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Demographic and evolutionary trends in ovarian function and aging

Triin Laisk[1,2], Olga Tšuiko[1,3], Tatjana Jatsenko[1], Peeter Hõrak[4], Marjut Otala[5], Mirkka Lahdenperä[6], Virpi Lummaa[6], Timo Tuuri[5], Andres Salumets[1,2,3,5,†], and Juha S. Tapanainen [5,7,†*]

[1]Competence Centre on Health Technologies, Tiigi 61b, 50410 Tartu, Estonia [2]Institute of Clinical Medicine, Department of Obstetrics and Gynaecology, University of Tartu, L. Puusepa 8, 50406 Tartu, Estonia [3]Institute of Biomedicine and Translational Medicine, Department of Biomedicine, University of Tartu, Ravila 19, 50411 Tartu, Estonia [4]Department of Zoology, University of Tartu, Vanemuise 46, 51003 Tartu, Estonia [5]Department of Obstetrics and Gynecology, University of Helsinki and Helsinki University Hospital, Haartmaninkatu 2, 00014 Helsinki, Finland [6]Department of Biology, University of Turku, Turun yliopisto, 20014 Turku, Finland [7]Department of Obstetrics and Gynecology, University Hospital of Oulu, University of Oulu, Medical Research Center Oulu and PEDEGO Research Unit, P.O BOX 23, FI-90029 OYS Oulu, Finland

*Correspondence address. E-mail: juha.tapanainen@helsinki.fi orcid.org/0000-0002-3139-9128

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BACKGROUND: The human female reproductive lifespan is regulated by the dynamics of ovarian function, which in turn is influenced by several factors: from the basic molecular biological mechanisms governing folliculogenesis, to environmental and lifestyle factors affecting the ovarian reserve between conception and menopause. From a broader point of view, global and regional demographic trends play an additional important role in shaping the female reproductive lifespan, and finally, influences on an evolutionary scale have led to the reproductive senescence that precedes somatic senescence in humans.

OBJECTIVE AND RATIONALE: The narrative review covers reproductive medicine, by integrating the molecular mechanisms of ovarian function and aging with short-term demographic and long-term evolutionary trends.

[†]Co-last authors.

SEARCH METHODS: PubMed and Google Scholar searches were performed with relevant keywords (menopause, folliculogenesis, reproductive aging, reproductive lifespan and life history theory). The reviewed articles and their references were restricted to those written in English.

OUTCOMES: We discuss and summarize the rapidly accumulating information from large-scale population-based and single-reproductive-cell genomic studies, their constraints and advantages in the context of female reproductive aging as well as their possible evolutionary significance on the life history trajectory from foetal-stage folliculogenesis until cessation of ovarian function in menopause. The relevant environmental and lifestyle factors and demographic trends are also discussed in the framework of predominant evolutionary hypotheses explaining the origin and maintenance of menopause.

WIDER IMPLICATIONS: The high speed at which new data are generated has so far raised more questions than it has provided solid answers and has been paralleled by a lack of satisfactory interpretations of the findings in the context of human life history theory. Therefore, the recent flood of data could offer an unprecedented tool for future research to possibly confirm or rewrite human evolutionary reproductive history, at the same time providing novel grounds for patient counselling and family planning strategies.

Key words: menopause / folliculogenesis / reproductive aging / reproductive lifespan / life history theory / ovarian aging / antagonistic pleiotropy

Introduction

Evolutionary logic predicts that any selection acting on post-reproductive lifespan would be weak (Williams, 1957). Consistent with this prediction, reproductive and somatic senescence occur in synchrony in all vertebrates, with the exception of humans and two species of toothed whales (Croft *et al.*, 2015). Why females of these species cease ovulation long before the end of their natural lifespan poses a major evolutionary puzzle. Age at menopause is moderately to highly heritable (Kirk *et al.*, 2001; de Bruin *et al.*, 2001a) and subject to natural selection in both pre-industrial (Lahdenperä *et al.*, 2004) and modern (Kirk *et al.*, 2001) societies, potentially permitting rapid evolutionary change. How menopause evolved and what maintains its current genetic variation are highly relevant questions for those aiming to understand the causes and consequences of reproductive senescence and associated pathologies.

The aim of this narrative review is to provide an overview of the basic molecular processes regulating folliculogenesis and summarize the changes in ovarian function accompanying female reproductive aging and their adverse impact on oocyte and embryo quality. Recent knowledge on the effect of events happening throughout the female life course on reproductive aging will also be reviewed, including the prenatal and early postnatal effects as well as lifestyle and environmental factors. Finally, the topic will be discussed in the context of short-term demographic and long-term evolutionary trends to better understand female fertility.

The first section aims to describe the process of folliculogenesis from birth to menopause, at the same time giving an overview of our current knowledge on the genetic and molecular regulators of follicular recruitment and development by summarizing studies on mouse models as well as large genetic association studies in humans. We then proceed to describe the aging of oocytes and its potential evolutionary significance. The following section summarizes the recent studies on the effects of various pre-birth and early-life events and lifestyle factors on reproductive aging. These chapters provide a background for the final chapter, in which we discuss the evolutionary origin and significance of menopause.

In the current review, we proceed from the life history theory that was developed to predict the coordinated evolution of the key traits (growth, survival and reproduction) contributing to fitness, i.e. the ability to leave viable offspring, including rate of maturation, number and quality of offspring, number of reproductive events, (reproductive) aging and lifespan. The theory views the evolution of these traits as the product of interactions between intrinsic constraints and trade-offs as well as extrinsic factors in the environment that affect mortality risk and resource availability (Stearns, 1989; Wells *et al.*, 2017). The trade-offs represent the costs paid in fitness when a beneficial change in one trait is linked to a detrimental change in another, at the genotype (e.g. antagonistic gene pleiotropy involved between fitness-related traits) and/or the phenotype level (e.g. the negative effect of high reproductive effort on lifespan). However, the life history theory has so far played a very small part in explaining ovarian function and aging, while the molecular and genetic mechanisms behind reproductive trade-offs have remained largely unknown.

In summary, this narrative review covers evolutionary reproductive biology and medicine, aiming at integrating the molecular mechanisms of ovarian function and aging with short-term demographic and long-term evolutionary trends.

Overview of ovarian biology: from foetal oogenesis to menopause

Stages of folliculogenesis and meiosis

Mammalian folliculogenesis is initiated during foetal life. In humans, primordial germ cells (PGCs) migrate from extraembryonic allantois into newly formed bipotential gonads at gestational week (GW) 8 and remain there as proliferative stem cells, multiplying their numbers for the following few weeks. At GWs 11–12, PGCs differentiate into oogonial stem cells that further proliferate and, starting at GWs 15–16, enter meiosis and recruit small numbers of flat pregranulosa cells, forming clusters of primordial follicles (Fig. 1). The number of primordial follicles is at highest at GW 20, peaking at up to 6–7 million. During the following weeks, most of the follicles vanish, leaving

