Human Reproduction, Vol.32, No.4 pp. 725–732, 2017
Advanced Access publication on March 2, 2017 doi:10.1093/humrep/dex043

human reproduction

MINI-REVIEW ***Developments in Reproductive Biology and Medicine***

Improving oocyte quality by transfer of autologous mitochondria from fully grown oocytes

Stine Gry Kristensen, Susanne Elisabeth Pors, and Claus Yding Andersen*

Laboratory of Reproductive Biology, Copenhagen University Hospital, Rigshospitalet, University of Copenhagen, Blegdamsvej 9, DK-2100 Copenhagen, Denmark

*Correspondence address: Laboratory of Reproductive Biology, Section 5712, Copenhagen University Hospital, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark. Tel: +45-35455822; Fax: +45-35455824; E-mail: yding@rh.dk

Submitted on October 26, 2016; resubmitted on January 14, 2017; accepted on February 17, 2017

ABSTRACT: Older women are often the most challenging group of patients in fertility clinics due to a decline in both number and overall quality of oocytes. The quality of oocytes has been linked to mitochondrial dysfunction. In this mini-review, we discuss this hypothesis and suggest alternative treatment options using autologous mitochondria to potentially augment pregnancy potential in ART. Autologous transfer of mitochondria from the patient's own germline cells has attracted much attention as a possible new treatment to revitalize deficient oocytes. IVF births have been reported after transfer of oogonial precursor cell-derived mitochondria; however, the source and quality of the mitochondria are still unclear. In contrast, fully grown oocytes are loaded with mitochondria which have passed the genetic bottleneck and are likely to be of high quality. An increased supply of such oocytes could potentially be obtained by *in vitro* follicle activation of ovarian cortical biopsies or from surplus immature oocytes collected from women undergoing ART or fertility preservation of ovarian tissue. Taken together, autologous oocytes are not necessarily a limiting resource in ART as fully grown oocytes with high quality mitochondria can be obtained from natural or stimulated ovaries and potentially be used to improve both quality and quantity of oocytes available for fertility treatment.

Key words: mitochondria / oocyte quality / fertility / *in vitro* activation / *in vitro* maturation / reproductive aged women / oocyte quantity / ART

Introduction

Many women in most high-income countries postpone childbearing until their thirties and forties (Mills *et al.*, 2011). However, female fecundity decreases with age, and the decline accelerates rapidly after 35 years of age (Menken *et al.*, 1986; Nelson *et al.*, 2013). Moreover, an age-dependent decline in the quality of oocytes occurs, mainly due to an increase in chromosomal aneuploidy of which the prevalence rises dramatically with advanced maternal age (Franasiak *et al.*, 2014).

Consequently, women of advanced reproductive age are often the most challenging population of patients in fertility centers as these women frequently present both low number and poor quality oocytes resulting in limited reproductive success (Stoop *et al.*, 2012).

In this mini-review, we discuss alternative treatment options using autologous mitochondria which potentially could augment pregnancy potential of oocytes from women of advanced reproductive age, including novel strategies that simultaneously provide both increased number and augmented quality of mature oocytes.

Mitochondria and oocytes

Mitochondria constitute the powerhouse of cells as they synthesize ATP by oxidative phosphorylation. They possess their own small genome in the form of mitochondrial DNA (mtDNA), but due to the lack of protective histones, introns and DNA-repair enzymes the mtDNA accumulates mutations and deletions to a higher degree than nuclear DNA (Satoh and Kuroiwa, 1991; Bentov and Casper, 2013; Scheibye-Knudsen *et al.*, 2015). Mitochondria are inherited uniparentally from the mother as sperm mitochondrial proteins are ubiquitinated and degraded after oocyte entry (Kaneda *et al.*, 1995; Song *et al.*, 2014).

Female germ cells and the genetic bottleneck of mtDNA

Mitochondrial replication starts in the primordial germ cells (PGCs) and continues during early oogenesis; however, a sharp increase in mitochondrial number is observed during later stages of oogenesis (St John, 2014). Thus, whereas PGCs only contain a few hundred copies of mtDNA, mature oocytes contain up to several hundred thousand copies (Wai *et al.*, 2008; Otten and Smeets, 2015). The fact that mitochondria on one hand undergo relatively rapid mutations and on the other hand are capable of maintaining an unmodified genome through generations has been explained by a genetic bottleneck theory (Wai *et al.*, 2008; Johnston *et al.*, 2015; reviewed in Mishra and Chan, 2014; Stewart and Chinnery, 2015), which postulates that only a small proportion of the total number of mitochondrial genomes are passed on from mother to offspring (Hauswirth and Laipis, 1985). The precise mechanism is not yet well defined, but originally it was thought that in PGCs the bottleneck resulted from selection of mitochondria with normal mtDNA while eliminating mutated mtDNA, which resulted in a more homogenous mtDNA population (Stewart and Chinnery, 2015; May-Panloup *et al.*, 2016). However, a murine study suggested that selection of mitochondria and mtDNA in the oocyte occurs randomly and no screening for the intact wild-type mitochondrial genome takes place (Inoue *et al.*, 2000). The selection of mtDNA nucleoids preferentially amplified during replication appears to occur during folliculogenesis and oocyte growth (Wai *et al.*, 2008; Bentov *et al.*, 2011).

It may be speculated that the actual bottleneck is occurring during fetal life, which is characterized by a massive loss of female germ cells during initiation of meiosis and follicular establishment (Baker, 1963; Gougeon *et al.*, 1994). Both of these processes are likely to require high energy consumption. Only around 1 in 10 of the oogonia makes it through fetal life and ends up in a functional follicle at birth. Thus, oocytes with preferentially normal mitochondria are likely to be better suited to undertake this process compared to those with abnormal mitochondria and insufficient energy production.

Mitochondria are major factors of oocyte quality

Mitochondria play vital roles in oocyte functions and are the major source of ATP during preimplantation embryonic development as glycolysis is limited after fertilization and mitochondrial replication is suppressed from metaphase II (MII) oocytes until the hatched blastocyst stage (Chappel, 2013).

