose the supplementation of IVM culture media with antioxidants, in order to enhance the mitochondrial function of *in vitro* matured oocytes, as well as to ameliorate the oocyte and embryonic competence upon IVM application. In fact, the supplementation of IVM media with the follicular fluid-derived melatonin has been shown to significantly increase the implantation rates in PCOS patients (Kim *et al.*, 2013), as well as the blastocyst formation rate in rescue-IVM oocytes (Hao *et al.*, 2017; Zou *et al.*, 2020). Other antioxidants that might improve IVM clinical outcomes include resveratrol, which resulted in improved spindle morphology and intact chromosomal localization in human rescue-IVM oocytes (Liu *et al.*, 2018), quercetin, which was proven to improve the mitochondrial function in porcine, mice, goat and human IVM oocytes (Kang *et al.*, 2013; Silva *et al.*, 2018; Cao *et al.*, 2020), leading to increased fertilization and blastocyst formation rates (Cao *et al.*, 2020). Finally, addition of the antioxidant anethole in IVM culture media for bovine oocytes has resulted in higher cleavage, better embryonic development and higher cell number per blastocyst rates (Sá *et al.*, 2019), while supplementation with the mitochondrial inner membrane coenzyme Q10 (CoQ10), is directly associated with increased mitochondrial function and better embryonic outcomes (Abdulhasan *et al.*, 2017; Heydarnejad *et al.*, 2019). Surprisingly, addition of CoQ10 in the IVM culture medium of human oocytes resulted in a 20% increase of the maturation rate, as well as a significant decrease in aneuploidy rates

https://doi.org/10.1017/S0967199424000182 Published online by Cambridge University Press

in the first polar body of patients with an advanced maternal age (Ma *et al.*, 2020). Taken together, supplementation of the IVM media with antioxidants should be taken into consideration and be applied in clinical practice, by selecting the right combination and concentration of the agents, to avoid possible detrimental effects on the oocytes and embryos.

Conclusion

To summarize, IVM represents a procedure where minimal or absence of ovarian stimulation is required in patients with specific indications. As such, it is supposed to be an advantageous technique, as it requires less time, minimal medical monitoring and fewer to no hormone injections and blood monitoring. Cost-effectiveness studies also suggest that IVM is a less expensive choice, compared to a conventional ovarian stimulation protocol, while these characteristics are associated with a better mental and psychological status of patients undergoing such procedures (Braam *et al.*, 2021; Practice Committees of the American Society for Reproductive Medicine, the Society of Reproductive Biologists and Technologists, and the Society for Assisted Reproductive Technology. Electronic address: jgoldstein@asrm.org, 2021).

If hormone-free protocols for both fertility preservation and *in vitro* fertilization represent the new era in ART, it still remains an open question. It is therefore crucial to highlight the importance of research and well-designed randomized controlled trials, in order to be able to resolve safety and effectiveness issues, as well as to improve protocols and culture conditions that are currently used during oocyte *in vitro* maturation. In conclusion, IVM requires specific expertise from both medical doctors and embryologists, while the follow-up of children born after IVM is urgently needed in order to ensure better clinical outcomes.

Competing interests. The authors declare no conflicts of interest.

Funding. Not applicable.

References

Abdulhasan, M.K., Li, Q., Dai, J., Abu-Soud, H.M., Puscheck, E.E. and Rappolee, D.A. (2017) CoQ10 increases mitochondrial mass and polarization, ATP and Oct4 potency levels, and bovine oocyte MII during IVM while decreasing AMPK activity and oocyte death. *Journal of Assisted Reproduction and Genetics* **34**, 1595–1607.

Al-Sunaidi, M., Tulandi, T., Holzer, H., Sylvestre, C., Chian, R.C. and Tan, S.L. (2007) Repeated pregnancies and live births after in vitro maturation treatment. *Fertility and Sterility* **87**, 1212.e9–12.

Bao, S., Obata, Y., Carroll, J., Domeki, I and Kono, T. (2000) Epigenetic modifications necessary for normal development are established during oocyte growth in mice. *Biology of Reproduction* **62**, 616–621.