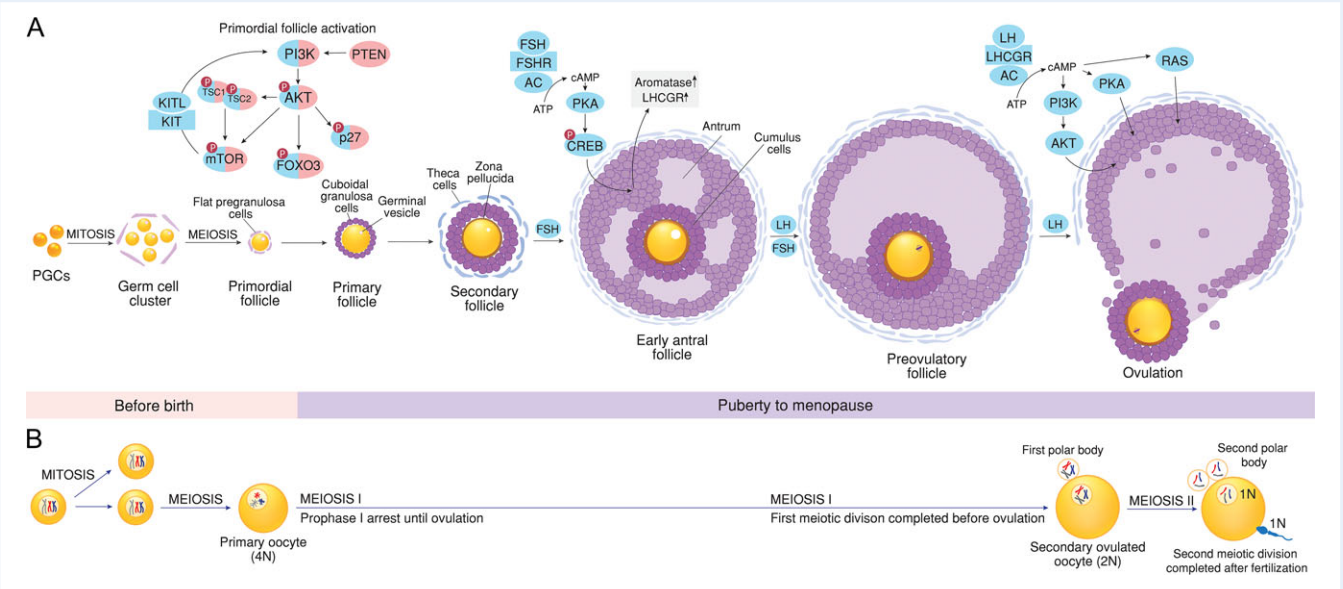

Figure 1 Mammalian folliculogenesis (**A**) and meiosis (**B**) summarizing the information from several species. (A) The dormancy of primordial follicles is maintained by the FOXO3, PTEN, p27, mTOR and TSC1/TSC2 proteins (indicated in pink). When phosphorylated (red P), these factors promote primordial follicle activation (indicated in blue). Activation of phosphoinositol-3-kinase (PI3K)-AKT kinase-signalling pathway initiates primordial follicle maturation. Phosphorylated AKT kinase mediates direct phosphorylation of transcription factor FOXO3, relocating it to cytoplasm and allowing primordial follicle growth. AKT also phosphorylates and inactivates inhibitors of the rapamycin complex (mTOR) pathway–tuberous sclerosis 1/tuberin (TSC1/TSC2) complex. Activated mTOR signalling promotes the expression in granulosa cells of KIT-ligand (KITL) that binds to its receptor KIT on the surface of resting oocytes, triggering follicle growth and activation of the PI3K-signalling cascade in oocytes. Activation of AKT kinase also leads to the phosphorylation of p27, diminishing its inhibitory effect on primordial follicle activation. In growing follicles FSH is needed for the transition into the antral follicle stage by its interaction with its G protein-coupled receptor (FSHR), expressed exclusively on granulosa cells. FSH binding to FSHR activates the adenylyl cyclase (AC)/cAMP/protein kinase A (PKA) cascade pathway and upregulates aromatase and LHCGR expression. Upon the LH surge, only one dominant follicle ovulates, following LH binding to LHCGR, leading to the activation of AC and accumulation of cAMP, and resulting in the activation of PKA pathway as well as PI3K-AKT and RAS downstream signalling cascades. As a result, LH triggers resumption of meiosis and ovulation of a mature secondary oocyte. (B) Meiosis. Oocytes initiate the first meiotic division before birth, while primary oocytes embedded within the primordial follicles remain arrested at prophase I of the first meiotic division. Gonadotrophins FSH and LH secreted after puberty trigger exit from the prophase arrest, and primary oocytes complete the first meiotic division at ovulation. The ovulated secondary oocyte arrests again at second meiotic metaphase, while fertilization triggers the completion of the second meiotic division. The resulting zygote contains two haploid genomes of maternal and paternal origin.

approximately 2 million left at the time of birth (Gougeon, 1996; Sun *et al.*, 2017).

Once activated to primary follicles, pregranulosa cells become cuboidal and the size of the follicle increases up to 50 µm (Gougeon and Chainy, 1987). Next, the number of granulosa cells increases and once at least two layers of cells are visible the follicles are known as secondary follicles. At this point, the size of the oocyte has also increased to ~50 µm, and the first flattened thecal cells can be observed around the expanding follicle. Within the next few weeks, small fluid-filled cavities appear between granulosa cells, forming the early antral follicle structure. After single antrum formation, the size of the follicle expands up to 1 mm. While granulosa cells continue to proliferate, most of the growth in follicular size now comes from enlargement of the antrum. Follicles sized about 2 mm at the time of selection during the menstrual cycle are recruited for final growth and ovulation (Gougeon, 1996), as summarized in Fig. 1.

In humans, some of the primordial follicles are activated to grow soon after their formation *in utero* and the first primary to small antral follicles are seen as early as GW 26. Since development from small antral follicles onward is strictly dependent on the correct hormonal milieu present only after menarche, all follicles growing before puberty become depleted through apoptotic cell death known as atresia. The onset of the growth of primordial follicles is gonadotropin-independent and sometimes called initial recruitment, distinguishing it from gonadotropin-dependent cyclic recruitment of antral follicles occurring after menarche and leading to selection of ovulating follicles (McGee and Hsueh, 2000).

Meiosis is the process of reducing the chromosomal number by half, and later the species-specific full set of diploid chromosomal complement is restored upon fertilization. In contrast to males, female meiosis is a temporally prolonged process, which is stopped twice at 'meiotic arrest', as indicated in Fig. 1. In humans, the first arrest occurs at mid-gestation at prophase I of the first meiotic division and may last for decades until the oocyte is ovulated. Although the evolutionary significance of the halted oocyte maturation at foetal development is not completely understood, it may help to minimize the mitotic divisions required to maintain the pool of the oocytes before their final post-pubertal maturation. Germ line mitotic cellular

divisions are known to raise mutations, which is a well-recognized problem in aged men after a number of spermatogonial divisions resulting in increased frequency of germ line gene mutations (Goriely and Wilkie, 2010; Lenormand et al., 2016). In contrast to the first arrest, the second arrest in oocyte maturation lasts for only a few hours, from release of the mature metaphase II oocyte at ovulation until sperm entry at fertilization; this arrest most likely evolved to prevent premature parthenogenetic activation and oocyte cleavage without the sperm-contributed paternal chromosomes (Lenormand et al., 2016).

Key molecular mechanisms of folliculogenesis

Oocytes are enclosed in dormant primordial follicles that are continuously recruited to develop towards fully fertilizable oocytes by removing inhibitory or adding activating signals (Fig. 1). The primordial follicle pool represents the fertility stockpile, i.e. the ovarian reserve that has to be sparingly maintained throughout reproductive life from puberty to the mid-40s. A move to commence development is taken in a minor fraction of silent primordial follicles at any time point by intra-oocyte signalling and close communication with follicular somatic cells. Although many intra- and intercellular molecular players have been found to tune folliculogenesis, the current review is focused predominantly on the mechanisms of early primordial follicle activation as the main gatekeeper determining the age-specific ovarian reserve and providing the fuel for folliculogenesis. Gonadotrophin-dependent follicular growth is only minimally mentioned as it remains largely outside the scope of the current review.

In mouse models, Foxo3 was one of the first well-proven key transcription factors preventing the recruitment of primordial follicles into the pool of growing follicles (Castrillon et al., 2003). Activation of the phosphoinositol-3-kinase (PI3K)-AKT kinase-signalling pathway in oocytes leads to Foxo3 phosphorylation and its transport from nucleus to cytoplasm, which is sufficient to initiate growth of the follicle (John et al., 2008). Indeed, constitutively active Foxo3 overexpressed in oocytes preserves the primordial follicle pool in aging mice (Pelosi et al., 2013). The role of FOXO3 in human remains controversial as in some studies it has not been found to be expressed in human or primate oocytes from primordial follicles (Tarnawa et al., 2013; Ting and Zelinski, 2017), while in some studies it has been localized in human oocytes (Li et al., 2010; McLaughlin et al., 2014). Functional studies regarding FOXO3 activity in human ovaries have not been published to our knowledge. However, the PI3K-AKT pathway itself is also under the regulation of several genes and proteins that limit the progressive activation of primordial follicles both in mouse and human. For instance, PTEN (phosphatase and tensin homologue deleted on chromosome 10), a phosphatase enzyme preventing the activation of AKT is a crucial factor in resting oocytes. In mice specifically lacking PTEN in oocytes, the entire pool of primordial follicles becomes simultaneously activated and soon depleted, causing premature ovarian failure (Reddy et al., 2008). Similarly, in human *in vitro* studies, inhibition of PTEN has led to activation of primordial follicles (Li et al., 2010; McLaughlin et al., 2014). In addition to PTEN, very recently, the receptor tyrosine kinase kit was also shown to be a key activator of the PI3K-signalling cascade in mouse oocytes (Saatcioglu et al., 2016). Conditional deletion of kit in oocytes at the time of birth leads to complete infertility, as primordial follicles resist activation. In contrast, if constitutively active kit is expressed, the whole follicular pool is activated and depleted immediately after birth. $p27^{Kip1}$ (p27), a member of the universal cyclin-dependent kinase inhibitor (CDKI) family, has also been shown to play an important role in preventing the premature activation of primordial follicles. In $p27^{Kip1}$-deficient mice, excessive activation of primordial follicles leads to exhaustion of the follicle pool and premature ovarian failure (Rajareddy et al., 2007).

Rapamycin complex 1 (mTORC1) signalling is another important regulatory pathway of primordial follicle activation. mTORC1 is a conserved serine/threonine kinase that controls cell growth, proliferation and survival in response to growth factors and nutrients. Primordial follicle development is dependent on at least two mTORC1 downstream targets; Kit-ligand (Kitl) expression in primordial follicle granulosa cells (Zhang et al., 2014) and its receptor Kit, activating the PI3K-AKT cascade in oocytes (Manova et al., 1993; Driancourt et al., 2000). Kitl is secreted from granulosa cells and binds to Kit receptor on the oocyte surface (Zhang and Liu, 2015), resulting in the activation of PI3K-AKT signalling in the oocyte (Reddy et al., 2008). This indicates that somatic cells initiate follicle activation in adulthood, as the enhanced mTORC1 signalling in granulosa cells promotes the expression of Kitl that, in turn, binds to the Kit receptor on resting oocytes to trigger their growth and follicle activation.