Dysfunction of oocyte mitochondria is believed to be a key factor involved in poor developmental competence of oocytes in older infertility patients (Babayev and Seli, 2015). The mtDNA content in oocytes of reproductive aged women or of women with diminished ovarian reserve is significantly lower than that of younger patients or those with normal ovarian reserve (Duran *et al.*, 2011; Murakoshi *et al.*, 2013). Furthermore, mtDNA content correlates with the ability of human oocytes to be fertilized as oocytes that remain unfertilized possess lower mtDNA copy number (Reynier *et al.*, 2001; Santos *et al.*, 2006). However, mitochondrial copy number does not seem to be crucial for oocyte maturation and fertilization in pigs and mice as alterations in mtDNA copy number only seem to interfere with embryo development (Ge *et al.*, 2012; Lee *et al.*, 2014). In humans, higher ATP levels in oocytes correlate with better embryo development and implantation rates (Van Blerkom *et al.*, 1995), thus it has been suggested that oocytes during maturation contain excessive amounts of mitochondria with the primary aim of supporting early embryonic development where mitochondrial replication is suppressed (Babayev and Seli, 2015).

Attenuated mitochondrial activity reduces the production of ATP, and failure of ATP production has been shown to have deleterious consequences on chromosome segregation and on embryonic development which could potentially lead to aneuploid embryos and reduced implantation (Van Blerkom, 2011; Eichenlaub-Ritter, 2012).

Fertility treatments using heterologous sources of mitochondria

Both pharmacological approaches and cytoplasmic-, nuclear- and mitochondrial transfer have been used to exchange and enhance the integrity, activity and number of mitochondria in quality-compromised oocytes.

In the 1990s, cytoplasmic transfer, or the augmentation of patient oocytes with a small volume (1–5%) of ooplasm from young donors was used by several IVF clinics in an effort to overcome repeated IVF failures in selected patients (Cohen *et al.*, 1998; Barritt *et al.*, 2001). The procedure essentially involved co-injection of donor ooplasm with sperm during ICSI. Heterologous ooplasmic transfer led to successful pregnancies with almost 50 live births. However, donor mtDNA was identified in the offspring ('three genetic parents') which raised both ethical and genetic questions and the procedure was suspended by the US Food and Drug Administration (FDA) in 2002 and remains so.

Mitochondrial manipulations involving the transfer of nuclear DNA have also been developed and were initially proposed to prevent vertical transmission of diseases caused by mutations in mtDNA (Craven *et al.*, 2010; Paull *et al.*, 2013). The procedure comprised removal of nuclear DNA from an unfertilized oocyte of a patient carrying abnormal mitochondria followed by transfer into an enucleated donor oocyte containing assumed healthy mitochondria. This constructed oocyte was subsequently fertilized by ICSI. However, such mitochondrial manipulations have come under criticism worldwide (Isasi *et al.*, 2016) as these techniques raise the risks of heteroplasmy i.e. the coexistence of two mtDNA genomes, linked to the use of mitochondria or ooplasm from a third donor. Intriguingly, non-pathogenic variants of mitochondrial heteroplasmy have been reported to alter physiological and cognitive functions in mice (Acton *et al.*, 2007; Sharpley *et al.*, 2012). Moreover, even though the nuclear genetic material is contributed by two parents 'only', and the oocyte donor who contributes the mtDNA provides only marginally to the total genetic make-up of the newborn, this cannot distract from the fact that there are three genetic parents. The techniques have therefore been ethically challenged and are currently considered controversial.

Autologous mitochondrial transfer

Injection of purified mitochondria instead of ooplasm is, however, a potential alternative approach to present much larger amounts of mitochondria into the oocyte (Liu *et al.*, 2014). Simple guidelines for mitochondrial injection demand that the mitochondria should be

obtained from the patient's own cells which optimally should be of ovarian or oocyte origin (Ferreira *et al.*, 2010; Takeda *et al.*, 2010; Koopman *et al.*, 2012; Wolf *et al.*, 2015; Darbandi *et al.*, 2016), and the mtDNA from these cells should have passed the mitochondrial bottleneck and be of high quality, without deletions or mutations (Darbandi *et al.*, 2016).

Use of autologous mitochondria from ovarian stem cells

The recent suggestion that adult oogonial stem cells (OSCs) might provide a source of patient-matched germline mitochondria to augment oocyte performance launched a new approach. In 2012, it was reported that human OSCs were isolated from cortical biopsies from ovaries of reproductive age women by a Ddx4 (Vasa) antibody-based protocol (White *et al.*, 2012). Recently, the American company 'OvaScience' reported improved fertility in women with previous poor reproductive performance by autologous mitochondrial injection treatment—also referred to as the AUGMENT^{SM} treatment (Fakih *et al.*, 2015; Woods and Tilly, 2015). By isolating OSCs from human cortical biopsies, they obtained autologous mitochondria by differential centrifugation. In connection with ICSI, the mitochondria were injected into oocytes to augment oocyte performance. As a result of this procedure, more than a dozen healthy live births or ongoing pregnancies have been reported (Fakih *et al.*, 2015; Meldrum *et al.*, 2016). However, this procedure has been challenged by the ESHRE SIG Stem Cells group, which have asked for RCT's with a proper control group, and highlighted methodological issues and the lack of information form animal models. Furthermore, it is not yet proven whether these germ cell-derived mitochondria actually have passed the genetic bottleneck and are suitable to pass on to the next generation (Gosden and Johnson, 2016). The current theory suggests that the mitochondrial bottleneck takes place during folliculogenesis (Wai *et al.*, 2008) which is long after the OSC development takes place.

Alternative approach; improving both oocyte quantity and quality

Since OSCs are difficult to obtain and contain relatively low numbers of mitochondria, alternative sources of oocytes that would have passed the genetic bottleneck and in theory would be loaded with uncompromised mitochondria should be considered. The number of autologous fully grown oocytes may be significantly augmented by *in vitro* follicle activation (IVA) of ovarian cortical biopsies or by using surplus immature oocytes collected when the woman undergoes ART or fertility preservation of ovarian tissue (Fig. 1). This alternative approach could potentially enhance the number of oocytes available for fertility treatment but also augment oocyte performance by autologous mitochondrial injection.

