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# Demographic and evolutionary trends in ovarian function and aging

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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.

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**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

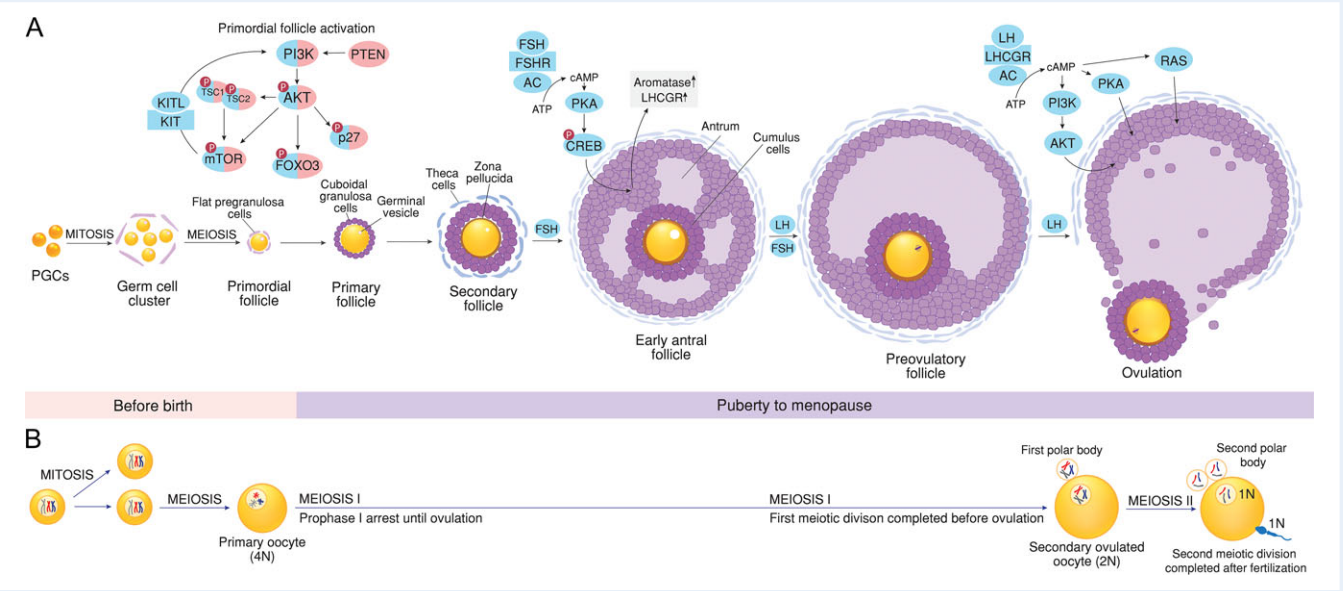


**Figure 1** Mammalian folliculogenesis (**A**) and meiosis (**B**) summarizing the information from several species. (A) The dormancy of primordial follicles is maintained by the FOXO3, PTEN, p27, mTOR and TSC1/TSC2 proteins (indicated in pink). When phosphorylated (red P), these factors promote primordial 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