An intriguing antagonistic pleiotropy theory has been formed around the mTOR pathway, linking the launch of female reproduction at puberty to its cessation at menopause (Blagosklonny, 2010). Antagonistic pleiotropy is the condition when a single gene or pathway defines more than one trait, where one of the traits is beneficial and the other has detrimental consequences. In female fertility, antagonistic pleiotropy is manifested if the same gene or biological pathway is essential for the onset of puberty and/or increased reproduction in early life, and loss of fertility later in life through augmented follicular depletion at menopause. Indeed, there is strong evidence indicating that mTOR is one of the regulators of puberty onset via modulation of the hypothalamic Kiss1 system, with the blockage of mTOR causing inhibition of the gonadotrophic axis at puberty and ovarian atrophy in rodents (Roa et al., 2009). Conversely, an opposite effect of mTOR has been found in adulthood, when its activity in follicles is negatively regulated by a heterodimeric protein complex consisting of tuberous sclerosis 1 (TSC1) and TSC2 (tuberin) (Laplante and Sabatini, 2012), maintaining the dormant state of primordial follicles. In knockout mice, lack of either of the TSC proteins causes overactivation of the mTOR pathway, accompanied by awakening of the primordial follicles, ending with accelerated follicular exhaustion in early adulthood and premature ovarian failure (Adhikari et al., 2009, 2010). As mammals show diverse female reproductive aging patterns, if happening at all (like in rodents), the mixing of the information obtained from different animal species poses a risk in building a generalizing theory that best describes the origin of menopause in humans. Still, collectively, this information indicates that menopause may not be biologically programmed and merely reflects the antagonistic pleiotropic relationship between uninterrupted mTOR overactivation indispensable for fertility at early ages, while becoming noxious for the remaining follicles during the pre-menopausal years in humans.

There are only a few other examples of possible antagonistic genetic pleiotropy concerning female fertility and menopausal timing. In a

recent large-scale genomic analysis of ~70 000 women, reproductive aging was linked to breast cancer susceptibility and *BRCA1*-mediated DNA repair (Day *et al.*, 2015b). *BRCA* mutations have been the most well-known and -characterized familial genetic mutations leading to an elevated cancer risk, with an estimated 40–85% lifetime risk of developing breast cancer and a 16–64% risk of ovarian cancer (Smith *et al.*, 2013). However, as *BRCA* mutations are relatively frequent, they have been thought to have conferred some benefits during our evolutionary history that may have enhanced the fitness of the mutation carriers and avoided exclusion of the mutations via natural selection. Indeed, female *BRCA* mutation carriers have demonstrated improved fitness owing to enhanced fertility, particularly in natural fertility conditions, but excess post-reproductive mortality due to an elevated cancer risk most evident in contemporary long-lived populations. For example, women carrying these mutations had on average two more children if they were born before 1930 in Utah and Idaho, USA (Smith *et al.*, 2012). The idea that the fertility benefits of the *BRCA* mutations appear to be confined to certain environments with overall high fertility but are not apparent in modern low-fertility environments is intriguing. Our recent review proposed that the mismatch between past adaptations and current environments where fertility levels are low overall and more individuals now live to the age where they experience the harmful effects of the genes means that gene variants linked to higher fitness in the past via improving fertility, through antagonistic pleiotropic effects, now predispose contemporary populations to non-communicable diseases such as cancer (Corbett *et al.*, 2018).

Albeit not entirely understood, the proposed mechanisms linking *BRCA* mutations to increased fertility have been centred on the possibility that these mutations may help to withstand the progressive age-related shortening of telomeres (Smith *et al.*, 2013), which might be particularly critical for oocytes that must survive the prolonged period between foetal life and mid-life ovulation. Disruption of *BRCA1* has been found to lead to telomere lengthening, while its overexpression reduces telomere length (French *et al.*, 2006; Ballal *et al.*, 2009), outcomes that point towards a favourable effect on fertility, as a positive association has been described between reproductive lifespan and telomere length (Aydos *et al.*, 2005). However, the entire concept still remains highly controversial, as the majority of studies have demonstrated an adverse or no fertility effect of being a *BRCA* mutation carrier, and the relationship between *BRCA* mutations, female fertility and telomere integrity have not yet been unequivocally established (as reviewed by Smith *et al.*, 2013).

Still, the majority of the molecular factors regulate folliculogenesis without any known pleiotropic antagonistic effects between onset and cessation of the female reproduction. For instance, the transforming growth factor beta (TGF-β) superfamily of growth and differentiation factors has been shown to play an important role in early folliculogenesis. Anti-Mullerian hormone (AMH) is a homodimeric glycoprotein that belongs to the TGF-β superfamily. AMH and its receptor AMHR2 are expressed in granulosa cells of primary and growing follicles in rodents and humans but not in the large antral and preovulatory follicles that have become responsive to follicle-stimulating hormone (FSH) (Weenen *et al.*, 2004). According to the current understanding, AMH controls follicular development at various steps and possibly also in a species-dependent manner. Most studies show it inhibits the recruitment of primordial follicles into the pool of growing follicles and also decreases the responsiveness of growing follicles to FSH (Durlinger *et al.*, 2002). At later stages, AMH has been shown to regulate at least follicular oestrogen synthesis (Dewailly *et al.*, 2014). Of the other TGF-β superfamily members, GDF9 and BMP15 are important paracrine growth factors regulating follicular growth beyond primary stage. Both of these factors are produced in oocytes and regulate various biological processes in granulosa cells (Persani *et al.*, 2014). Activins and inhibins, also members of the TGF-β superfamily, are other important granulosa-derived factors controlling ovarian functions and follicular growth mostly by regulating the biosynthesis and secretion of FSH from pituitary cells. In the ovarian context, they regulate many different cellular processes from granulosa cell proliferation to oocyte maturation (Wijayarathna and de Kretser, 2016).

Before acquisition of a mature hypothalamic–pituitary–gonadal axis, follicles from the primordial follicle pool are recruited to grow, but they cannot form large antral follicles. For this to occur, the gonadotrophins FSH and luteinizing hormone (LH) are needed, which are mostly responsible for follicular growth and ovulation triggering, respectively. FSH binds to its G protein-coupled receptor (FSHR), initiating the classical adenylyl cyclase (AC)/cAMP/protein kinase A (PKA) pathway. This activates the transcription factor cAMP response element binding protein (CREB), which further upregulates a number of target genes, such as those for aromatase and the LH receptor (Ulloa-Aguirre *et al.*, 2007). The dominant follicle thereby gradually becomes less dependent on FSH and more responsive to LH (Baerwald *et al.*, 2012). Following the LH-surge, LH receptors in the granulosa cells activate the downstream AC/cAMP/PKA pathway, as well as the PI3K-AKT and RAS-signalling cascades that are critical for ovulation (Richards and Pangas, 2010).

In contrast to natural conception, described above, where the cycle is a fine-tuned physiological event and orchestrated by a plethora of auto-, para- and endocrine mediators to produce a single ovulated oocyte, in *in vitro* fertilization ovaries are stimulated with exogenous FSH above the physiological threshold to ensure development of multiple follicles. This ovarian stimulation overrules the natural selection of a single ovulated oocyte, as it is crucial that many oocytes are obtained at ovarian puncture for further *in vitro* manipulations. Altering the selective pressure placed on oocytes by means of IVF and its pharmacological and technological interventions has raised the provocative question of what are the implications of assisted reproduction from the perspective of evolution? Although IVF is believed to remove several biological barriers in follicular and oocyte selection, its long-term consequences will only be judged adequately after IVF has been practised for a longer period of time to allow evaluation of the health and fertility profiles of future generations of IVF offspring. However, it has been pointed out that IVF is unlikely to change the most tightly regulated filter in follicular development that ensures that only ~0.005% of oocytes pass through the stringent selection process to avoid atresia and become fertilizable oocytes (Hanevik *et al.*, 2016).

Genetics of folliculogenesis

Folliculogenesis and the accompanying concept of the ovarian reserve form the basis of female fertility and are the main limiting steps of female reproductive potential. Therefore, it is only natural that the

genetic control of folliculogenesis has attracted considerable interest, as an improved understanding of the natural variation would also help to better understand several folliculogenesis-associated pathologies, such as polycystic ovary syndrome (PCOS). PCOS is relatively widespread despite the harmful effect on fertility, due to ovarian androgen excess and chronic anovulation, raising the question about its evolutionary origin and the mechanisms through which it remains prevalent in the population. Among the suggested selective advantages of PCOS is delayed ovarian aging, a theory which received support from a recent PCOS genome-wide association study (GWAS), where it was demonstrated that increased susceptibility to PCOS is associated with later menopause (Day et al., 2015a). The same study also showed that alleles increasing susceptibility to PCOS were also associated with higher AMH levels in girls, leading the authors to propose that since higher AMH levels inhibit primordial follicle recruitment, the ovarian reserve is more efficiently used in PCOS patients, or alternatively, they have a larger primordial follicle pool to begin with (Day et al., 2015a).