Increasing oocyte quantity by IVA

The PI3K-AKT signaling pathway is responsible for regulation of follicle quiescence, and upregulation of the pathway has been demonstrated to stimulate activation and growth of dormant primordial follicles (Li *et al.*, 2010; Adhikari *et al.*, 2012; Kawamura *et al.*, 2013; Novella-Maestre *et al.*, 2015; Suzuki *et al.*, 2015; Cheng *et al.*, 2015a,b). The lipid phosphatase PTEN inhibits the PI3K-AKT signal transduction pathway and acts as a break on follicle activation (Reddy *et al.*, 2008). In 2013, a Japanese group were the first to test IVA in a clinical setting (Kawamura *et al.*, 2013). In this study, ovarian cortical tissue from premature ovarian insufficiency patients were activated *in vitro* using a PTEN inhibitor, bpv(pic), together with an AKT stimulator, and after grafting they found rapid follicle growth in some patients. Mature oocytes were successfully retrieved and have now resulted in three live births in Japan and China and one pregnancy in Spain (Kawamura *et al.*, 2013; Suzuki *et al.*, 2015; Kawamura *et al.*, 2015; Zhai *et al.*, 2016).

As an alternative, the ovarian biopsy excised for OSCs production could be used for IVA instead (Fig. 1). Following a short treatment to stimulate PI3K-AKT signaling, ovarian tissue should be transplanted back into the woman—without cryopreservation of the tissue as performed in Japan. Three to six months later, the patient will potentially develop a large cohort of follicles containing fully grown oocytes. These oocytes would be available for ART, but their competence would probably be compromised due to the age of the woman. If this approach results in extended number of mature oocytes, some of these oocytes may be used as a source of mitochondria to augment performance of the remaining cohort of oocytes. Delivery of the mitochondria can be performed in connection with ICSI as previously shown. Thus, this strategy would not only enhance oocyte numbers but also potentially improve oocyte quality, and may represent an alternative strategy especially in women with poor reproductive performance.

However, good evidence for the safe use of IVA in humans are currently lacking as only few studies on human ovarian tissue have been performed and no RCT has yet been conducted. PTEN has a well-known role as a tumor suppressor and is commonly mutated in human cancer diseases (Cully *et al.*, 2006; Song *et al.*, 2012). Thus, deletion or inhibition of PTEN could potentially lead to oncogenic transformation of cells in the ovary (Kim *et al.*, 2016). Moreover, PTEN inhibitors have been shown be unspecific and cytotoxic when used in high (micro-molar) concentrations (Schmid *et al.*, 2004). However, no toxicity, tumorigenesis or effects on fertility were observed when injecting female mice directly with PTEN inhibitors or in first- and second-generation offspring (Li *et al.*, 2010; Adhikari *et al.*, 2012). Nonetheless, recent human studies have raised concerns about IVA treatment as human follicular survival was severely compromised in *in vitro* studies and significant deleterious effects were found (Lerer-Serfaty *et al.*, 2013; McLaughlin *et al.*, 2014). Therefore, potential tumorigenic effects of PTEN inhibition should thoroughly be tested in animal models, preferentially non-human primates, before applied in a clinical setting. Different targets of the PI3K-AKT and other signaling pathways are now being investigated as potential new candidates for IVA to establish more safe, reproducible and valid methods (Cheng *et al.*, 2015a,b; Sun *et al.*, 2015; Saatcioglu *et al.*, 2016).

IVM oocytes available in connection with ovarian tissue freezing and ART

Cryopreservation of ovarian tissue differs from conventional oocyte and embryo cryopreservation by restoring the ovarian organ function (von Wolff *et al.*, 2009). Current practice usually involves the excision of one ovary and the cortical tissue with its dense population of resting primordial follicles is frozen (Rosendahl *et al.*, 2011). A number of