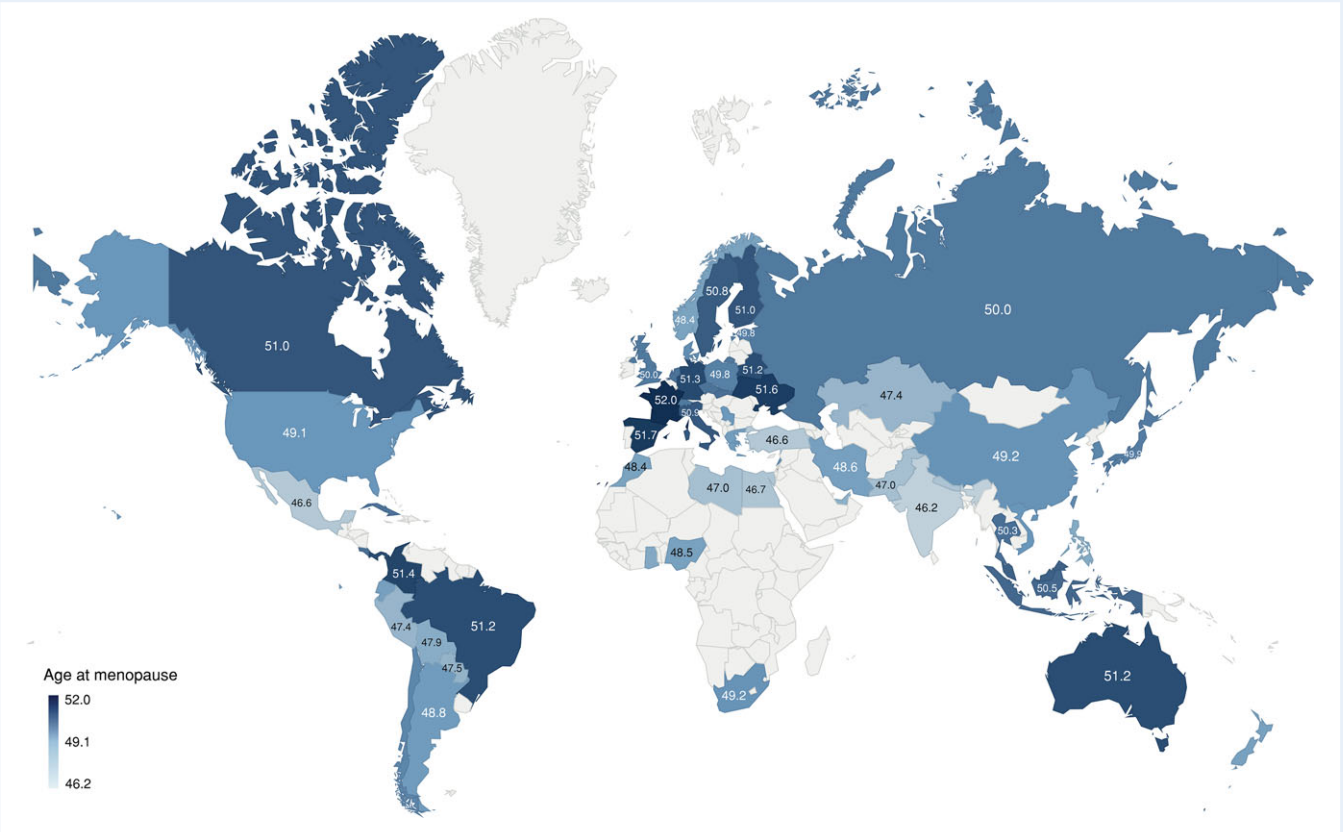


**Figure 2** Variation in menopause timing according to geographical region. Details and data sources are given in Supplementary Table 1.

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).

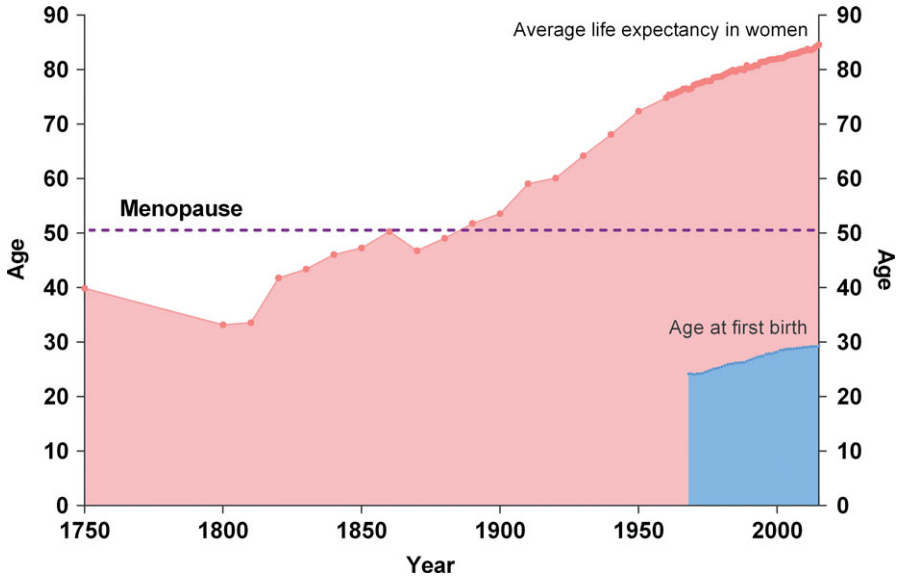


**Figure 3** Age at menopause in the context of demographic trends. Data from Sweden were used for graphic representation of female life expectancy at birth (https://www.clio-infra.eu/), age at first birth (http://www.humanfertility.org/) and age at menopause that was demonstrated to be stable, because 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