Beilby, K.H., Kneebone, E., Roseboom, T.J., van Marrewijk, I.M., Thompson, J.G., Norman, R.J., Robker, R.L., Mol, B.W.J. and Wang, R. (2023) Offspring physiology following the use of IVM, IVF and ICSI: a systematic review and meta-analysis of animal studies. *Human Reproduction Update* **29**, 272–290.

Belva, F., Roelants, M., Vermaning, S., Desmyttere, S., De Schepper, J., Bonduelle, M., Tournaye, H., Hes, F. and De Vos, M. (2020) Growth and other health outcomes of 2-year-old singletons born after IVM versus controlled ovarian stimulation in mothers with polycystic ovary syndrome. *Human Reproduction Open* **2020**, hoz043.

Bertolini, M., Mason, J.B., Beam, S.W., Carneiro, G.F., Sween, M.L., Kominek, D.J., Moyer, A.L., Famula, T.R., Sainz, R.D. and Anderson, G.B. (2002) Morphology and morphometry of in vivo- and in vitro-produced bovine concepti from early pregnancy to term and association with high birth weights. *Theriogenology* **58**, 973–994.

Bock, F.J. and Tait, S.W.G. (2020) Mitochondria as multifaceted regulators of cell death. *Nature Reviews Molecular Cell Biology* **21**, 85–100.

Boyman, L., Karbowski, M. and Lederer, W.J. (2020) Regulation of Mitochondrial ATP production: Ca2+ signaling and quality control. *Trends in Molecular Medicine* **26**, 21–39.

Braam, S.C., Ho, V.N.A., Pham, T.D., Mol, B.W, Van Wely, M. and Vuong, L.N. (2021) In-vitro maturation versus IVF: a cost-effectiveness analysis. *Reproductive Biomedicine Online* **42**, 143–149.

Braga, D.P.D.A.F., Figueira, R.D.C.S., Ferreira, R.C., Pasqualotto, F.F., Iaconelli, A. and Borges, E. (2010) Contribution of in-vitro maturation in ovarian stimulation cycles of poor-responder patients. *Reproductive Biomedicine Online* **20**, 335–340.

Buckett, W.M., Chian, R.C., Holzer, H., Dean, N., Usher, R. and Tan, S.L. (2007) Obstetric outcomes and congenital abnormalities after in vitro maturation, in vitro fertilization, and intracytoplasmic sperm injection. *Obstet Gynecol* **110**, 885–891.

Buckett, W.M., Chian, R.C. and Tan, S.L. (2004) Human chorionic gonadotropin for in vitro oocyte maturation: does it improve the endometrium or implantation? *The Journal of Reproductive Medicine* **49**, 93–98.

Cao, Y., Zhao, H., Wang, Z., Zhang, C., Bian, Y., Liu, X., Zhang, C., Zhang, X. and Zhao, Y. (2020) Quercetin promotes in vitro maturation of oocytes from humans and aged mice. *Cell Death & Disease* **11**, 965.

Casper, R.F. (2015). Basic understanding of gonadotropin-releasing hormone–agonist triggering. *Fertility and sterility* **103**, 867–869.

Cha, K.Y., Chung, H.M., Lee, D.R., Kwon, H., Chung, M.K., Park, L.S., Choi, D.H. and Yoon, T.K. (2005) Obstetric outcome of patients with polycystic ovary syndrome treated by in vitro maturation and in vitro fertilization-embryo transfer. *Fertility and sterility* **83**, 1461–1465.

Cha, K.Y., Han, S.Y., Chung, H.M., Choi, D.H., Lim, J.M., Lee, W.S., Ko, J.J. and Yoon, T.K. (2000) Pregnancies and deliveries after in vitro maturation culture followed by in vitro fertilization and embryo transfer without stimulation in women with polycystic ovary syndrome. *Fertility and sterility* **73**, 978–983.