Genetic factors modify folliculogenesis in two distinct ways. First, there are the genetic factors involved in establishment of the primordial follicle pool, and then those influencing the recruitment of follicles from this pool. While the first factors exert their effects pre-birth, the latter take a dominant role once active folliculogenesis starts at puberty. The genetic fertility potential is further modified by its interactions with environmental and lifestyle factors, such as smoking. Current knowledge on the genetics of folliculogenesis and ovarian reserve has been comprehensively reviewed previously (Wood and Rajkovic, 2013), and partly in previous sections, and will not be discussed here in detail. One of the defining characteristics of the current data is the fact that it is largely derived from animal models (with interspecies differences in folliculogenesis) or from human monogenic conditions that also affect folliculogenesis (e.g. primary ovarian insufficiency, POI). GWA studies on the age at menopause have also provided additional clues about the mechanisms behind (ovarian) aging, and interestingly, the genetic signals for age at menopause are not enriched in genes related to ovarian function (e.g. those coding for the aforementioned FOXO3, PTEN, KIT, p27, AMH/AMHR, GDF9, BMP15 and inhibin proteins), but instead in DNA damage response and monogenic POI-associated genes (Day et al., 2015b).

Large GWA studies also provide researchers with excellent data to estimate genetic correlations (using for example the linkage disequilibrium score method (Bulik-Sullivan et al., 2015)) between different traits and conditions. In this spirit, it was demonstrated that menopausal age is genetically negatively correlated to adult obesity and body mass index (BMI), while positive genetic correlations were observed for age at menarche (Day et al., 2015b) and age at first birth (Zheng et al., 2016). According to a recent study, there is also evidence that menopause and epigenetic aging are genetically correlated, and furthermore, menopause accelerates epigenetic aging of blood (Levine et al., 2016), suggesting that menopausal hormonal changes accelerate aging in women (Levine et al., 2016).

At the same time, GWA studies for traits that are directly used to measure ovarian function (FSH, AMH, follicle count and COS outcome in IVF) have been less successful, probably due to smaller sample sizes (van Disseldorp et al., 2011; Schuh-Huerta et al., 2012a, 2012b). Interestingly, variants near the *AMH* gene are associated with AMH levels in males, but not in females (Perry et al., 2016), where *AMH* polymorphisms explained very little of the variance in AMH levels in females. In a combined sample of both men and women, plasma FSH levels were associated with a genetic variant (rs11031005) near the *FSHB* gene (Ruth et al. 2016b). The same locus was also associated with LH levels in the same study and is in linkage disequilibrium with a variant associated with menopause timing (Stolk et al., 2012). The rs11031005 variant is in perfect linkage disequilibrium with rs110310056, which has also been associated with FSH levels, and also with earlier age at menarche, first birth and menopause, higher lifetime parity and dizygotic twinning (Mbarek et al., 2016), traits characteristic of individuals with fast life history trajectories (Wells et al., 2017). Genetic variation in the *FSHB* promoter leads to longer menstrual cycles and later menopause and at the same time is protective against endometriosis (a common gynaecological disease), as shown in recent studies utilizing the large-scale UK Biobank resource (Ruth, et al., 2016a; Laisk et al., 2018).

Depletion of the follicular pool

During the reproductive lifespan, roughly 450 oocytes are ovulated, while the rest become depleted by atresia via apoptosis at different stages of development (Pelosi et al., 2015). However, due to the irrevocable demographic trend towards increased contraceptive prevalence worldwide, a cut to <300 in numbers of fertilizable oocytes released from the ovaries over the reproductive years can be assumed, considering that ca 40% of European women aged 15–49 years who are married or in a union are using some form of ovulation-supressing contraception according to United Nations (www.un.org). Only a small fraction of the early primordial follicles is activated at a fairly constant rate at any age before menopause. In a recent study, it was estimated that, at the time of menarche, ~800–900 primordial follicles are recruited in a month, a number which then decreases steadily to <100 at the time of menopause (Wallace and Kelsey, 2010). At the time of menopause, the ovaries contain <1000 primordial follicles (Broekmans et al., 2009). There has also been some debate on whether or not the use of oral contraceptives has any effect on menopausal timing via interfering with the recruitment process of primordial follicles and/or depletion of the follicular reserve. Indeed, some studies have shown that the use of oral contraceptives is associated with later menopause (Gold et al., 2001; Palmer et al., 2003), while others have observed results inconsistent with this hypothesis (de Vries et al., 2001).

Until recently the common dogma was that new oocytes are not formed in mammalian ovaries after the foetal period and female fertility relies only on the fixed stockpile of oocytes available at birth until complete attrition of the ovarian reserve at menopause. However, this view has lately been challenged by several studies suggesting the existence of ovarian stem cells found even in adult ovaries and capable of initiating follicular development in various mammalian species including humans, as recently thoroughly reviewed (Truman et al., 2017). More detailed analyses of the nature of murine ovarian stem cells known as female germ line stem cells (FGSCs) or oogonial stem cells suggest that they are distinct from embryonic PGCs but share common features with spermatogonial stem cells, for example in cell culture behaviour and transcriptional profiles (Xie et al., 2014). FGSCs have been transplanted into adult ovaries, where they have initiated follicular development, yielding mature oocytes capable of being fertilized, producing healthy offspring in mice (Zou et al., 2009;

Zhang *et al.*, 2011; Xiong *et al.*, 2015) and rats (Zhou *et al.*, 2014), and a very recent genetic study strongly suggests that *de novo* folliculogenesis contributes to the murine follicular pool under normal physiological conditions (Wang *et al.*, 2017a). Nevertheless, the role of adult ovarian regeneration in humans, if any, is believed to be negligible as regards maintenance of fertility and endocrine function, since they decline as a result of age-related follicular depletion.

Reproductive aging and ovarian function

Evolutionary perspective on oocyte and embryonic aneuploidy

Age-associated events in oocytes will undoubtedly jeopardize embryo development. Numerous cellular defects occurring in the aging oocyte will have a negative impact on fertilization rates and reduce embryo development potential (Miao *et al.*, 2009). Moreover, the incidence of oocyte aneuploidy rises abruptly in the mid-30s and reaches 80% by the age of 45 (Franasiak *et al.*, 2014). When fertilized, aneuploid oocytes will unavoidably give rise to aneuploid embryos, which will either fail to implant, result in miscarriage or lead to birth of an infant with congenital disorders (Hassold and Hunt, 2001).

As a woman ages, her meiotic recombination machinery becomes less efficient, because it is constantly influenced by the environmental and age-related factors that accumulate in the ovary. In turn, this has been proposed to lead to altered genomic recombination patterns that have been identified as risk factors of trisomy 21 (Lamb *et al.*, 2005a,b). In general, meiotic recombination in oocytes is surprisingly error-prone, as oocytes are inefficient in establishing crossovers, while lower genome-wide recombination rates can increase the risk of developing vulnerable crossover configurations that can interfere with proper segregation of sister chromatids (Ottolini *et al.*, 2015; Wang *et al.*, 2017b). However, age-related oocyte aneuploidy is multifactorial, originating from diminished cell cycle regulation, aberrant spindle formation, loss of proper checkpoint signalling and compromised centromeric cohesion between sister chromatids (as recently reviewed by Webster and Schuh, 2017). In humans and mice, the age-related loss of cohesin leads to an inability to stabilize crossover sites or hold sister chromatids together, causing premature chromosome separation (Chiang *et al.*, 2011; Tsutsumi *et al.*, 2014). Defective spindle assembly can also be attributed to the age-related chaotic microtubule dynamics that result in multipolar spindles and predispose the oocyte to chromosome segregation errors (Nakagawa and FitzHarris, 2017). This, in combination with the oocyte-specific spindle assembly checkpoint-mediated control of meiosis, which is not able to block meiotic progression upon misalignment of a single chromosome, results in increased aneuploid oocyte formation in older women and propagation of aneuploidy in the developing embryo (Nagaoka *et al.*, 2011).

One of the reasons for the age-related decrease in cellular function in the oocyte might be the influence of ovarian aging on mitochondria biogenesis, morphology and function. The association between mitochondrial dysfunction and oocyte aging has been extensively reviewed elsewhere (May-Panloup *et al.*, 2016). Importantly, cell division is energy-demanding and alterations in mitochondrial dynamics and metabolic pathways can impair the process (Salazar-Roa and Malumbres, 2017). In support of this, a deficit in mitochondrial-produced ATP causes spindle instability and impaired chromosome segregation in MII mouse oocytes (Zhang *et al.*, 2006). Because mitochondrial DNA (mtDNA) content in oocytes decreases with age (May-Panloup *et al.*, 2005; Fragouli *et al.*, 2015; Babayev *et al.*, 2016), failure to synthesize sufficient amounts of ATP can potentially lead to aneuploidy in oocytes and future embryos. In addition, embryos seem to possess a compensatory mechanism to deal with inherited mitochondrial deficiency by initiating premature mitochondrial biogenesis, which itself can lead to reduced developmental potential (Fragouli *et al.*, 2015).