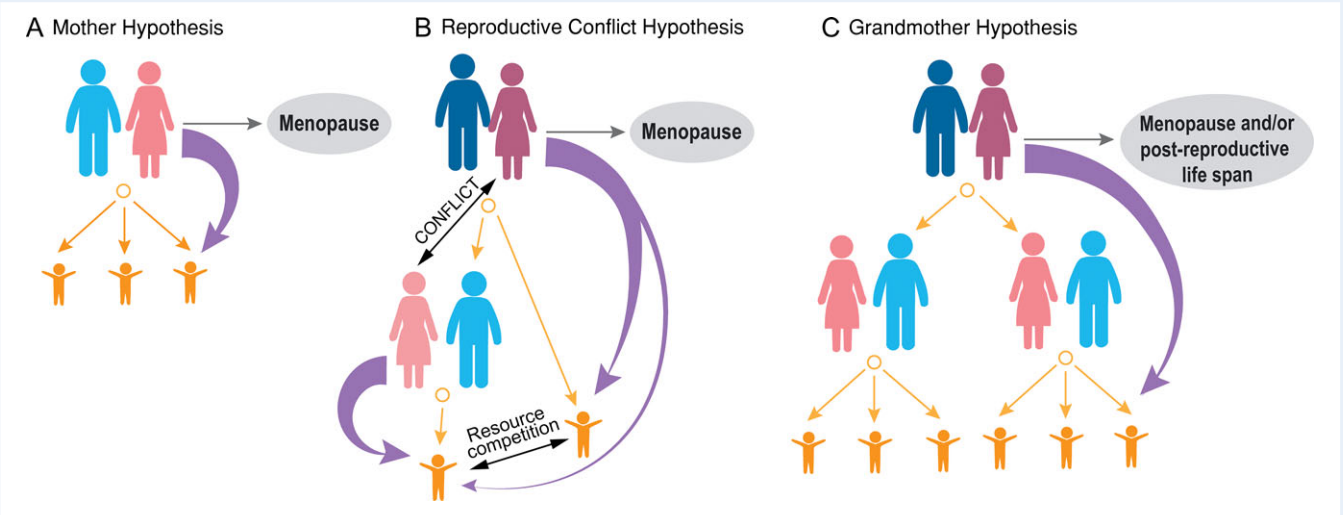


**Figure 4** The three most popular adaptive explanations for the evolution and maintenance of menopause and/or prolonged lifespan after menopause. The purple arrows denote resource flow from the menopausal woman to her children and grandchildren or to the children of her son and his wife. (**A**) According 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

All authors participated in study design, critical discussion and manuscript drafting. Figures were prepared by TJ.

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## Conflict of interest

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

## References

Adhikari D, Flohr G, Gorre N, Shen Y, Yang H, Lundin E, Lan Z, Gambello MJ, Liu K. Disruption of Tsc2 in oocytes leads to overactivation of the entire pool of primordial follicles. *Mol Hum Reprod* 2009;**15**:765–770.

Adhikari D, Zheng W, Shen Y, Gorre N, Hämäläinen T, Cooney AJ, Huhtaniemi I, Lan Z-J, Liu K. Tsc/mTORC1 signaling in oocytes governs the quiescence and activation of primordial follicles. *Hum Mol Genet* 2010;**19**:397–410.

Anderson RA, McIlwain L, Coutts S, Kinnell HL, Fowler PA, Childs AJ. Activation of the aryl hydrocarbon receptor by a component of cigarette smoke reduces germ cell proliferation in the human fetal ovary. *Mol Hum Reprod* 2014;**20**:42–48.

Aydos SE, Elhan AH, Tükün A. Is telomere length one of the determinants of reproductive life span? *Arch Gynecol Obstet* 2005;**272**:113–116.

Babayev E, Wang T, Szigeti-Buck K, Lowther K, Taylor HS, Horvath T, Seli E. Reproductive aging is associated with changes in oocyte mitochondrial dynamics, function, and mtDNA quantity. *Maturitas* 2016;**93**:121–130.

Baerwald AR, Adams GP, Pierson RA. Ovarian antral folliculogenesis during the human menstrual cycle: a review. *Hum Reprod Update* 2012;**18**:73–91.

Ballal RD, Saha T, Fan S, Haddad BR, Rosen EM. BRCA1 localization to the telomere and its loss from the telomere in response to DNA damage. *J Biol Chem* 2009;**284**:36083–36098.

Bell R. Trends in birthweight in the north of England. *Hum Fertil* 2008;**11**:1–8.

Ben-Eliezer I, Pomerantz Y, Galiani D, Nevo N, Dekel N. Appropriate expression of Ube2C and Ube2S controls the progression of the first meiotic division. *FASEB J* 2015;**29**:4670–4681.

Blagosklonny M V. Why men age faster but reproduce longer than women: mTOR and evolutionary perspectives. *Aging (Albany NY)* 2010;**2**:265–273.

Bolund E, Hayward A, Pettay JE, Lummaa V. Effects of the demographic transition on the genetic variances and covariances of human life-history traits. *Evolution* 2015;**69**:747–755.

Broekmans FJ, Soules MR, Fauser BC. Ovarian aging: mechanisms and clinical consequences. *Endocr Rev* 2009;**30**:465–493.