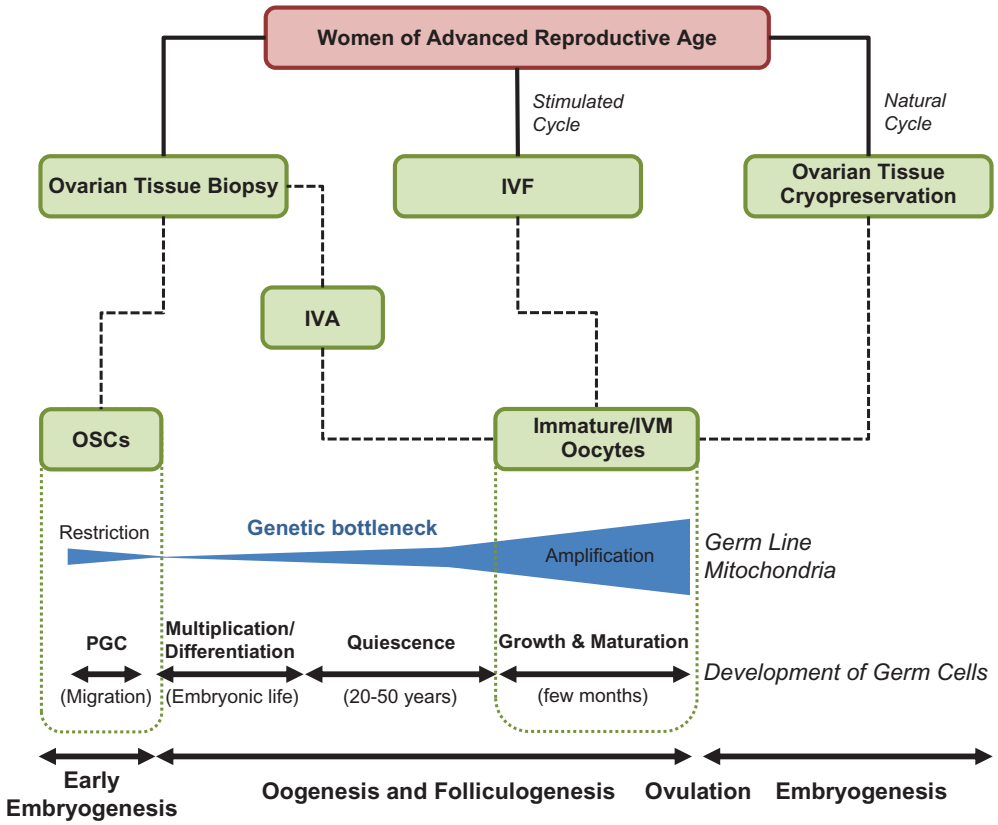

Figure 1 Alternative sources of autologous oocytes. Autologous transfer of mitochondria from the patient's own germline cells has attracted much attention as a new possible treatment to revitalize deficient oocytes in women of advanced reproductive age. Oogonial stem cells (OSCs) and oocytes from fully grown oocytes are potential sources of autologous mitochondria which could enhance oocyte performance. IVF births have been reported by the use of mitochondria from OSCs, but the OSCs are difficult to obtain and contain relatively low numbers of mitochondria with a questionable quality as they probably have not passed the genetic bottleneck. In contrast, fully grown oocytes are loaded with mitochondria which have passed the genetic bottleneck and may represent high quality mitochondria. Fully grown oocytes constitute an alternative source of autologous oocytes, which could potentially be obtained by *in vitro* activation (IVA) of ovarian cortical biopsies or from surplus immature oocytes collected from women undergoing ART or fertility preservation of ovarian tissue. Lower panel of the figure has been modified from May-Panloup *et al.* (2016). IVM, *in vitro* maturation; PGC, primordial germ cells.

studies have shown that immature oocytes can be collected from antral follicles visible on the surface of the ovary or released to the dissection medium during preparation of the tissue (Fig. 2A and B) (Wilken-Jensen *et al.*, 2014; Yin *et al.*, 2016). These oocytes may be matured, vitrified and used to augment fertility to the patient herself (Fasano *et al.*, 2011; Gonzalez *et al.*, 2011; Imesch *et al.*, 2013; Yin *et al.*, 2016). The first two successful pregnancies resulting from cryopreserved embryos obtained from *in vitro* matured (IVM) oocytes collected after oophorectomy were recently reported (Prasath *et al.*, 2014; Segers *et al.*, 2015). In a recent study, we demonstrated that surprisingly many immature oocytes can be retrieved during the process of ovarian cortex preparation (Fig. 2C) (Yin *et al.*, 2016). On average, 11 oocytes were collected per patient, ranging from 0 to 43, and ~30% of the oocytes matured to the MII stage. The remaining oocytes or potentially all these fully grown oocytes may serve as an autologous source of mitochondria to augment deficient oocytes (Fig. 1). Isolated cryo-stored mitochondria could be used in subsequent IVF treatment following oocyte collection after transplantation of frozen/thawed ovarian tissue. Furthermore, these IVM oocytes are from unstimulated ovaries and they have a fairly high maturation rate (Yin *et al.*, 2016), which suggest that they are healthier than immature oocytes from stimulated IVF cycles.

Traditional IVF regimes use gonadotrophins to stimulate the development of a large number of follicles, and oocytes are then recovered by transvaginal ultrasound-guided follicle aspiration, 34–36 h after hCG administration. Only those oocytes that reach MII are used for conventional IVF. However, between 5 and 20% of recovered oocytes are immature (Cha and Chian, 1998) and are usually discarded due to attenuated developmental competence. These immature oocytes may be compromised, but the mitochondria have passed the genetic bottleneck and may represent a new unexploited source of mitochondria to improve oocyte performance in ART (Fig. 1). In addition, small antral follicles with diameters <12–14 mm may even represent an extra source of immature oocytes that is currently not aspirated in connection with IVF and which may be used to prepare mitochondria.

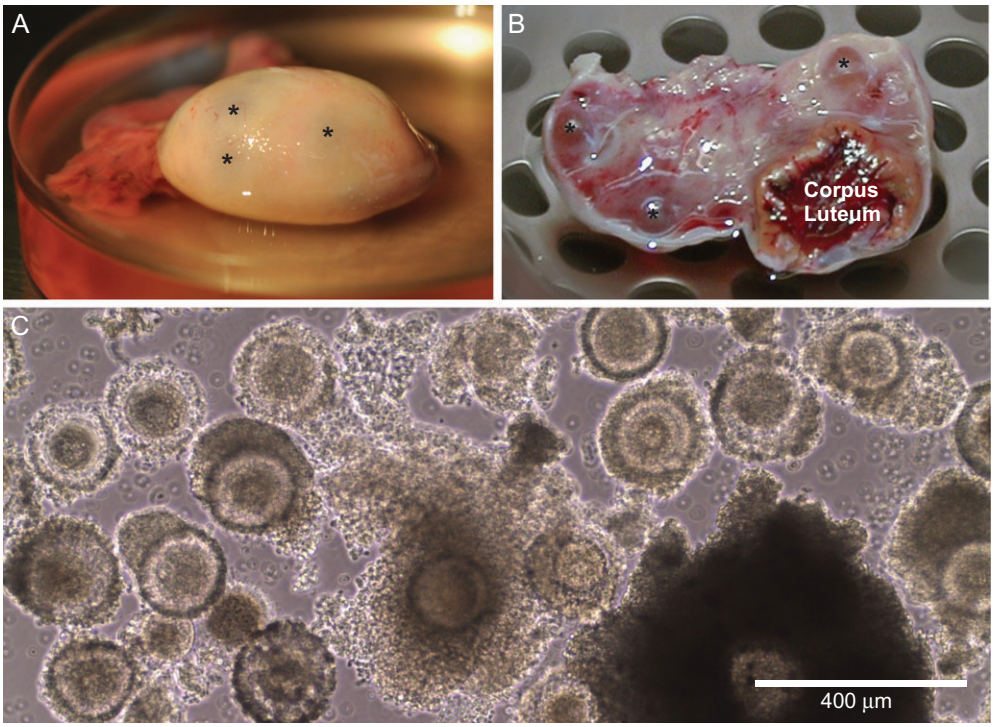

Figure 2 Immature oocytes available in women undergoing fertility preservation of ovarian tissue. A human ovary surgically removed for fertility preservation (**A**) and cut into half for cortical preparation (**B**). Asterisks show small antral follicles visible on the surface of the ovary and on the inside of the ovary. (**C**) Human *in vitro* matured (IVM) oocytes collected from the dissection medium at the time of the cryopreservation procedure.