Apart from mitochondrial biology, aging also has a deleterious effect on oocyte transcriptional activity, necessary to guide cytoplasmic maturation, meiotic divisions and post-translational processes (Steuerwald *et al.*, 2007; Grøndahl *et al.*, 2010). Notably, age-related downregulation of protein ubiquitination in mammalian oocytes can lead to breakdown of the ubiquitin–proteasome pathway, resulting in failure to support meiosis, cell cycle progression and polar body I extrusion (Sun *et al.*, 2004; Ben-Eliezer *et al.*, 2015; Yu *et al.*, 2015). The altered function of genes and proteins involved in the meiotic cell cycle and metaphase spindle formation may partially explain the elevated prevalence of three-pro-nuclear (3PN) zygotes in older women undergoing IVF treatment, resulting from the compromised ability to extrude polar body II (Grøndahl *et al.*, 2017). In addition, both the zona pellucida and plasma membrane of aged oocytes undergo structural changes that might predispose the aged oocyte to fertilization abnormalities, resulting in either reduced fertilization rates or an increased incidence of polyspermy due to a compromised membrane block (Wortzman and Evans, 2005). In addition, dispermy can result in diandric (presence of two paternal genomes) triploid embryos that adversely affect embryonic/foetal and placental development (Philipp *et al.*, 2004) or lead to the creation of an androgenetic cell lineage with highly proliferative potential within a single embryo (Destouni *et al.*, 2016). As such, the age-associated reduced ability to prevent polyspermy can also be one of the reasons why women at advanced age are at risk of developing molar pregnancy of androgenetic origin (Sebire *et al.*, 2002; Savage *et al.*, 2013).

The recent rapid progress in high-throughput single-cell genomic technologies has revealed the astonishing fact that only one among 10 IVF embryos even in young couples is completely chromosomally normal (Vanneste *et al.*, 2009), which is partially a result of suboptimal oocyte quality. The high level of genomic aberrations is referred to as chromosomal instability (CIN), which is considered to be one of the major reasons why human IVF embryos fail to implant or why the pregnancy is miscarried at early stages. The frequency of aneuploidy/CIN is especially high in women of advanced age due to an increased rate of meiotic errors in the oocytes. In order to avoid transfer of a genetically abnormal embryo, cells are taken from IVF embryos to confirm genomic integrity in preimplantation genetic screening, currently termed as preimplantation genetic testing for aneuploidy (PGT-A). Although the potential benefit of PGT-A is yet to be established by extensive randomized controlled studies, performed in academic settings, transfer of euploid embryos seems to reduce the negative effect of advanced maternal age on implantation rates. Hence, maternal aging does not impair the implantation

potential of euploid embryos but rather greatly reduces the numbers of chromosomally normal embryos due to diminished ovarian function and oocyte quality. Paradoxically, PGT-A is supposed to reveal the genomic aberrations in embryos from the IVF procedure, which itself is known to induce CIN due to adverse *in vitro* conditions and manipulations, as shown in our recent study comparing *in vivo*- and *in vitro*-produced bovine embryos (Tšuiko *et al.*, 2017).

The increasingly popular PGT-A procedure has created a predominant view that human IVF embryos are often genetically abnormal and selection of a normal embryo is required to attain pregnancy. For that reason, we rate chromosomally abnormal embryos as the developmental deadlock that excludes livebirth and thus will be eliminated by natural selection. However, the view that genetically unbalanced embryos are not viable has been recently challenged, when healthy babies were born after the transfer of chromosomally mosaic embryos to the uterus, due to the possible self-correction processes (Greco *et al.*, 2015). Moreover, the evolutionary view on CIN in oocytes and early embryos may wholly differ from the clinical point of view, as chromosomal rearrangements in natural conception may also drive evolution by speciation (i.e. the formation of new and distinct species in the course of evolution), as chromosomal mutations may, albeit rarely, provide an unexpected evolutionary advantage to an organism (as reviewed by Carbone and Chavez, 2015). Although the extent of CIN in human oocytes and embryos in natural conception is not known and will probably remain unstudied for ethical reasons, the possibly elevated rate of CIN in human early reproduction, especially in the context of ovarian aging and its potential role in genome evolution itself, awaits to be discussed, at least at a theoretical level.

Life course events and reproductive aging

Large cohort studies and data accumulated in national biobanks have made it possible to study the genetic and non-genetic determinants of menopausal timing on an unprecedented scale. As a result, it is now clear that even pre-birth and early-life events can have an impact which manifests at later stages of the reproductive lifespan.

Pre-birth events and ovarian aging

As the oocyte pool is established in GW 8, pre-birth events (in this context, defined as occurring before/at the time of birth or affecting the gestational period), such as maternal smoking during pregnancy, could affect the earliest phases of folliculogenesis, and therefore also influence reproductive potential and the timing of menopause. Indeed, this hypothesis is supported by several studies (Strohsnitter *et al.*, 2008; Tawfik *et al.*, 2015). The largest study to date addressing this question used the self-reported data from more than 180 000 UK Biobank participants (Ruth, *et al.*, 2016c) and reported that maternal smoking during pregnancy had a significant effect on the risk of early menopause, when the used statistical model was adjusted for subject's socioeconomic status, BMI and smoking status but was not significant in the full model, which additionally included her alcohol intake frequency, smoking pack-years, number of live births, educational level and whether the participant ate meat (Ruth, *et al.*, 2016c). This indicates that although pre-birth events can influence age at menopause, the effect is further modified by lifestyle factors in later stages of life.

In addition to chemicals in cigarette smoke, others such as diethylstilbestrol, a synthetic oestrogen and known endocrine-disrupting chemical (EDC), have been associated with earlier menopause (Hatch *et al.*, 2006; Steiner *et al.*, 2010), with *in utero*-exposed individuals entering menopause approximately a year earlier (Steiner *et al.*, 2010). Similar substances can also affect menopausal age post-birth, as a recent cross-sectional study on 31 575 women analysed for 111 EDCs revealed that phthalates, bisphenol A, pesticides and tobacco are the most common substances having a negative impact on ovarian function and are associated with an earlier age of menopause (1.9–3.8 years earlier) after adjustment for age, race/ethnicity, smoking and BMI. Furthermore, a dose–response relationship was demonstrated for 14 of the 111 EDCs, suggesting that exposure to these long-life chemicals could increase the speed of follicular depletion (Vabre *et al.*, 2017).

In addition to the above, the same study on UK Biobank participants revealed a strong association between being part of a multiple birth and the risk of early menopause, with an impact comparable to that of being a current smoker (Ruth, *et al.*, 2016c). This is further supported by studies showing a higher POI risk in both mono and dizygotic twins (Gosden *et al.*, 2007). Although it could be argued that since multiple birth is usually accompanied by lower individual birthweight, which could be the true causal factor, the association between being part of a multiple birth and earlier menopause remained significant after adjusting for several early-life factors, including birthweight (Ruth, *et al.*, 2016c). Moreover, studies evaluating the effect of birthweight have yielded inconsistent results (Cresswell *et al.*, 1997; Treloar *et al.*, 2000; Steiner *et al.*, 2010; Tom *et al.*, 2010; Ruth, *et al.*, 2016c) (see the next sub-section). Although multiple pregnancies are more common in older mothers, which could indicate a reduced ovarian reserve in the offspring, studies have actually shown that older maternal age at birth is associated with later menopausal age (Steiner *et al.*, 2010). Therefore, Ruth *et al.* concluded that the shared intra-uterine/early-life environment plays a role, or alternatively, there is an overlap in the genetics of twinning and menopausal timing (Ruth *et al.*, 2016c). Interestingly, a recent GWAS on dizygotic twinning demonstrated that the G-allele of a variant near *FSHB* increases both the odds of twinning and the risk of early menopause (Mbarek *et al.*, 2016). The occurrence of such pleiotropic effects is consistent with the predictions of the life history theory that early reproductive senescence co-evolves with high reproductive rates and lower offspring quality, characteristic of twinning (Wells *et al.*, 2017).

In conclusion, it is clear that pre-birth events are closely intertwined and it is difficult to pick apart true causal associations. Further studies using, for example, Mendelian randomization with genetic instruments might be able to provide some additional insight.

Early-life events and oocyte biology

Early-life events, in this context those affecting childhood, can similarly influence the rate of follicular atresia and thus have an effect on the ovarian reserve and the age at menopause.