Bulik-Sullivan B, Finucane HK, Anttila V, Gusev A, Day FR, Loh P-R, Duncan L, Perry JRB, Patterson N, Robinson EB *et al*. An atlas of genetic correlations across human diseases and traits. *Nat Genet* 2015;**47**:1236–1241.

Byars SG, Ewbank D, Govindaraju DR, Stearns SC. Natural selection in a contemporary human population. *Proc Natl Acad Sci* 2010;**107**:1787–1792.

Cant MA, Johnstone RA. Reproductive conflict and the separation of reproductive generations in humans. *Proc Natl Acad Sci* 2008;**105**:5332–5336.

Carbone L, Chavez SL. Mammalian pre-implantation chromosomal instability: species comparison, evolutionary considerations, and pathological correlations. *Syst Biol Reprod Med* 2015;**61**:321–335.

Carty CL, Spencer KL, Setiawan VW, Fernandez-Rhodes L, Malinowski J, Buyske S, Young A, Jorgensen NW, Cheng I, Carlson CS *et al*. Replication of genetic loci for ages at menarche and menopause in the multi-ethnic Population Architecture using Genomics and Epidemiology (PAGE) study. *Hum Reprod* 2013;**28**:1695–1706.

Caserta D, Bordi G, Di Segni N, D'Ambrosio A, Mallozzi M, Moscarini M. The influence of cigarette smoking on a population of infertile men and women. *Arch Gynecol Obstet* 2013;**287**:813–818.

Caspari R, Lee S-H. Older age becomes common late in human evolution. *Proc Natl Acad Sci USA* 2004;**101**:10895–10900.

Castrillon DH, Miao L, Kollipara R, Horner JW, DePinho RA. Suppression of ovarian follicle activation in mice by the transcription factor Foxo3a. *Science* 2003;**301**:215–218.

Chen CTL, Fernández-Rhodes L, Brzyski RG, Carlson CS, Chen Z, Heiss G, North KE, Woods NF, Rajkovic A, Kooperberg C *et al*. Replication of loci influencing ages at menarche and menopause in Hispanic women: the Women's Health Initiative SHARe Study. *Hum Mol Genet* 2012;**21**:1419–1432.

Chen CTL, Liu C-T, Chen GK, Andrews JS, Arnold AM, Dreyfus J, Franceschini N, Garcia ME, Kerr KF, Li G *et al*. Meta-analysis of loci associated with age at natural menopause in African-American women. *Hum Mol Genet* 2014;**23**:3327–3342.

Chiang T, Schultz RM, Lampson MA. Age-dependent susceptibility of chromosome cohesion to premature separase activation in mouse oocytes. *Biol Reprod* 2011;**85**:1279–1283.

Cloutier CT, Coxworth JE, Hawkes K. Age-related decline in ovarian follicle stocks differ between chimpanzees (Pan troglodytes) and humans. *Age (Dordr)* 2015;**37**:9746.

Corbett S, Courtiol A, Lummaa V, Moorad J, Stearns S. The transition to modernity and chronic disease: mismatch and natural selection. *Nat Rev Genet* 2018;**19**:419–430.

Cresswell JL, Egger P, Fall CH, Osmond C, Fraser RB, Barker DJ. Is the age of menopause determined in-utero? *Early Hum Dev* 1997;**49**:143–148.

Croft DP, Brent LJN, Franks DW, Cant MA. The evolution of prolonged life after reproduction. *Trends Ecol Evol* 2015;**30**:407–416.

Day FR, Hinds DA, Tung JY, Stolk L, Styrkarsdottir U, Saxena R, Bjonnes A, Broer L, Dunger DB, Halldorsson B V *et al*. Causal mechanisms and balancing selection inferred from genetic associations with polycystic ovary syndrome. *Nat Commun* 2015a;**6**:8464.

Day FR, Ruth KS, Thompson DJ, Lunetta KL, Pervjakova N, Chasman DI, Stolk L, Finucane HK, Sulem P, Bulik-Sullivan B *et al*. Large-scale genomic analyses link reproductive aging to hypothalamic signaling, breast cancer susceptibility and BRCA1-mediated DNA repair. *Nat Genet* 2015b;**47**:1294–1303.

de Bruin JP, Bovenhuis H, van Noord PA, Pearson PL, van Arendonk JA, te Velde ER, Kuurman WW, Dorland M. The role of genetic factors in age at natural menopause. *Hum Reprod* 2001a;**16**:2014–2018.

de Bruin JP, Nikkels PG, Bruinse HW, van Haaften M, Looman CW, te Velde ER. Morphometry of human ovaries in normal and growth-restricted fetuses. *Early Hum Dev* 2001b;**60**:179–192.

de Vries E, den Tonkelaar I, van Noord PA, van der Schouw YT, te Velde ER, Peeters PH. Oral contraceptive use in relation to age at menopause in the DOM cohort. *Hum Reprod* 2001;**16**:1657–1662.