Cha, K.Y., Koo, J.J., Ko, J.J., Choi, D.H., Han, S.Y. and Yoon, T.K. (1991) Pregnancy after in vitro fertilization of human follicular oocytes collected from nonstimulated cycles, their culture in vitro and their transfer in a donor oocyte program. *Fertility and sterility* **55**, 109–113.

Chang, E.M., Song, H.S., Lee, D.R., Lee, W.S. and Yoon, T.K. (2014) In vitro maturation of human oocytes: its role in infertility treatment and new possibilities. *Clinical and Experimental Reproductive Medicine* **41**, 41–46.

Child, T.J, Abdul-Jalil, A.K., Gulekli, B. and Tan, S.L. (2001) In vitro maturation and fertilization of oocytes from unstimulated normal ovaries, polycystic ovaries, and women with polycystic ovary syndrome. *Fertility and sterility* **76**, 936–942.

Cobley, J.N. (2020) Mechanisms of mitochondrial ROS production in assisted reproduction: the known, the unknown, and the intriguing. *Antioxidants* **9**, 933.

Coticchio, G., Dal Canto, M., Fadini, R., Mignini Renzini, M., Guglielmo, M.C., Miglietta, S., Palmerini, M.G., Macchiarelli, G. and Nottola, S.A. (2016) Ultrastructure of human oocytes after in vitro maturation. *Molecular Human Reproduction* **22**, 110–118.

De Vos, A., Van de Velde, H., Joris, H. and Van Steirteghem, A. (1999) In-vitro matured metaphase-I oocytes have a lower fertilization rate but similar embryo quality as mature metaphase-II oocytes after intracytoplasmic sperm injection. *Human Reproduction* **14**, 1859–1863.

De Vos, M., Grynberg, M., Ho, T.M., Yuan, Y., Albertini, D.F. and Gilchrist, R.B. (2021) Perspectives on the development and future of oocyte IVM in clinical practice. *Journal of Assisted Reproduction and Genetics* **38**, 1265–1280.

De Vos, M., Ortega-Hrepich, C., Albuz, F.K., Guzman, L., Polyzos, N.P., Smitz, J. and Devroey, P. (2011) Clinical outcome of non-hCG-primed oocyte in vitro maturation treatment in patients with polycystic ovaries and polycystic ovary syndrome. *Fertility and sterility* **96**, 860–864.

De Vos, M., Smitz, J., Thompson, J.G. and Gilchrist, R.B. (2016) The definition of IVM is clear-variations need defining. *Human Reproduction* **31**, 2411–2415.

Dumollard, R., Campbell, K., Halet, G., Carroll, J. and Swann, K. (2008) Regulation of cytosolic and mitochondrial ATP levels in mouse eggs and zygotes. *Developmental Biology* **316**, 431–440.

https://doi.org/10.1017/S0967199424000182 Published online by Cambridge University Press

Edwards, R.G. (1962) Meiosis in ovarian oocytes of adult mammals. *Nature* **196**, 446–450.

Edwards, R.G. (1965) Maturation in vitro of human ovarian oöcytes. *Lancet* **2**, 926–929.

Edwards, R.G., Bavister, B.D. and Steptoe, P.C. (1969) Early stages of fertilization in vitro of human oocytes matured in vitro. *Nature* **221**, 632–635.

ESHRE Add-ons working group, **Lundin, K., Bentzen, J.G., Bozdag, G., Ebner, T., Harper, J., Le Clef, N., Moffett, A., Norcross, S., Polyzos, N.P. and Rautakallio-Hokkanen, S.** (2023) Good practice recommendations on add-ons in reproductive medicine†. *Human Reproduction* **38**, 2062–2104.

ESHRE Guideline Group on Female Fertility Preservation, **Anderson, R.A., Amant, F., Braat, D., D'Angelo, A., Chuva de Sousa Lopes, S.M., Demeestere, I., Dwek, S., Frith, L., Lambertini, M. and Maslin, C.** (2020) ESHRE guideline: female fertility preservation. *Human Reproduction Open* **2020**, hoaa052.