Finding the balance

Constant maintenance of a healthy mitochondrial pool is necessary for normal aging and depends on a precisely coordinated balance of mitochondrial biogenesis and selective cellular degradation. While too few mitochondria impede generation of energy, too many mitochondria also compromise cellular function (Ylikallio *et al.*, 2010). This might be reflected in a recent study showing that mtDNA levels are higher in embryos from reproductive aged women, in aneuploid embryos (independent of age) and in euploid blastocysts that failed to implant (Fragouli *et al.*, 2015). Thus, this study is in contrast to the studies showing that a high level of mtDNA content improve oocyte maturation and fertilization (Reynier *et al.*, 2001; Santos *et al.*, 2006). Therefore, the relationship between mtDNA content, female age, oocyte maturation, fertilization and embryo development warrants further investigations to elucidate the precise role of mitochondria in reproduction.

Moreover, the movement of mitochondria to high energy consumption areas is crucial for oocyte maturation (Dumollard *et al.*, 2006; Mao *et al.*, 2014). In immature oocytes, mitochondria are aggregated around the germinal vesicle (GV) and usually absent from the cortical part of the cytoplasm (Sathananthan and Trounson, 2000; Familiari *et al.*, 2006), whereas in MI and MII oocytes mitochondria become more numerous and are spread out in the ooplasm (Motta *et al.*, 2000; Sathananthan and Trounson, 2000). Unlike other species, mitochondria of human oocytes form large aggregates with smooth endoplasmic reticulum tubular membranes and vesicles at the end of the maturation process (Motta *et al.*, 2000; Familiari *et al.*, 2006; Mao *et al.*, 2014). Whether or not isolated donor or autologous mitochondria are actually capable to function properly, establish the necessary physical connections with other organelles and augment oocyte performance in connection with fertility treatment requires further studies.

Finally, proper animal studies and RCTs are needed in order to substantiate the legitimacy of this potential new treatment option. Moreover, a better characterization and understanding of mitochondrial integrity, content and activity from 'natural', IVF and *in vitro* activated oocytes are required to advance these techniques to clinical practice.

Conclusion

To overcome ethical considerations on genetic origin and the risk of heteroplasmy; transfer of autologous mitochondria from ovarian cells has attracted much attention as a new possible treatment to improve pregnancy potential of deficient oocytes from reproductive aged women. Both OSCs and oocytes from fully grown oocytes are potential sources of autologous mitochondria which could enhance oocyte performance. The advantages of using surplus fully grown oocytes from IVF, IVM or IVA are that they are fairly easy to obtain and would already have passed the mitochondrial genetic bottleneck and are likely to represent high quality mitochondria (Fig. 1). In contrast, OSCs

are difficult to obtain and contain relatively low numbers of mitochondria of unknown quality.

If this potential new treatment becomes an option, it is not necessarily appropriate for all women of advanced reproductive age as dysfunctional mitochondria may be one among many factors that cause developmental failure in oocytes of reproductive aged women. Future studies should try to identify a suitable group of patients for whom the transfer of autologous mitochondria would be the most beneficial.

In conclusion, autologous oocytes are not necessarily a limiting resource in ART for women of advanced reproductive age, and high quality mitochondria could potentially be transferred from natural and stimulated surplus fully grown oocytes to revitalize deficient oocytes, thereby both augmenting quantity and quality of the available cohort of oocytes for fertility.

Authors' roles

S.G.K., S.E.P. and C.Y.A. all contributed to the conception and writing of this paper.

Funding

The Research Pools of Rigshospitalet and the EU interregional project ReproUnion are thanked for having funded this study. They had no role in the study design, collection and analysis of data, data interpretation or in writing the report.

Conflict of interest

None declared.

References

Acton B, Lai I, Shang X, Jurisicova A, Casper R. Neutral mitochondrial heteroplasmy alters physiological function in mice. *Biol Reprod* 2007;**77**:569–576.

Adhikari D, Gorre N, Risal S, Zhao Z, Zhang H, Shen Y, Liu K. The safe use of a PTEN inhibitor for the activation of dormant mouse primordial follicles and generation of fertilizable eggs. *PLoS One* 2012;**7**:e39034.

Babayev E, Seli E. Oocyte mitochondrial function and reproduction. *Curr Opin Obstet Gynecol* 2015;**27**:175–181.

Baker TG. A quantitative and cytological study of germ cells in human ovaries. *Proc R Soc Lond B Biol Sci* 1963;**158**:417–433.

Barritt JA, Brenner CA, Malter HE, Cohen J. Mitochondria in human offspring derived from ooplasmic transplantation. *Hum Reprod* 2001;**16**:513–516.

Bentov Y, Casper RF. The aging oocyte—can mitochondrial function be improved? *Fertil Steril* 2013;**99**:18–22.

Bentov Y, Yavorska T, Esfandiari N, Jurisicova A, Casper RF. The contribution of mitochondrial function to reproductive aging. *J Assist Reprod Genet* 2011;**28**:773–783.

Cha KY, Chian RC. Maturation in vitro of immature human oocytes for clinical use. *Hum Reprod Update* 1998;**4**:103–120.

Chappel S. The role of mitochondria from mature oocyte to viable blastocyst. *Obstet Gynecol Int* 2013;**2013**:183024.

Cheng Y, Feng Y, Jansson L, Sato Y, Deguchi M, Kawamura K, Hsueh AJ. Actin polymerization—enhancing drugs promote ovarian follicle growth mediated by the Hippo signaling effector YAP. *FASEB J* 2015a;**29**:2423–2430.

Cheng Y, Kim J, Li XX, Hsueh AJ. Promotion of ovarian follicle growth following mTOR activation: synergistic effects of AKT stimulators. *PLoS One* 2015b;**10**:e0117769.