Destouni A, Zamani Esteki M, Catteeuw M, Tšuiko O, Dimitriadou E, Smits K, Kurg A, Salumets A, Van Soom A, Voet T *et al*. Zygotes segregate entire parental genomes in distinct blastomere lineages causing cleavage-stage chimerism and mixoploidy. *Genome Res* 2016;**26**:567–578.

Dewailly D, Andersen CY, Balen A, Broekmans F, Dilaver N, Fanchin R, Griesinger G, Kelsey TW, La Marca A, Lambalk C *et al*. The physiology and clinical utility of anti-Mullerian hormone in women. *Hum Reprod Update* 2014;**20**:370–385.

Driancourt MA, Reynaud K, Cortvrindt R, Smitz J. Roles of KIT and KIT LIGAND in ovarian function. *Rev Reprod* 2000;**5**:143–152.

Durlinger ALL, Visser JA, Themmen APN. Regulation of ovarian function: the role of anti-Müllerian hormone. *Reproduction* 2002;**124**:601–609.

Ellis S, Franks DW, Nattrass S, Cant MA, Bradley DL, Giles D, Balcomb KC, Croft DP. Postreproductive lifespans are rare in mammals. *Ecol Evol* 2018;**8**:2482–2494.

Fragouli E, Spath K, Alfarawati S, Kaper F, Craig A, Michel C-E, 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. Kim SK (ed). *PLoS Genet* 2015;**11**:e1005241.

Franasiak JM, Forman EJ, Hong KH, Werner MD, Upham KM, Treff NR, Scott RT. 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.e1.

French JD, Dunn J, Smart CE, Manning N, Brown MA. Disruption of BRCA1 function results in telomere lengthening and increased anaphase bridge formation in immortalized cell lines. *Genes Chromosomes Cancer* 2006;**45**:277–289.

Gold EB, Bromberger J, Crawford S, Samuels S, Greendale GA, Harlow SD, Skurnick J. Factors associated with age at natural menopause in a multiethnic sample of midlife women. *Am J Epidemiol* 2001;**153**:865–874.

Goriely A, Wilkie AOM. Missing heritability: paternal age effect mutations and selfish spermatogonia. *Nat Rev Genet* 2010;**11**:589.

Gosden RG, Treloar SA, Martin NG, Cherkas LF, Spector TD, Faddy MJ, Silber SJ. Prevalence of premature ovarian failure in monozygotic and dizygotic twins. *Hum Reprod* 2007;**22**:610–615.

Gougeon A. Regulation of ovarian follicular development in primates: facts and hypotheses. *Endocr Rev* 1996;**17**:121–155.

Gougeon A, Chainy GB. Morphometric studies of small follicles in ovaries of women at different ages. *J Reprod Fertil* 1987;**81**:433–442.

Greco E, Minasi MG, Fiorentino F. Healthy Babies after Intrauterine Transfer of Mosaic Aneuploid Blastocysts. *N Engl J Med* 2015;**373**:2089–2090.

Grøndahl ML, Christiansen SL, Kesmodel US, Agerholm IE, Lemmen JG, Lundstrøm P, Bogstad J, Raaschou-Jensen M, Ladelund S. Effect of women's age on embryo morphology, cleavage rate and competence-A multicenter cohort study. Sturmey R (ed). *PLoS One* 2017;**12**:e0172456.

Grøndahl ML, Yding Andersen C, Bogstad J, Nielsen FC, Meinertz H, Borup R. Gene expression profiles of single human mature oocytes in relation to age. *Hum Reprod* 2010;**25**:957–968.

Gurven M, Kaplan H. Longevity among hunter-gatherers: a cross-cultural examination. *Popul Dev Rev* 2007;**33**:321–365.

Hanevik HI, Hessen DO, Sunde A, Breivik J. Can IVF influence human evolution? *Hum Reprod* 2016;**31**:1397–1402.

Hardy R, Kuh D. Does early growth influence timing of the menopause? Evidence from a British birth cohort. *Hum Reprod* 2002;**17**:2474–2479.

Hassold T, Hunt P. To err (meiotically) is human: the genesis of human aneuploidy. *Nat Rev Genet* 2001;**2**:280–291.

Hatch EE, Troisi R, Wise LA, Hyer M, Palmer JR, Titus-Ernstoff L, Strohsnitter W, Kaufman R, Adam E, Noller KL *et al*. Age at natural menopause in women exposed to diethylstilbestrol in utero. *Am J Epidemiol* 2006;**164**:682–688.