Fadini, R., Comi, R., Mignini Renzini, M., Coticchio, G., Crippa, M., De Ponti, E. and Dal Canto, M. (2011) Anti-mullerian hormone as a predictive marker for the selection of women for oocyte in vitro maturation treatment. *Journal of Assisted Reproduction and Genetics* **28**, 501–508.

Galvão, A., Segers, I., Smitz, J., Tournaye, H. and De Vos, M. (2018) In vitro maturation (IVM) of oocytes in patients with resistant ovary syndrome and in patients with repeated deficient oocyte maturation. *Journal of Assisted Reproduction and Genetics* **35**, 2161–2171.

Ge, H., Tollner, T.L., Hu, Z., Da, M., Li, X., Guan, H., Shan, D., Lu, J., Huang, C. and Dong, Q. (2012) Impaired mitochondrial function in murine oocytes is associated with controlled ovarian hyperstimulation and in vitro maturation. *Reproduction, Fertility and Development* **24**, 945–952.

Gilchrist, R.B. (2011) Recent insights into oocyte-follicle cell interactions provide opportunities for the development of new approaches to in vitro maturation. *Reproduction, Fertility and Development* **23**, 23–31.

Gilchrist, R.B., Luciano, A.M., Richani, D., Zeng, H.T., Wang, X., De Vos, M., ... and Thompson, J.G. (2016) Oocyte maturation and quality: role of cyclic nucleotides. *Reproduction* **152**, R143–R157.

Gilchrist, R.B. and Smitz, J. (2023) Oocyte in vitro maturation: physiological basis and application to clinical practice. *Fertility and sterility* **119**, 524–539.

Gilchrist, R.B., Ho, T.M., De Vos, M., Sanchez, F., Romero, S., Ledger, W.L., Anckaert, E., Vuong, L.N. and Smitz, J. (2024) A fresh start for IVM: capacitating the oocyte for development using pre-IVM. *Human Reproduction Update* **30**, 3–25.

Gonen, Y.A.E.L., Balakier, H., Powell, W. and Casper, R.F. (1990) Use of gonadotropin-releasing hormone agonist to trigger follicular maturation for in vitro fertilization. *The Journal of Clinical Endocrinology & Metabolism* **71**, 918–922.

Hao, Y., Zhang, Z., Han, D., Cao, Y., Zhou, P., Wei, Z., Lv, M. and Chen, D. (2017) Gene expression profiling of human blastocysts from in vivo and 'rescue IVM' with or without melatonin treatment. *Molecular Medicine Reports* **16**, 1278–1288.

El Hachem, H., Sonigo, C., Benard, J., Presse, M., Sifer, C., Sermondade, N. and Grynberg, M. (2018) Comparison of GnRH agonist and hCG for priming in vitro maturation cycles in cancer patients undergoing urgent fertility preservation. *PLoS One* **13**, e0208576.

Heydarnejad, A., Ostadhosseini, S., Varnosfaderani, S.R., Jafarpour, F., Moghimi, A. and Nasr-Esfahani, M.H. (2019) Supplementation of maturation medium with CoQ10 enhances developmental competence of ovine oocytes through improvement of mitochondrial function. *Molecular Reproduction and Development* **86**, 812–824.

Hourvitz, A., Maman, E., Brengauz, M., Machtinger, R. and Dor, J. (2010) In vitro maturation for patients with repeated in vitro fertilization failure due to "oocyte maturation abnormalities." *Fertility and sterility* **94**, 496–501.

Huhtaniemi, I. and Alevizaki, M. (2006) Gonadotrophin resistance. *Best Pract Res Clin Endocrinol Metab* **20**, 561–576.

Jacobsen, H., Schmidt, M., Hom, P., Sangild, P.T., Greve, T. and Callesen, H. (2000) Ease of calving, blood chemistry, insulin and bovine growth hormone of newborn calves derived from embryos produced in vitro in culture systems with serum and co-culture or with PVA. *Theriogenology* **54**, 147–158.

Jansen, R.P. and De Boer, K. (1998) The bottleneck: mitochondrial imperatives in oogenesis and ovarian follicular fate. *Molecular and Cellular Endocrinology* **145**, 81–88.