Birthweight, especially low birthweight has been associated with earlier menopause (Steiner *et al.*, 2010; Ruth *et al.*, 2016c), as has

been poor weight gain during early childhood (Cresswell *et al.*, 1997; Hardy and Kuh, 2002; Mishra *et al.*, 2007), and some studies have even suggested that women at the extremes of birthweight reach menopause earlier (Tom *et al.*, 2010). As discussed in an extensive review (Richardson and Guo, 2014), maternal feed and foetal growth restrictions, low birthweight and poor weight gain in newborns have been associated with lower ovarian reserve in female offspring in cattle, pigs, sheep and humans. At the same time, others have found no such associations between birthweight and menopause, as in a recent systematic review it was concluded that while poor weight gain in early childhood and exposure to famine (early-life nutritional stress) are associated with earlier menopause, birthweight as such is not (Sadrzadeh *et al.* 2018). This is also supported by the lack of a genetic correlation between age at menopause and birthweight (Zheng *et al.*, 2016). Therefore, while there is a trend towards declining birthweight in some developed countries (Oken, 2013; Takemoto *et al.*, 2016) and an increase in birthweights over 3500 g in others (Bell, 2008), it is unlikely that it has a remarkable effect on menopausal age, and rather, a shorter reproductive lifespan can be expected in populations where early-life nutritional stress is an issue (Sadrzadeh *et al.* 2018). This is in line with the results of a study by de Bruin and others showing that intra-uterine growth restriction *per se* does not disturb ovarian development (de Bruin, *et al.*, 2001b).

Lifestyle determinants of age at menopause

Out of all the lifestyle and environmental factors studied in relation to menopausal timing, smoking has shown the most consistent associations and relatively constant effect estimates: smokers enter menopause a year earlier, on average (Mikkelsen *et al.*, 2007; Henderson *et al.*, 2008a; Ruth, *et al.*, 2016c). As a potential mechanism, it has been proposed that polycyclic aromatic hydrocarbons, components of smoke, act via the aryl hydrocarbon receptor and activate the pro-apoptotic *BAX* gene, causing oocyte demise (Anderson *et al.*, 2014), and consequently leading to an earlier age at the birth of the last child and an earlier onset of menopause (Richardson and Guo, 2014). This is reflected by a premature increase in serum FSH levels (Caserta *et al.*, 2013) and a decrease in AMH levels (Plante *et al.*, 2010). On a positive note, there has been a trend towards decreased smoking among women in recent decades in many, although not all, developed countries (www.who.int).

Although on a genetic level there appears to be a negative correlation between age at menopause and BMI, on an epidemiological level the association is not as clear, with some studies showing a positive association between weight/BMI and age at menopause, and others showing no significant association (Ruth, *et al.*, 2016c). Differences in results could be partly caused by differences in study design, such as the time point when BMI is reported, is before menopause, at the time of menopause or perhaps even years after menopause, when data are collected retrospectively. Although globally there is an overall trend towards increasing BMI (the worldwide prevalence of obesity has tripled since 1975; WHO Obesity and Overweight factsheet; http://www.who.int/mediacentre/factsheets/fs311/en/), considering the lack of sound proof of causality, it is difficult to estimate how this will affect menopausal timing. However, even in studies showing an association between anthropometric parameters and age at menopause, the reported effect sizes have been extremely small and not comparable to those reported for smoking, for example.

Several investigators have observed a correlation between nulliparity and earlier age at menopause (Gold *et al.*, 2001; Henderson *et al.*, 2008b; Morris *et al.*, 2012; Ruth, *et al.*, 2016c; Mishra *et al.*, 2017), indicating possible underlying sub-fertility or infertility. In fact, a recent large study combining data from multiple populations revealed that nulliparous women are at twice the risk of early menopause, compared with women with two or more children (Mishra *et al.*, 2017). Interestingly, later menopause in turn is associated with an increased lifespan (Ossewaarde *et al.*, 2005) and the demonstrated epidemiological link between female fertility and longevity has resulted in the conclusion that extended fertility (increased maternal age at last birth) correlates positively with longevity. Further, it was found that men whose sisters gave birth at a late age lived longer, suggesting that the link between extended fertility and longevity has a genetic component that is independent of physiological changes as a result of having offspring (Smith *et al.*, 2009). Again, the occurrence of such a genetic link is predicted by the life history theory, proposing that life expectancy and ability to maintain reproductive capacity at older age evolve in a coordinated manner (Wells *et al.*, 2017). Eventually, such coevolution would result in genetic variants enabling longer life and those supporting slower reproductive aging ending up in the same individuals.

Ethnicity, demographics and menopause

Age at menopause shows variation from 44.6 to 54.5 years across different geographic regions (Fig. 2, Supplementary Table 1), with earlier menopause in women from African, Middle Eastern, some Latin American and some Asian countries, and later menopause in Europe, Australia, Canada and the USA (Schoenaker *et al.*, 2014). However, it is unclear whether this variation is caused by genetic, or socioeconomic and environmental/lifestyle factors. Large-scale GWA studies concerning menopausal age have been conducted mostly among populations of European ancestry, with other ancestry efforts partially replicating the original findings (Carty *et al.*, 2013; Shen *et al.*, 2013; Chen *et al.*, 2012, 2014), pointing to an overlap between the genetic determinants of menopausal age in different populations. At the same time, a recent large GWAS in ~67 000 Japanese women (Horikoshi *et al.*, 2017) showed considerable differences in effect estimates between Japanese and European populations, underlining the importance of well-powered studies in different populations to fully understand the genetic factors contributing to geographic differences in menopause timing.

Considerable variation in menopause timing has also been seen across different birth cohorts, with an ~1- to 2-month increase in menopausal age per later year of birth, resulting in a 1-year increase in menopausal age per every 10-year period (Rödström *et al.*, 2003; Ruth, *et al.*, 2016c). This can probably be attributed to an overall improvement of health and nutrition. Historically, selection (in terms of increased offspring number) for advanced age at last reproduction (ALR) has been demonstrated in a 17th–19th century Sami population (Helle *et al.*, 2005). Here, we suggested that providing there is no other kind of balancing selection favouring earlier end to the reproductive career, this selection pressure can lead to evolution of later age at menopause because age at last birth is likely to correlate

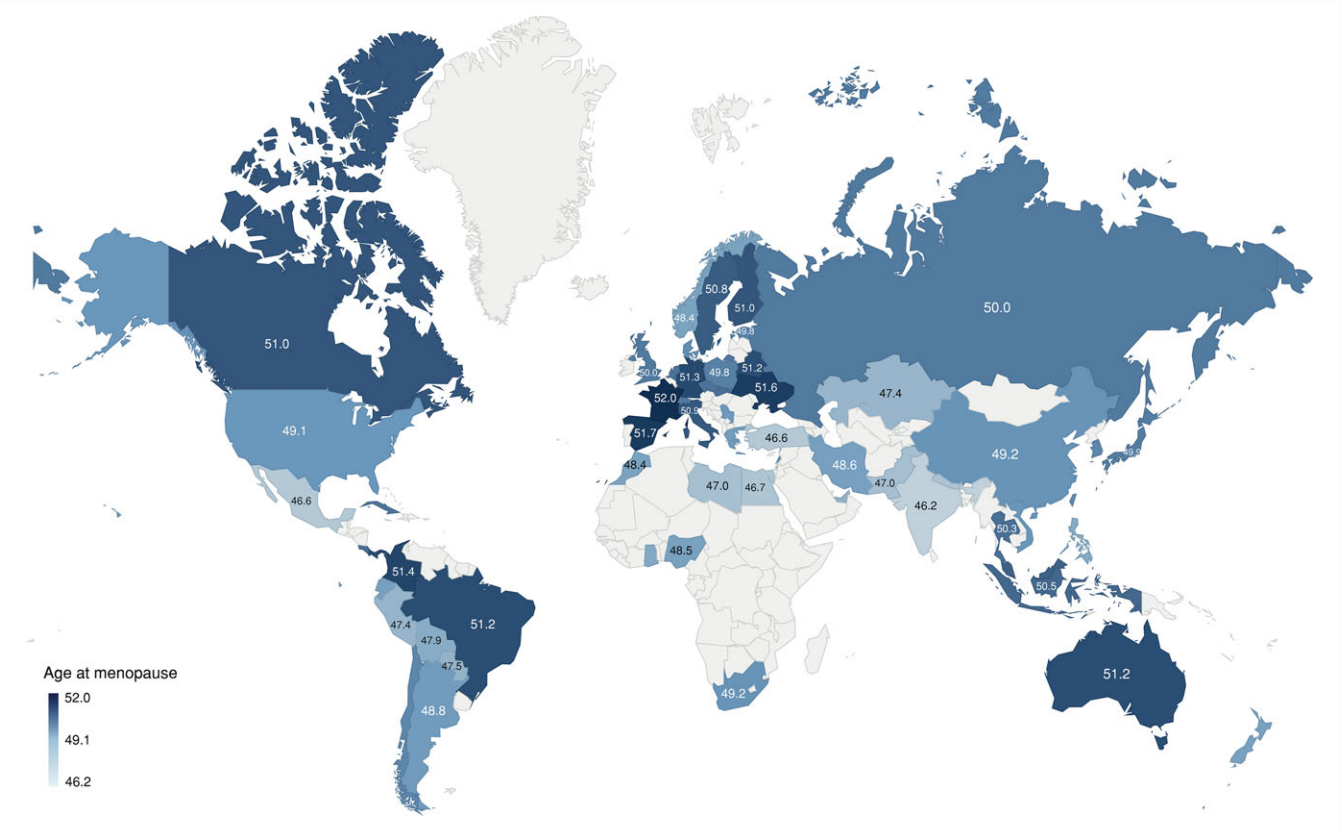

Figure 2 Variation in menopause timing according to geographical region. Details and data sources are given in Supplementary Table 1.