Hawkes K. The grandmother effect. *Nature* 2004;**428**:128–129.

Hawkes K, O'Connell JF, Jones NG, Alvarez H, Charnov EL. Grandmothering, menopause, and the evolution of human life histories. *Proc Natl Acad Sci USA* 1998;**95**:1336–1339.

Helle S, Lummaa V, Jokela J. Are reproductive and somatic senescence coupled in humans? Late, but not early, reproduction correlated with longevity in historical Sami women. *Proc Biol Sci* 2005;**272**:29–37.

Henderson KD, Bernstein L, Henderson B, Kolonel L, Pike MC. Predictors of the timing of natural menopause in the multiethnic cohort study. *Am J Epidemiol* 2008a;**167**:1287–1294.

Henderson KD, Bernstein L, Henderson B, Kolonel L, Pike MC. Predictors of the timing of natural menopause in the multiethnic cohort study. *Am J Epidemiol* 2008b;**167**:1287–1294.

Horikoshi M, Day F, Kamatani Y, Hirata M, Akiyama M, Matsuda K, Wright H, Toro C, Ojeda S, Lomniczi A *et al*. Elucidating the genetic architecture of reproductive ageing in the Japanese population. *Nat Commun* 2018;**9**:1997.

John GB, Gallardo TD, Shirley LJ, Castrillon DH. Foxo3 is a PI3K-dependent molecular switch controlling the initiation of oocyte growth. *Dev Biol* 2008;**321**:197–204.

Jones OR, Scheuerlein A, Salguero-Gómez R, Camarda CG, Schaible R, Casper BB, Dahlgren JP, Ehrlén J, García MB, Menges ES *et al*. Diversity of ageing across the tree of life. *Nature* 2014;**505**:169–173.

Kirk KM, Blomberg SP, Duffy DL, Heath AC, Owens IP, Martin NG. Natural selection and quantitative genetics of life-history traits in Western women: a twin study. *Evolution* 2001;**55**:423–435.

Lahdenperä M, Gillespie DOS, Lummaa V, Russell AF. Severe intergenerational reproductive conflict and the evolution of menopause. Sorci G (ed). *Ecol Lett* 2012;**15**:1283–1290.

Lahdenperä M, Lummaa V, Helle S, Tremblay M, Russell AF. Fitness benefits of prolonged post-reproductive lifespan in women. *Nature* 2004;**428**:178–181.

Lahdenperä M, Mar KU, Lummaa V. Reproductive cessation and post-reproductive lifespan in Asian elephants and pre-industrial humans. *Front Zool* 2014;**11**:54.

Lahdenperä M, Mar KU, Lummaa V. Nearby grandmother enhances calf survival and reproduction in Asian elephants. *Sci Rep* 2016;**6**:27213.

Laisk T, Kukushkina V, Palmer D, Laber S, Chen C-Y, Ferreira T, Rahmioglu N, Zondervan K, Becker C, Smoller JW *et al*. GWAS meta-analysis highlights the hypothalamic-pituitary-gonadal axis (HPG axis) in the genetic regulation of menstrual cycle length. *Hum Mol Genet* 2018. doi:10.1093/hmg/ddy317.

Lamb NE, Sherman SL, Hassold TJ. Effect of meiotic recombination on the production of aneuploid gametes in humans. *Cytogenet Genome Res* 2005a;**111**:250–255.

Lamb NE, Yu K, Shaffer J, Feingold E, Sherman SL. Association between maternal age and meiotic recombination for trisomy 21. *Am J Hum Genet* 2005b;**76**:91–99.

Laplante M, Sabatini DM. mTOR signaling in growth control and disease. *Cell* 2012;**149**:274–293.

Lenormand T, Engelstädter J, Johnston SE, Wijnker E, Haag CR. Evolutionary mysteries in meiosis. *Philos Trans R Soc B Biol Sci* 2016;**371**:20160001.

Levine ME, Lu AT, Chen BH, Hernandez DG, Singleton AB, Ferrucci L, Bandinelli S, Salfati E, Manson JE, Quach A *et al*. Menopause accelerates biological aging. *Proc Natl Acad Sci USA* 2016;**113**:9327–9332.

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

Lummaa V. Life history theory, reproduction and longevity in humans. In: Louise Barrett and Robin Dunbar (eds). *The Oxford Handbook of Evolutionary Psychology*. USA: Oxford University Press, 2007, 397–414.

Manova K, Huang EJ, Angeles M, De Leon V, Sanchez S, Pronovost SM, Besmer P, Bachvarova RF. The expression pattern of the c-kit ligand in gonads of mice supports a role for the c-kit receptor in oocyte growth and in proliferation of spermatogonia. *Dev Biol* 1993;**157**:85–99.