Kang, J.T., Kwon, D.K., Park, S.J., Kim, S.J., Moon, J.H., Koo, O.J., Jang, G. and Lee, B.C. (2013) Quercetin improves the in vitro development of porcine oocytes by decreasing reactive oxygen species levels. *Journal of Veterinary Science* **14**, 15–20.

Khoueiry, R., Ibala-Rhomdane, S., Méry, L., Blachère, T., Guérin, J.F., Lornage, J. and Lefèvre, A. (2008) Dynamic CpG methylation of the KCNQ1OT1 gene during maturation of human oocytes. *Journal of Medical Genetics* **45**, 583–588.

Kim, M.K., Park, E.A., Kim, H.J., Choi, W.Y., Cho, J.H., Lee, W.S., Cha, K.Y., Kim, Y.S., Lee, D.R. and Yoon, T.K. (2013) Does supplementation of in-vitro culture medium with melatonin improve IVF outcome in PCOS? *Reprod Biomed Online* **26**, 22–29.

Krisher, R.L. (2022) Present state and future outlook for the application of in vitro oocyte maturation in human infertility treatment. *Biology of Reproduction* **106**, 235–242.

Kuhtz, J., Romero, S., De Vos, M., Smitz, J., Haaf, T. and Anckaert, E. (2014) Human in vitro oocyte maturation is not associated with increased imprinting error rates at LIT1, SNRPN, PEG3 and GTL2. *Human Reproduction* **29**, 1995–2005.

Li, J., Chen, J., Sun, T., Zhang, S., Jiao, T., Chian, R.C., Li, Y. and Xu, Y. (2021) Chromosome aneuploidy analysis in embryos derived from in vivo and in vitro matured human oocytes. *Journal of Translational Medicine* **19**, 416.

Li, Y., Jin, L., Tian, W., Yan, E., Li, Y., Ren, X. and Guo, N. (2024) The ploidy of blastocysts from in-vitro-matured metaphase I oocytes. *Reproductive BioMedicine Online* **48**, 103571.

Le, F., Lou, H.Y., Wang, Q.J., Wang, N., Wang, L.Y., Li, L.J., Yang, X.Y., Zhan, Q.T., Lou, Y.Y. and Jin, F. (2019) Increased hepatic INSIG-SCAP-SREBP expression is associated with cholesterol metabolism disorder in assisted reproductive technology-conceived aged mice. *Reproductive Toxicology* **84**, 9–17.

Lill, R. and Freibert, S.A. (2020) Mechanisms of mitochondrial iron-sulfur protein biogenesis. *Annual Review of Biochemistry* **89**, 471–499.

Liu, M.J., Sun, A.G., Zhao, S.G., Liu, H., Ma, S.Y., Li, M., Huai, Y.X., Zhao, H. and Liu, H.B. (2018) Resveratrol improves in vitro maturation of oocytes in aged mice and humans. *Fertility and sterility* **109**, 900–907.

Liu, S., Li, Y., Gao, X., Yan, J.H. and Chen, Z.J. (2010) Changes in the distribution of mitochondria before and after in vitro maturation of human oocytes and the effect of in vitro maturation on mitochondria distribution. *Fertility and sterility* **93**, 1550–1555.

Ma, L., Cai, L., Hu, M., Wang, J., Xie, J., Xing, Y., Shen, J., Cui, Y., Liu, X.J. and Liu, J. (2020) Coenzyme Q10 supplementation of human oocyte in vitro maturation reduces postmeiotic aneuploidies. *Fertility and sterility* **114**, 331–337.

Market-Velker, B.A., Zhang, L., Magri, L.S., Bonvissuto, A.C. and Mann, M.R.W. (2010) Dual effects of superovulation: loss of maternal and paternal imprinted methylation in a dose-dependent manner. *Human Molecular Genetics* **19**, 36–51.

May-Panloup, P., Chrétien, M.F., Jacques, C., Vasseur, C., Malthièry, Y. and Reynier, P. (2005) Low oocyte mitochondrial DNA content in ovarian insufficiency. *Human Reproduction* **20**, 593–597.