strongly with age at menopause in such natural fertility populations. Whether or not selection pressures on age at menopause have remained similar in contemporary societies practising family planning remain open. In this context, it is notable that after demographic transition (i.e. among Finns born between 1880 and 1971) from high birth and death rates to lower birth and death rates, the ALR was nearly 10 years earlier (34.7 *vs.* 40.5 years) than among those born between 1705 and 1879 (Bolund *et al.*, 2015). Despite such a secular trend to an earlier end to reproduction, driven by changes in desired family size, availability of contraception and lifestyle factors, selection is at the genetic level actually acting to delay age at menopause in contemporary human populations (Byars *et al.*, 2010). Such mismatches can occur because delayed ability to conceive is linked to higher overall offspring number in contemporary populations, even if most women now prefer to not use that potential towards increasing their family size. Concurrently, an increase in age at first birth is now also observed in many Western countries (Fig. 3; http://www.humanfertility.org/). Given the proposed ~10-year gap between the onset of sterility and beginning of menopause (Broekmans *et al.*, 2009), women at the earlier end of the reproductive aging continuum are also at risk of earlier reproductive decline, and hence, at some point the advance in reproductive age will begin to overlap with their inevitably declined fertility levels, leading to more cases of involuntary infertility.

The evolutionary origin and significance of menopause

Experiencing menopause well before the end of life is not a recent phenomenon for our species that only affects women in recently established affluent conditions. First, archaeological evidence suggests that the exceptional longevity in humans compared with related species likely evolved already during late Pleistocene (Caspari and Lee, 2004). Second, in historical and current hunter-gatherer populations with low life expectancy at birth and no access to modern medical care, a third or more of all women surviving past childhood continue to live beyond menopause (Gurven and Kaplan, 2007; Lahdenperä *et al.*, 2014). Indeed, the increase in life expectancy during the last century (Fig. 3) reflects a reduction in childhood mortality, not in post-menopausal survival which was already frequent in historical times (Hawkes, 2004). This raises two evolutionary justified questions: (i) why and how did menopause emerge in our evolutionary history and (ii) why do the lifespan and reproductive span not match in women, or why life does not end at menopause? Maintaining traits and functions that result in wasting time and energy for anything that does not serve the purpose of passing genes to the next generations does not make sense in evolutionary terms, because natural selection generally cannot directly favour remaining vigorous beyond the age when reproduction is completed (Williams, 1957).

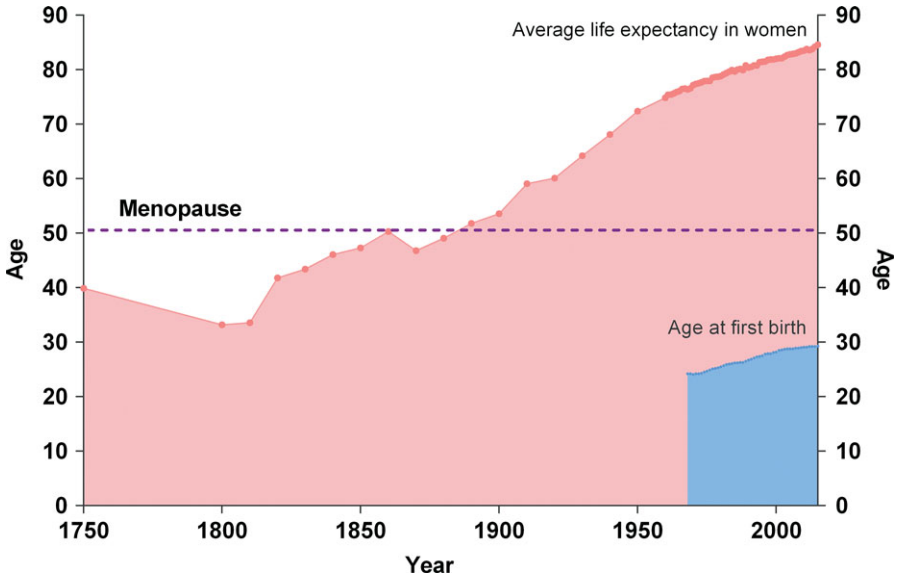

Figure 3 Age at menopause in the context of demographic trends. Data from Sweden were used for graphic representation of female life expectancy at birth (https://www.clio-infra.eu/), age at first birth (http://www.humanfertility.org/) and age at menopause that was demonstrated to be stable, because of lack of the data about its dynamics (depicted as a constant at 50.8 years, mean age at menopause for a Swedish cohort from Schoenaker *et al.*, (2014)).

General declines in reproductive function with age are not surprising as such, because senescence is widespread across the entire tree of life: animals as diverse as nematodes, insects, birds or mammals all show declining fertility as they age (Jones *et al.*, 2014). Such reproductive senescence can evolve via antagonistic pleiotropy if the genes that cause increased reproduction in early life also cause (or are inherited together with genes that cause) aging, as discussed earlier. Such pleiotropic genes are favoured because they have positive effects at younger ages, even though they have negative effects later in life (Williams, 1957; Peccei, 2001a). For instance, it has been hypothesized that because follicular depletion is essential for the maintenance of regular menstrual/ovarian cycles at younger reproductive ages, it is the follicular depletion system that is subject to strong selection, not menopause *per se* (Wood *et al.*, 2000). Under this scenario, the selective advantage of a particular combination of initial follicle stock and its rate of atresia in maintaining cycles at earlier ages more than offsets the selective costs of exhaustion of ovarian reserve at older age. This hypothesis is in line with the finding suggesting that the rate of follicular atresia is much steeper in women after age 35, compared with the mechanism of reproductive senescence in chimpanzees (Cloutier *et al.*, 2015). Such an explanation does not, however, address why selection for follicular depletion to maintain regular ovarian cycles led to the evolution of complete reproductive cessation well before the end of potential lifespan in women, but not in the vast majority of other species including the chimpanzees or elephants whose lifespan closely resembles that of humans without complete loss of reproductive ability (Lahdenperä *et al.*, 2014). Indeed, reproductive and somatic senescence occur in synchrony as predicted by evolutionary theory in all vertebrates with the exception of humans and few species of toothed whales (Ellis *et al.*, 2018).

The most popular adaptive explanations for the evolution (and maintenance) of menopause and prolonged post-reproductive lifespan are the Mother, the Reproductive Conflict and the Grandmother Hypotheses (Fig. 4). Human children require exceptionally long parental care compared with most other species and, according to the Mother Hypothesis, menopause evolved to ensure prolonged investment in offspring already born when confronted with the rising risk of death at childbirth with age (Williams, 1957; Peccei, 2001b; Lummaa, 2007). According to the Mother Hypothesis, menopause thus evolved in order to reduce the chances that the mother dies before all her children become independent. However, fitness calculations (Rogers, 1993; Lahdenperä *et al.*, 2012) or tests have found little support for this hypothesis. Analyses with historical Finnish data have concluded that offspring were adversely affected by maternal loss only in their first 2 years, suggesting that although mothers are required to ensure offspring survival in humans, maternal loss thereafter can be compensated by other family members, and maternal effects on dependent offspring are unlikely to explain the evolution or maintenance of menopause or prolonged post-reproductive lifespan in women (Lummaa, 2007).

An alternative idea for how menopause evolved and is maintained is termed the Reproductive Conflict Hypothesis, which relies on the premise that given the long reproductive lifespan in humans with overlapping generations that live in close proximity, women in patrilocal societies compete with their mothers-in-law for the resources required for their offspring if their reproductive schedules overlap. The daughter-in-law is unrelated to the children of her mother-in-law, but in contrast, the mother-in-law has 25% of genes common with children of her daughter-in-law and has thus lower genetic motivation to prefer her own reproduction to that of her son's wife. In a formal game-theoretical model, Cant and Johnstone (Cant and Johnstone, 2008) demonstrated that such an inter-generational