May-Panloup P, Boucret L, Chao de la Barca J-M, 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.

May-Panloup P, Chrétien MF, Jacques C, Vasseur C, Malthièry Y, Reynier P. Low oocyte mitochondrial DNA content in ovarian insufficiency. *Hum Reprod* 2005;**20**:593–597.

Mbarek H, Steinberg S, Nyholt DR, Gordon SD, Miller MB, McRae AF, Hottenga JJ, Day FR, Willemsen G, de Geus EJ *et al*. Identification of common genetic variants influencing spontaneous dizygotic twinning and female fertility. *Am J Hum Genet* 2016;**98**:898–908.

McGee EA, Hsueh AJ. Initial and cyclic recruitment of ovarian follicles. *Endocr Rev* 2000;**21**:200–214.

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.

Miao Y-L, Kikuchi K, Sun Q-Y, Schatten H. Oocyte aging: cellular and molecular changes, developmental potential and reversal possibility. *Hum Reprod Update* 2009;**15**:573–585.

Mikkelsen TF, Graff-Iversen S, Sundby J, Bjertness E. Early menopause, association with tobacco smoking, coffee consumption and other lifestyle factors: a cross-sectional study. *BMC Public Health* 2007;**7**:149.

Mishra GD, Cooper R, Tom SE, Kuh D. Early life circumstances and their impact on menarche and menopause. *Womens Health (Lond Engl)* 2009;**5**:175–190.

Mishra G, Hardy R, Kuh D. Are the effects of risk factors for timing of menopause modified by age? Results from a British birth cohort study. *Menopause* 2007;**14**:717–724.

Mishra GD, Pandeya N, Dobson AJ, Chung H-F, Anderson D, Kuh D, Sandin S, Giles GG, Bruinsma F, Hayashi K *et al*. Early menarche, nulliparity and the risk for premature and early natural menopause. *Hum Reprod* 2017;**32**:679–686.

Morris DH, Jones ME, Schoemaker MJ, McFadden E, Ashworth A, Swerdlow AJ. Body mass index, exercise, and other lifestyle factors in relation to age at natural menopause: analyses from the breakthrough generations study. *Am J Epidemiol* 2012;**175**:998–1005.

Nagaoka SI, Hodges CA, Albertini DF, Hunt PA. Oocyte-specific differences in cell-cycle control create an innate susceptibility to meiotic errors. *Curr Biol* 2011;**21**:651–657.

Nakagawa S, FitzHarris G. Intrinsically defective microtubule dynamics contribute to age-related chromosome segregation errors in mouse oocyte meiosis-I. *Curr Biol* 2017;**27**:1040–1047.

Oken E. Secular trends in birthweight. *Nestle Nutr Inst Workshop Ser* 2013;**71**:103–114.

Ossewaarde ME, Bots ML, Verbeek ALM, Peeters PHM, van der Graaf Y, Grobbee DE, van der Schouw YT. Age at menopause, cause-specific mortality and total life expectancy. *Epidemiology* 2005;**16**:556–562.

Ottolini CS, Newnham L, Capalbo A, Natesan SA, Joshi HA, Cimadomo D, Griffin DK, Sage K, Summers MC, Thornhill AR *et al*. Genome-wide maps of recombination and chromosome segregation in human oocytes and embryos show selection for maternal recombination rates. *Nat Genet* 2015;**47**:727–735.

Palmer JR, Rosenberg L, Wise LA, Horton NJ, Adams-Campbell LL. Onset of natural menopause in African American women. *Am J Public Health* 2003;**93**:299–306.

Peccei JS. Menopause: adaptation or epiphenomenon? *Evol Anthropol Issues, News, Rev* 2001a;**10**:43–57.

Peccei JS. A critique of the grandmother hypotheses: old and new. *Am J Hum Biol* 2001b;**13**:434–452.

Pelosi E, Forabosco A, Schlessinger D. Genetics of the ovarian reserve. *Front Genet* 2015;**6**:308.

Pelosi E, Omari S, Michel M, Ding J, Amano T, Forabosco A, Schlessinger D, Ottolenghi C. Constitutively active Foxo3 in oocytes preserves ovarian reserve in mice. *Nat Commun* 2013;**4**:1843.

Perry JRB, McMahon G, Day FR, Ring SM, Nelson SM, Lawlor DA. Genome-wide association study identifies common and low-frequency variants at the *AMH* gene locus that strongly predict serum AMH levels in males. *Hum Mol Genet* 2016;**25**:382–388.

Persani L, Rossetti R, Di Pasquale E, Cacciatore C, Fabre S. The fundamental role of bone morphogenetic protein 15 in ovarian function and its involvement in female fertility disorders. *Hum Reprod Update* 2014;**20**:869–883.