Cohen J, Scott R, Alikani M, Schimmel T, Munne S, Levron J, Wu L, Brenner C, Warner C, Willadsen S. Ooplasmic transfer in mature human oocytes. *Mol Hum Reprod* 1998;**4**:269–280.

Craven L, Tuppen HA, Greggains GD, Harbottle SJ, Murphy JL, Cree LM, Murdoch AP, Chinnery PF, Taylor RW, Lightowlers RN. Pronuclear transfer in human embryos to prevent transmission of mitochondrial DNA disease. *Nature* 2010;**465**:82–85.

Cully M, You H, Levine AJ, Mak TW. Beyond PTEN mutations: the PI3K pathway as an integrator of multiple inputs during tumorigenesis. *Nat Rev Cancer* 2006;**6**:184–192.

Darbandi S, Darbandi M, Khorshid HRK, Sadeghi MR, Al-Hasani S, Agarwal A, Shirazi A, Heidari M, Akhondi MM. Experimental strategies towards increasing intracellular mitochondrial activity in oocytes: a systematic review. *Mitochondrion* 2016;**30**:8–17.

Dumollard R, Duchen M, Sardet C. Calcium signals and mitochondria at fertilisation. *Semin Cell Dev Biol* 2006;**17**:314–323.

Duran HE, Simsek-Duran F, Oehninger SC, Jones HW Jr, Castora FJ. The association of reproductive senescence with mitochondrial quantity, function, and DNA integrity in human oocytes at different stages of maturation. *Fertil Steril* 2011;**96**:384–388.

Eichenlaub-Ritter U. Oocyte ageing and its cellular basis. *Int J Dev Biol* 2012;**56**:841–852.

Fakih MHSM, Szeptycki J, dela Cruz DB, Lux C, Verjee S, Burgess CM, Cohn GM, Casper RF. The AUGMENT treatment: physician reported outcomes of the initial global patient experience. *JFIV Reprod Med Genet* 2015;**3**:154.

Familiari G, Heyn R, Relucenti M, Nottola SA, Sathananthan AH. Ultrastructural dynamics of human reproduction, from ovulation to fertilization and early embryo development. *Int Rev Cytol* 2006;**249**:53–141.

Fasano G, Moffa F, Dechène J, Englert Y, Demeestere I. Vitrification of in vitro matured oocytes collected from antral follicles at the time of ovarian tissue cryopreservation. *Reprod Biol Endocrinol* 2011;**9**:150.

Ferreira CR, Burgstaller JP, Perecin F, Garcia JM, Chiaratti MR, Méo SC, Müller M, Smith LC, Meirelles FV, Steinborn R. Pronounced segregation of donor mitochondria introduced by bovine ooplasmic transfer to the female germ-line. *Biol Reprod* 2010;**82**:563–571.

Fragouli E, Spath K, Alfarawati S, Kaper F, Craig A, Michel CE, Kokocinski F, Cohen J, Munne S, Wells D. Altered levels of mitochondrial DNA are associated with female age, aneuploidy, and provide an independent measure of embryonic implantation potential. *PLoS Genet* 2015;**11**:e1005241.

Franasiak JM, Forman EJ, Hong KH, Werner MD, Upham KM, Treff NR, Scott RT Jr. The nature of aneuploidy with increasing age of the female partner: a review of 15,169 consecutive trophectoderm biopsies evaluated with comprehensive chromosomal screening. *Fertil Steril* 2014;**101**:656–663.

Ge H, Tollner TL, Hu Z, Dai M, Li X, Guan H, Shan D, Zhang X, Lv J, Huang C *et al*. The importance of mitochondrial metabolic activity and mitochondrial DNA replication during oocyte maturation in vitro on oocyte quality and subsequent embryo developmental competence. *Mol Reprod Dev* 2012;**79**:392–401.

Gonzalez C, Devesa M, Boada M, Coroleu B, Veiga A, Barri PN. Combined strategy for fertility preservation in an oncologic patient: vitrification of in vitro matured oocytes and ovarian tissue freezing. *J Assist Reprod Genet* 2011;**28**:1147–1149.

Gosden RG, Johnson MH. Can oocyte quality be augmented? *Reprod Biomed Online* 2016;**32**:551–555.

Gougeon A, Ecochard R, Thalabard JC. Age-related changes of the population of human ovarian follicles: increase in the disappearance rate of

non-growing and early-growing follicles in aging women. *Biol Reprod* 1994;**50**:653–663.

Hauswirth W, Laipis P. Transmission genetics of mammalian mitochondria: a molecular model and experimental evidence. In: Quagliarello E (ed). *Achievements and Perspectives of Mitochondrial Research*. Amsterdam: Elsevier Biomedical, 1985, 49–59.

Imesch P, Scheiner D, Xie M, Fink D, Macas E, Dubey R, Imthurn B. Developmental potential of human oocytes matured in vitro followed by vitrification and activation. *J Ovarian Res* 2013;**6**:30.

Inoue K, Nakada K, Ogura A, Isobe K, Goto Y, Nonaka I, Hayashi JI. Generation of mice with mitochondrial dysfunction by introducing mouse mtDNA carrying a deletion into zygotes. *Nat Genet* 2000;**26**: 176–181.

Isasi R, Kleiderman E, Knoppers BM. Genetic technology regulation. editing policy to fit the genome? *Science* 2016;**351**:337–339.

Johnston IG, Burgstaller JP, Havlicek V, Kolbe T, Rulicke T, Brem G, Poulton J, Jones NS. Stochastic modelling, Bayesian inference, and new in vivo measurements elucidate the debated mtDNA bottleneck mechanism. *Elife* 2015;**4**:e07464.

Kaneda H, Hayashi J, Takahama S, Taya C, Lindahl KF, Yonekawa H. Elimination of paternal mitochondrial DNA in intraspecific crosses during early mouse embryogenesis. *Proc Natl Acad Sci USA* 1995;**92**:4542–4546.

Kawamura K, Cheng Y, Sun YP, Zhai J, Diaz-Garcia C, Simon C, Pellicer A, Hsueh AJ. Ovary transplantation: to activate or not to activate. *Hum Reprod* 2015;**30**:2457–2460.