Menkin, M.F. and Rock, J. (1948) In vitro fertilization and cleavage of human ovarian eggs. *American Journal of Obstetrics and Gynecology* **55**, 440–452.

Mikkelsen, A.L., Smith, S.D. and Lindenberg, S. (1999) In-vitro maturation of human oocytes from regularly menstruating women may be successful without follicle stimulating hormone priming. *Human Reproduction* **14**, 1847–1851.

Mostinckx, L., Segers, I., Belva, F., Buyl, R., Santos-Ribeiro, S., Blockeel, C., Smitz, J., Anckaert, E., Tournaye, H. and De Vos, M.0020(2019) Obstetric and neonatal outcome of ART in patients with polycystic ovary syndrome: IVM of oocytes versus controlled ovarian stimulation. *Human Reproduction* **34**, 1595–1607.

Mostinckx, L., Goyens, E., Mackens, S., Roelens, C., Boudry, L., Uvin, V., Segers, I., Schoemans, C., Drakopoulos, P., Blockeel, C. and De Vos, M. (2024) Clinical outcomes from ART in predicted hyperresponders: in vitro maturation of oocytes versus conventional ovarian stimulation for IVF/ICSI. *Human Reproduction* **39**, 586–594.

Ortega-Hrepich, C., Drakopoulos, P., Bourgain, C., Van Vaerenbergh, I., Guzman, L., Tournaye, H., Smitz, J. and De Vos, M. (2019) Aberrant

https://doi.org/10.1017/S0967199424000182 Published online by Cambridge University Press

endometrial steroid receptor expression in in-vitro maturation cycles despite hormonal luteal support: A pilot study. *Reprod Biol* **19**, 210–217.

Pincus, G. and Enzmann, E.V. (1935) The comparative behavior of mammalian eggs in vivo and in vitro: I. The activation of ovarian eggs. *The Journal of Experimental Medicine* **62**, 665–675.

Pinyopummintr, T. and Bavister, B.D. (1995) Optimum gas atmosphere for in vitro maturation and in vitro fertilization of bovine oocytes. *Theriogenology* **44**, 471–477.

Pliushch, G., Schneider, E., Schneider, T., El Hajj, N., Rösner, S., Strowitzki, T. and Haaf, T. (2015) In vitro maturation of oocytes is not associated with altered deoxyribonucleic acid methylation patterns in children from in vitro fertilization or intracytoplasmic sperm injection. *Fertility and sterility* **103**, 720–727.e1.

Practice Committees of the American Society for Reproductive Medicine, the Society of Reproductive Biologists and Technologists, and the Society for Assisted Reproductive Technology. (2021) Electronic address: jgoldstein@asrm.org. In vitro maturation: a committee opinion. *Fertility and sterility* **115**, 298–304.

Reynier, P., May-Panloup, P., Chrétien, M.F., Morgan, C.J., Jean, M., Savagner, F., Barrière, P. and Malthièry, Y. (2001) Mitochondrial DNA content affects the fertilizability of human oocytes. *Molecular Human Reproduction* **7**, 425–429.

Richani, D. and Gilchrist, R.B. (2022) Approaches to oocyte meiotic arrest in vitro and impact on oocyte developmental competence. *Biology of Reproduction* **106**, 243–252.

Rock, J. and Menkin, M.F. (1944) In vitro fertilization and cleavage of human ovarian eggs. *Science* **100**, 105–107.

Rossi, A., Pizzo, P. and Filadi, R. (2019) Calcium, mitochondria and cell metabolism: A functional triangle in bioenergetics. *Biochimica et Biophysica Acta, Molecular Cell Research* **1866**, 1068–1078.

Sá, N.A.R., Vieira, L.A., Ferreira, A.C.A., Cadenas, J., Bruno, J.B., Maside, C., Sousa, F.G.C., Cibin, F.W.S., Alves, B.G., Rodrigues, A.P.R. and Leal-Cardoso, J.H. (2019) Anethole supplementation during oocyte maturation improves in vitro production of bovine embryos. *Reproductive Sciences*. doi: 10.1177/1933719119831783.