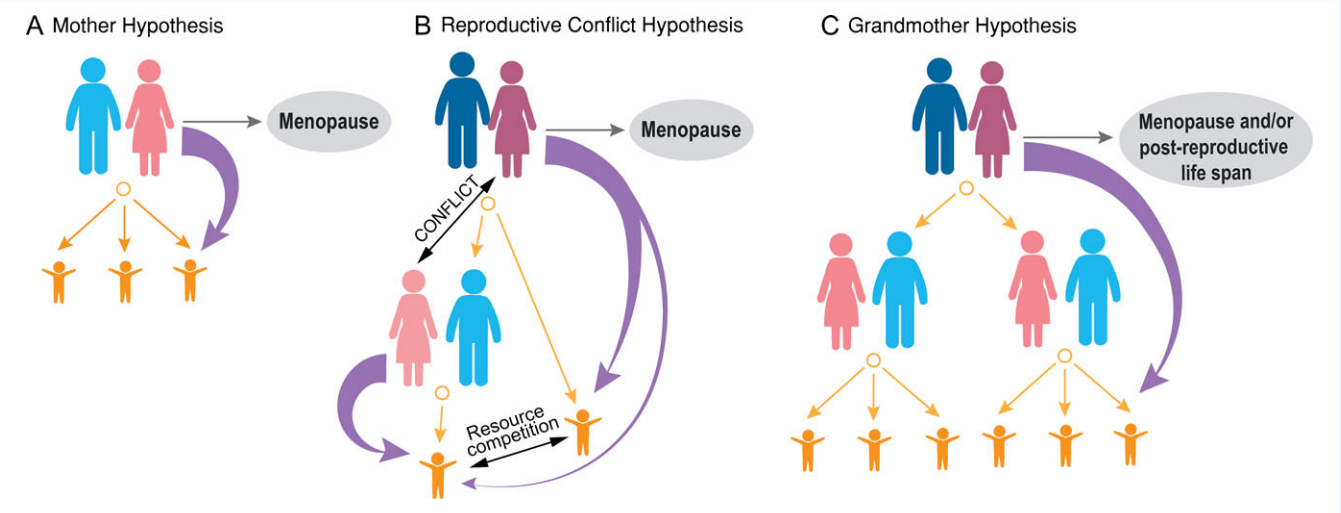

Figure 4 The three most popular adaptive explanations for the evolution and maintenance of menopause and/or prolonged lifespan after menopause. The purple arrows denote resource flow from the menopausal woman to her children and grandchildren or to the children of her son and his wife. (**A**) According to the Mother Hypothesis, menopause is meant to ensure prolonged investment in offspring already born; (**B**) the Reproductive Conflict Hypothesis postulates that women compete with their mothers-in-law for the resources required for their offspring if their reproductive schedules overlap. According to this hypothesis, menopause evolves under patrilocal conditions where the couple co-resides with the husband's mother and the daughter-in-law and her husband's mother compete for the limited resources. This conflict is asymmetric because the mother-in-law has 25% of genes common to the children of her daughter-in-law. Ceasing reproduction would thus result in a smaller fitness cost for the mother-in-law than for the daughter-in-law (who lacks common genetic interests with her husband's mother), potentially permitting the evolution of menopause in mothers-in-law and (**C**) the Grandmother Hypothesis states that menopause or prolonged lifespan after menopause evolved as an adaptation that favours helping adult children and grandchildren.

conflict yields an evolutionarily stable solution in which the mother-in-law commits irreversibly to zero reproduction when daughter-in-law starts to reproduce. This hypothesis may explain why humans exhibit an extraordinarily low degree of reproductive overlap between generations, despite their lifespan potentially allowing several generations to reproduce simultaneously. However, to date only a few empirical tests of the hypothesis exist. Whereas data from ancestrally patrilocal and matrilocal Indonesians (Snopkowski *et al.*, 2014) provided no support for the hypothesis, a study using a 200-year data set on pre-industrial Finns showed that simultaneous reproduction by daughters-in-law and mothers-in-law was associated with declines in offspring survivorship (Lahdenperä *et al.*, 2012). Such costs of two women reproducing simultaneously in the same household were sufficient to generate selection against continued reproduction beyond 51 years. At present, the available evidence therefore supports that menopause evolved, in part, because of age-specific increases in reproductive competition under ecological scarcity.

Why then do women continue living after reaching these 'conflict ages' as sterile rather than dying? The Grandmother Hypothesis (Hawkes *et al.*, 1998) postulates that the exceptionally prolonged post-reproductive lifespan in our species following menopause evolved as an adaptation that favours helping kin: typical families in the past contained multiple dependent offspring per mother whose caretaking required assistance also from other persons besides the mother herself. Such kin selection can operate only in situations where grandmothers can transfer resources (including information, expertise and prestige) to their grandchildren. Depending on the residence pattern in our evolutionary history and currently around the world, the post-menopausal grandmothers may help to raise the grandchildren from either their sons or daughters, or both. In line with this, there is now overwhelming evidence from very different kinds of societies to demonstrate that a grandmother's presence improves the grandchild's nutritional condition, development and survival probability to adulthood (Tanskanen and Danielsbacka, 2018). Such benefits of continued lifespan after menopause also translate to significant benefits to evolutionary fitness: in the 17th- and 18th-century farming/fishing communities of Finns and Canadians, women gained two extra grandchildren for every 10 years they survived beyond menopause until their mid-70 s, showing that post-reproductive lifespan can be under positive selection at least until this age (Lahdenperä *et al.*, 2004).

The Reproductive Conflict and the Grandmother Hypotheses explain the evolution and maintenance of menopause and extended post-reproductive longevity, respectively. The current evidence, however, raises the question of why women have not been selected to maintain the ability to conceive late in life should suitable opportunities for this arise (e.g. plentiful conditions with no shortage of food even for multiple women in the household to reproduce simultaneously), as do many other long-lived species (e.g. elephants) that also help their relatives to reproduce more successfully (Lahdenperä *et al.*, 2014, 2016). One explanation is that the fitness accrued through grandmothering during the post-reproductive period, although lower, is more predictable than that gained through late-life reproduction when conflict or death in childbirth might arise unpredictably (Lahdenperä *et al.*, 2012). In such a scenario, the shutting down of an energetically costly system that was seldom used

beneficially led to the evolution of menopause accompanied by a significant post-reproductive lifespan allocated towards grandmothering.

To date, most evolutionary research on menopause and post-reproductive longevity has focused on the ultimate causes that led to the origins of these traits, as highlighted above. In contrast, less attention has been paid to the 'micro-evolutionary' processes that affect selection on menopause age and general reproductive senescence patterns in contemporary human populations. In this light, research lines from the perspective of evolutionary reproductive biology may offer interesting insights into medicine where rich data on contemporary human populations is available. Mechanistically, the evolution of traits such as the age at menopause depend on their heritability, genetic structure and contribution to reproductive success (i.e. genetic representation in the following generations). Age at menopause is moderately to highly heritable (Kirk *et al.*, 2001; de Bruin *et al.*, 2001a) permitting potentially rapid evolutionary change. Thus, it is pertinent to ask what maintains its current genetic variation and would future selection weed out genetic variants associated with its early onset. These are highly relevant questions for those aiming to understand the causes and consequences of reproductive senescence and associated pathologies. Specifically, one might predict that genome-level analyses would reveal pleiotropic links between genes affecting early fertility and genes affecting the onset of menopause, as demonstrated by genetic correlations between timings of menarche or age at first birth and menopause (as discussed earlier). Research in reproductive senescence would thus benefit from finding out whether and under what (environmental) conditions such hypothesized pleiotropic links will be expressed as phenotypic correlations. For instance, it is possible that between-population differences under environmental conditions (likely related to welfare) explain the obscure relationships between the ages at menarche and menopause: some studies have found positive and others have found negative phenotypic correlations, or no relationship at all, between these traits (Mishra *et al.*, 2009). The current improvement of living conditions, boosting contraceptive use and demographic changes witnessed in age at first and last deliveries, together with large, multigenerational, prospective cohort studies (that allow direct measurements of genetic variation and selection intensity for the physiological traits controlling fertility), offer exciting opportunities to investigate how ecological, epidemiological, demographic and evolutionary changes interact to determine fertility patterns across the female lifespan. Such studies should be performed in a range of nutritional, cultural and geographic settings, ideally using longitudinal genomic and medical information on individuals, to further our understanding of the antagonistic pleiotropic effects that contribute to the ovarian function in short-term demographic and long-term evolutionary perspectives. Only by combining research on the ultimate mechanisms promoting the evolution of menopause with studies that allow addressing the evolutionary (genetic) mechanisms underlying the observed patterns in reproductive senescence and variation in menopause age will be able to predict future patterns and responses to our changing lifestyle, environment and family living.

Conclusions

The current narrative review on evolutionary reproductive biology and medicine is aimed at integrating the molecular mechanisms of ovarian function and aging with short-term demographic and long-term evolutionary trends. However, in the present review we do not pretend to discuss the full list of all demographic trends interfering with female reproductive aging. We also avoided describing all evolutionary theories of menopausal aging that have been advanced in the context of life history theory, which is supposed to explain how human reproduction and post-reproductive behaviour have been shaped by natural selection. Rather we were more focussed on integrating the recently obtained information from large population-wide genome-screening projects and from high-throughput single-cell (oocyte or embryonal cell) genomic technologies with a possible evolutionary significance. The high speed at which we are generating new data has, so far, raised more questions than it has provided solid answers and has been paralleled by a lack of satisfactory interpretations of the findings in the context of human life history theory. Therefore, the recent flood of 'big data' could offer an unprecedented tool for future research to possibly confirm or rewrite human evolutionary reproductive history.

Supplementary data

Supplementary data are available at *Human Reproduction* Update online.

Authors' roles

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Conflict of interest

The authors have no potential conflicts of interest relevant to this article.

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