Kawamura K, Cheng Y, Suzuki N, Deguchi M, Sato Y, Takae S, Ho CH, Kawamura N, Tamura M, Hashimoto S *et al*. Hippo signaling disruption and Akt stimulation of ovarian follicles for infertility treatment. *Proc Natl Acad Sci USA* 2013;**110**:17474–17479.

Kim SY, Ebbert K, Cordeiro MH, Romero MM, Whelan KA, Suarez AA, Woodruff TK, Kurita T. Constitutive activation of PI3K in oocyte induces ovarian granulosa cell tumors. *Cancer Res* 2016;**76**:3851–3861.

Koopman WJ, Willems PH, Smeitink JA. Monogenic mitochondrial disorders. *N Engl J Med* 2012;**366**:1132–1141.

Lee SK, Zhao MH, Kwon JW, Li YH, Lin ZL, Jin YX, Kim NH, Cui XS. The association of mitochondrial potential and copy number with pig oocyte maturation and developmental potential. *J Reprod Dev* 2014;**60**:128–135.

Lerer-Serfaty G, Samara N, Fisch B, Shachar M, Kossover O, Seliktar D, Ben-Haroush A, Abir R. Attempted application of bioengineered/biosynthetic supporting matrices with phosphatidylinositol-trisphosphate—enhancing substances to organ culture of human primordial follicles. *J Assist Reprod Genet* 2013;**30**:1279–1288.

Li J, Kawamura K, Cheng Y, Liu S, Klein C, Liu S, Duan EK, Hsueh AJW. Activation of dormant ovarian follicles to generate mature eggs. *Proc Natl Acad Sci USA* 2010;**107**:10280–10284.

Liu CS, Chang JC, Kuo SJ, Liu KH, Lin TT, Cheng WL, Chuang SF. Delivering healthy mitochondria for the therapy of mitochondrial diseases and beyond. *Int J Biochem Cell Biol* 2014;**53**:141–146.

Mao L, Lou H, Lou Y, Wang N, Jin F. Behaviour of cytoplasmic organelles and cytoskeleton during oocyte maturation. *Reprod Biomed Online* 2014; **28**:284–299.

May-Panloup P, Boucret L, Chao de la Barca JM, Desquiret-Dumas V, Ferré-L'Hotellier V, Morinière C, Descamps P, Procaccio V, Reynier P. Ovarian ageing: the role of mitochondria in oocytes and follicles. *Hum Reprod Update* 2016;**22**:725–743.

McLaughlin M, Kinnell HL, Anderson RA, Telfer EE. Inhibition of phosphatase and tensin homologue (PTEN) in human ovary in vitro results in increased activation of primordial follicles but compromises development of growing follicles. *Mol Hum Reprod* 2014;**20**:736–744.

Meldrum DR, Casper RF, Diez-Juan A, Simon C, Domar AD, Frydman R. Aging and the environment affect gamete and embryo potential: can we intervene? *Fertil Steril* 2016;**105**:548–559.

Menken J, Trussell J, Larsen U. Age and infertility. *Science* 1986;**233**: 1389–1394.

Mills M, Rindfuss RR, McDonald P, Velde Te E. Why do people postpone parenthood reasons and social policy incentives. *Hum Reprod Update* 2011;**17**:848–860.

Mishra P, Chan DC. Mitochondrial dynamics and inheritance during cell division, development and disease. *Nat Rev Mol Cell Biol* 2014;**15**: 634–646.

Motta PM, Nottola SA, Makabe S, Heyn R. Mitochondrial morphology in human fetal and adult female germ cells. *Hum Reprod* 2000;**15**: 129–147.

Murakoshi Y, Sueoka K, Takahashi K, Sato S, Sakurai T, Tajima H, Yoshimura Y. Embryo developmental capability and pregnancy outcome are related to the mitochondrial DNA copy number and ooplasmic volume. *J Assist Reprod Genet* 2013;**30**:1367–1375.

Nelson SM, Telfer EE, Anderson RA. The ageing ovary and uterus: new biological insights. *Hum Reprod Update* 2013;**19**:67–83.

Novella-Maestre E, Herraiz S, Rodríguez-Iglesias B, Díaz-García C, Pellicer A. Short-term PTEN inhibition improves in vitro activation of primordial follicles, preserves follicular viability, and restores AMH levels in cryopreserved ovarian tissue from cancer patients. *PLoS One* 2015;**10**:e0127786.

Otten AB, Smeets HJ. Evolutionary defined role of the mitochondrial DNA in fertility, disease and ageing. *Hum Reprod Update* 2015;**21**:671–689.

Paull D, Emmanuele V, Weiss KA, Treff N, Stewart L, Hua H, Zimmer M, Kahler DJ, Goland RS, Noggle SA *et al*. Nuclear genome transfer in human oocytes eliminates mitochondrial DNA variants. *Nature* 2013; **493**:632–637.

Prasath EB, Chan ML, Wong WH, Lim CJ, Tharmalingam MD, Hendricks M, Loh SF, Chia YN. First pregnancy and live birth resulting from cryopreserved embryos obtained from in vitro matured oocytes after oophorectomy in an ovarian cancer patient. *Hum Reprod* 2014;**29**:276–278.

Reddy P, Liu L, Adhikari D, Jagarlamudi K, Rajareddy S, Shen Y, Du C, Tang W, Hämäläinen T, Peng SL *et al*. Oocyte-specific deletion of Pten causes premature activation of the primordial follicle pool. *Science* 2008; **319**:611–613.

Reynier P, May-Panloup P, Chretien MF, Morgan CJ, Jean M, Savagner F, Barrière P, Malthièry Y. Mitochondrial DNA content affects the fertilizability of human oocytes. *Mol Hum Reprod* 2001;**7**:425–429.

Rosendahl M, Schmidt KT, Ernst E, Rasmussen PE, Loft A, Byskov AG, Andersen AN, Andersen CY. Cryopreservation of ovarian tissue for a decade in Denmark: a view of the technique. *Reprod Biomed Online* 2011;**22**:162–171.

Saatcioglu HD, Cuevas I, Castrillon DH. Control of oocyte reawakening by kit. *PLoS Genet* 2016;**12**:e1006215.