Saenz-de-Juano, M.D., Ivanova, E., Romero, S., Lolicato, F., Sánchez, F., Van Ranst, H., Krueger, F., Segonds-Pichon, A., De Vos, M., Andrews, S. and Smitz, J. (2019) DNA methylation and mRNA expression of imprinted genes in blastocysts derived from an improved in vitro maturation method for oocytes from small antral follicles in polycystic ovary syndrome patients. *Human Reproduction* **34**, 1640–1649.

Sánchez, F., Romero, S., De Vos, M., Verheyen, G. and Smitz, J. (2015) Human cumulus-enclosed germinal vesicle oocytes from early antral follicles reveal heterogeneous cellular and molecular features associated with in vitro maturation capacity. *Human Reproduction* **30**, 1396–1409.

Sangild, P.T., Schmidt, M., Jacobsen, H., Fowden, A.L., Forhead, A., Avery, B. and Greve, T. (2000) Blood chemistry, nutrient metabolism, and organ weights in fetal and newborn calves derived from in vitro-produced bovine embryos. *Biology of Reproduction* **62**, 1495–1504.

Silva, A.A.A., Silva, M.N.P., Figueiredo, L.B.F., Gonçalves, J.D., Silva, M.J.S., Loiola, M.L.G., Bastos, B.D.M., Oliveira, R.A., Ribeiro, L.G.M., Barberino, R.S. and Gouveia, B.B. (2018) Quercetin influences in vitro maturation, apoptosis and metabolically active mitochondria of goat oocytes. *Zygote* **26**, 465–470.

Söderström-Anttila, V., Mäkinen, S., Tuuri, T. and Suikkari, A.M. (2005) Favourable pregnancy results with insemination of in vitro matured oocytes from unstimulated patients. *Human Reproduction* **20**, 1534–1540.

Söderström-Anttila, V., Salokorpi, T., Pihlaja, M., Serenius-Sirve, S. and Suikkari, A.M. (2006) Obstetric and perinatal outcome and preliminary results of development of children born after in vitro maturation of oocytes. *Human Reproduction* **21**, 1508–1513.

Son, W.Y., Chung, J.T., Chian, R.C., Herrero, B., Demirtas, E., Elizur, S., Gidoni, Y., Sylvestre, C., Dean, N. and Tan, S.L. (2008) A 38 h interval between hCG priming and oocyte retrieval increases in vivo and in vitro oocyte maturation rate in programmed IVM cycles. *Human Reproduction* **23**, 2010–2016.

Spinelli, J.B., Haigis, M.C. (2018) The multifaceted contributions of mitochondria to cellular metabolism. *Nature Cell Biology* **20**, 745–754.

Strowitzki, T., Bruckner, T. and Roesner, S. (2021) Maternal and neonatal outcome and children's development after medically assisted reproduction with in-vitro matured oocytes-a systematic review and meta-analysis. *Human Reproduction Update* **27**, 460–473.

Takahashi, Y., Hashimoto, S., Yamochi, T., Goto, H., Yamanaka, M., Amo, A., Matsumoto, H., Inoue, M., Ito, K., Nakaoka, Y. and Suzuki, N. (2016) Dynamic changes in mitochondrial distribution in human oocytes during meiotic maturation. *Journal of Assisted Reproduction and Genetics* **33**, 929–938.

Talbert, L.M., Raj, M.H., Hammond, M.G. and Greer, T. (1984) Endocrine and immunologic studies in a patient with resistant ovary syndrome. *Fertility and sterility* **42**, 741–744.

Tao, X., Landis, J.N., Krisher, R.L., Duncan, F.E., Silva, E., Lonczak, A., Scott, R.T., Zhan, Y., Chu, T., Scott, R.T. and Treff, N.R. (2017) Mitochondrial DNA content is associated with ploidy status, maternal age, and oocyte maturation methods in mouse blastocysts. *Journal of Assisted Reproduction and Genetics* **34**, 1587–1594.

Thompson, J. and Gilchrist, R. Improving oocyte maturation in vitro. 2013; Cambridge University Press Available from: https://digital.library.adelaide.edu.au/dspace/handle/2440/81932.

Trebichalská, Z., Kyjovská, D., Kloudová, S., Otevřel, P., Hampl, A. and Holubcová, Z. (2021) Cytoplasmic maturation in human oocytes: an ultrastructural study †. *Biology of Reproduction* **104**, 106–116.

Walls, M.L., Hunter, T., Ryan, J.P., Keelan, J.A., Nathan, E. and Hart, R.J. (2015) In vitro maturation as an alternative to standard in vitro fertilization for patients diagnosed with polycystic ovaries: a comparative analysis of fresh, frozen and cumulative cycle outcomes. *Human Reproduction* **30**, 88–96.

Van Wagtendonk-de Leeuw, A.M., Mullaart, E., De Roos, A.P., Merton, J.S., den Daas, J.H., Kemp, B. and De Ruigh, L. (2000) Effects of different reproduction techniques: AI MOET or IVP, on health and welfare of bovine offspring. *Theriogenology* **53**, 575–597.

Whitty, A., Kind, K.L., Dunning, K.R. and Thompson, J.G. (2021) Effect of oxygen and glucose availability during in vitro maturation of bovine oocytes on development and gene expression. *Journal of Assisted Reproduction and Genetics* **38**, 1349–1362.

Vuong, L.N., Ho, V.N.A., Ho, T.M., Dang, V.Q., Phung, T.H., Giang, N.H., Le, A.H., Pham, T.D., Wang, R., Smitz, J. and Gilchrist, R.B. (2020) In-vitro maturation of oocytes versus conventional IVF in women with infertility and a high antral follicle count: a randomized non-inferiority controlled trial. *Human Reproduction* **35**, 2537–2547.

Vuong, L.N., Nguyen, L.K., Le, A.H., Pham, H.H., Ho, V.N., Le, H.L., Pham, T.D., Dang, V.Q., Phung, T.H., Smitz, J. and Ho, T.M. (2021) Fresh embryo transfer versus freeze-only after in vitro maturation with a pre-maturation step in women with high antral follicle count: a randomized controlled pilot study. *Journal of Assisted Reproduction and Genetics* **38**, 1293–1302.

Vuong, L.N., Nguyen, M.H.N., Nguyen, N.A., Ly, T.T., Tran, V.T.T., Nguyen, N.T., Hoang, H.L.T., Le, X.T.H., Pham, T.D., Smitz, J.E.J. and Mol, B.W. (2022) Development of children born from IVM versus IVF: 2-year follow-up of a randomized controlled trial. *Human Reproduction* **37**, 1871–1879.

Vuong, L.N., Pham, T.D., Ho, T.M. and De Vos, M. (2023). Outcomes of clinical in vitro maturation programs for treating infertility in hyper responders:a systematic review. *Fertility and Sterility* **119**, 540–549.

Yang, S.H., Patrizio, P., Yoon, S.H., Lim, J.H. and Chian, R.C. (2012) Comparison of pregnancy outcomes in natural cycle IVF/M treatment with or without mature oocytes retrieved at time of egg collection. *Systems Biology in Reproductive Medicine* **58**, 154–159.

Zhao, H., Li, T., Zhao, Y., Tan, T., Liu, C., Liu, Y., Chang, L., Huang, N., Li, C., Fan, Y. and Yu, Y. (2019) Single-Cell Transcriptomics of Human Oocytes: Environment-Driven Metabolic Competition and Compensatory Mechanisms During Oocyte Maturation. *Antioxidants & Redox Signaling* **30**, 542–559.

Zou, H., Chen, B., Ding, D., Gao, M., Chen, D., Liu, Y., Hao, Y., Zou, W., Ji, D., Zhou, P. and Wei, Z. (2020) Melatonin promotes the development of immature oocytes from the COH cycle into healthy offspring by protecting mitochondrial function. *Journal of Pineal Research* **68**, e12621.

https://doi.org/10.1017/S0967199424000182 Published online by Cambridge University Press