Santos TA, El Shourbagy S, St John JC. Mitochondrial content reflects oocyte variability and fertilization outcome. *Fertil Steril* 2006;**85**: 584–591.

Sathananthan AH, Trounson AO. Mitochondrial morphology during preimplantational human embryogenesis. *Hum Reprod* 2000;**15**: 148–159.

Satoh M, Kuroiwa T. Organization of multiple nucleoids and DNA molecules in mitochondria of a human cell. *Exp Cell Res* 1991;**196**:137–140.

Scheibye-Knudsen M, Fang EF, Croteau DL, Wilson DM III, Bohr VA. Protecting the mitochondrial powerhouse. *Trends Cell Biol* 2015;**25**: 158–170.

Schmid AC, Byrne RD, Vilar R, Woscholski R. Bisperoxovanadium compounds are potent PTEN inhibitors. *FEBS Lett* 2004;**566**:35–38.

Segers I, Mateizel I, Van Moer E, Smitz J, Tournaye H, Verheyen G, De Vos M. In vitro maturation (IVM) of oocytes recovered from ovariectomy specimens in the laboratory: a promising "ex vivo" method of oocyte cryopreservation resulting in the first report of an ongoing pregnancy in Europe. *J Assist Reprod Genet* 2015;**32**:1221–1231.

Sharpley MS, Marciniak C, Eckel-Mahan K, McManus M, Crimi M, Waymire K, Lin CS, Masubuchi S, Friend N, Koike M *et al*. Heteroplasmy of mouse mtDNA is genetically unstable and results in altered behavior and cognition. *Cell* 2012;**151**:333–343.

Song MS, Salmena L, Pandolfi PP. The functions and regulation of the PTEN tumour suppressor. *Nat Rev Mol Cell Biol* 2012;**13**:283–296.

Song WH, Ballard JW, Yi YJ, Sutovsky P. Regulation of mitochondrial genome inheritance by autophagy and ubiquitin-proteasome system: implications for health, fitness, and fertility. *Biomed Res Int* 2014; **2014**:981867.

St John J. The control of mtDNA replication during differentiation and development. *Biochim Biophys* Acta 2014;**1840**:1345–1354.

Stewart JB, Chinnery PF. The dynamics of mitochondrial DNA heteroplasmy: implications for human health and disease. *Nat Rev Genet* 2015;**16**: 530–542.

Stoop D, Ermini B, Polyzos NP, Haentjens P, De Vos M, Verheyen G, Devroey P. Reproductive potential of a metaphase II oocyte retrieved after ovarian stimulation: an analysis of 23 354 ICSI cycles. *Hum Reprod* 2012;**27**:2030–2035.

Sun X, Su Y, He Y, Zhang J, Liu W, Zhang H, Hou Z, Liu J, Li J. New strategy for in vitro activation of primordial follicles with mTOR and PI3K stimulators. *Cell Cycle* 2015;**14**:721–731.

Suzuki N, Yoshioka N, Takae S, Sugishita Y, Tamura M, Hashimoto S, Morimoto Y, Kawamura K. Successful fertility preservation following ovarian tissue vitrification in patients with primary ovarian insufficiency. *Hum Reprod* 2015;**30**:608–615.

Takeda K, Tasai M, Akagi S, Matsukawa K, Takahashi S, Iwamoto M, Srirattana K, Onishi A, Tagami T, Nirasawa K. Microinjection of serum-starved mitochondria derived from somatic cells affects parthenogenetic development of bovine and murine oocytes. *Mitochondrion* 2010;**10**: 137–142.

Van Blerkom J. Mitochondrial function in the human oocyte and embryo and their role in developmental competence. *Mitochondrion* 2011;**11**: 797–813.

Van Blerkom J, Davis PW, Lee J. ATP content of human oocytes and developmental potential and outcome after in-vitro fertilization and embryo transfer. *Hum Reprod* 1995;**10**:415–424.

von Wolff M, Donnez J, Hovatta O, Keros V, Maltaris T, Montag M, Salle B, Sonmezer M, Andersen CY. Cryopreservation and autotransplantation of human ovarian tissue prior to cytotoxic therapy—a technique in its infancy but already successful in fertility preservation. *Eur J Cancer* 2009; **45**:1547–1553.

Wai T, Teoli D, Shoubridge EA. The mitochondrial DNA genetic bottleneck results from replication of a subpopulation of genomes. *Nat Genet* 2008;**40**:1484–1488.

White YA, Woods DC, Takai Y, Ishihara O, Seki H, Tilly JL. Oocyte formation by mitotically active germ cells purified from ovaries of reproductive-age women. *Nat Med* 2012;**18**:413–421.

Wilken-Jensen HN, Kristensen SG, Jeppesen JV, Yding Andersen C. Developmental competence of oocytes isolated from surplus medulla tissue in connection with cryopreservation of ovarian tissue for fertility preservation. *Acta Obstet Gynecol Scand* 2014;**93**:32–37.

Wolf DP, Mitalipov N, Mitalipov S. Mitochondrial replacement therapy in reproductive medicine. *Trends Mol Med* 2015;**21**:68–76.

Woods DC, Tilly JL. Autologous Germline Mitochondrial Energy Transfer (AUGMENT) in human assisted reproduction. *Semin Reprod Med* 2015; **33**:410–421.

Yin H, Jiang H, Kristensen SG, Andersen CY. Vitrification of in vitro matured oocytes collected from surplus ovarian medulla tissue resulting from fertility preservation of ovarian cortex tissue. *J Assist Reprod Genet* 2016;**33**:741–746.

Ylikallio E, Tyynismaa H, Tsutsui H, Ide T, Suomalainen A. High mitochondrial DNA copy number has detrimental effects in mice. *Hum Mol Genet* 2010;**19**:2695–2705.

Zhai J, Yao G, Dong F, Bu Z, Cheng Y, Sato Y, Hu L, Zhang Y, Wang J, Dai S *et al*. In vitro activation of follicles and fresh tissue auto-transplantation in primary ovarian insufficiency patients. *J Clin Endocrinol Metab* 2016;**29**: jc20161589.