Philipp T, Grillenberger K, Separovic ER, Philipp K, Kalousek DK. Effects of triploidy on early human development. *Prenat Diagn* 2004;**24**:276–281.

Plante BJ, Cooper GS, Baird DD, Steiner AZ. The impact of smoking on antimüllerian hormone levels in women aged 38 to 50 years. *Menopause* 2010; **17**:571–576.

Rajareddy S, Reddy P, Du C, Liu L, Jagarlamudi K, Tang W, Shen Y, Berthet C, Peng SL, Kaldis P *et al*. p27kip1 (cyclin-dependent kinase inhibitor 1B) controls ovarian development by suppressing follicle endowment and activation and promoting follicle atresia in mice. *Mol Endocrinol* 2007;**21**:2189–2202.

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.

Richards JS, Pangas SA. The ovary: basic biology and clinical implications. *J Clin Invest* 2010;**120**:963–972.

Richardson MC, Guo M, Fauser BC, Macklon NS. Environmental and developmental origins of ovarian reserve. *Hum Reprod Update* 2014;**20**:353–369.

Roa J, Garcia-Galiano D, Varela L, Sánchez-Garrido MA, Pineda R, Castellano JM, Ruiz-Pino F, Romero M, Aguilar E, López M *et al*. The mammalian target of rapamycin as novel central regulator of puberty onset via modulation of hypothalamic Kiss1 system. *Endocrinology* 2009;**150**:5016–5026.

Rogers AR. Why menopause? *Evol Ecol* 1993;**7**:406–420.

Ruth KS, Beaujmont RN, Tyrrell J, Jones SE, Tuke MA, Yaghootkar H, Wood AR, Freathy RM, Weedon MN, Frayling TM *et al*. Genetic evidence that lower circulating FSH levels lengthen menstrual cycle, increase age at menopause and impact female reproductive health. *Hum Reprod* 2016a;**31**:473–481.

Ruth KS, Campbell PJ, Chew S, Lim EM, Hadlow N, Stuckey BG, Brown SJ, Feenstra B, Joseph J, Surdulescu GL *et al*. Genome-wide association study with 1000 genomes imputation identifies signals for nine sex hormone-related phenotypes. *Eur J Hum Genet* 2016b;**24**:284–290.

Ruth KS, Perry JRB, Henley WE, Melzer D, Weedon MN, Murray A. Events in early life are associated with female reproductive ageing: a UK Biobank study. *Sci Rep* 2016c;**6**:24710.

Rödström K, Bengtsson C, Milsom I, Lissner L, Sundh V, Bjoürkelund C. Evidence for a secular trend in menopausal age: a population study of women in Gothenburg. *Menopause* 2003;**10**:538–543.

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

Sadrzadeh S, Verschuuren M, Schoonmade LJ, Lambalk CB, Painter RC. The effect of adverse intrauterine conditions, early childhood growth and famine exposure on age at menopause: a systematic review. *J Dev Orig Health Dis* 2018;**9**:127–136.

Salazar-Roa M, Malumbres M. Fueling the cell division cycle. *Trends Cell Biol* 2017; **27**:69–81.

Savage PM, Sita-Lumsden A, Dickson S, Iyer R, Everard J, Coleman R, Fisher RA, Short D, Casalboni S, Catalano K *et al*. The relationship of maternal age to molar pregnancy incidence, risks for chemotherapy and subsequent pregnancy outcome. *J Obstet Gynaecol* 2013;**33**:406–411.

Schoenaker DA, Jackson CA, Rowlands J V, Mishra GD. Socioeconomic position, lifestyle factors and age at natural menopause: a systematic review and meta-analyses of studies across six continents. *Int J Epidemiol* 2014;**43**:1542–1562.

Schuh-Huerta SM, Johnson NA, Rosen MP, Sternfeld B, Cedars MI, Reijo Pera RA. Genetic variants and environmental factors associated with hormonal markers of ovarian reserve in Caucasian and African American women. *Hum Reprod* 2012a; **27**:594–608.

Schuh-Huerta SM, Johnson NA, Rosen MP, Sternfeld B, Cedars MI, Reijo Pera RA. Genetic markers of ovarian follicle number and menopause in women of multiple ethnicities. *Hum Genet* 2012b;**131**:1709–1724.

Sebire NJ, Foskett M, Fisher RA, Rees H, Seckl M, Newlands E. Risk of partial and complete hydatidiform molar pregnancy in relation to maternal age. *BJOG* 2002; **109**:99–102.

Shen C, Delahanty RJ, Gao Y-T, Lu W, Xiang Y-B, Zheng Y, Cai Q, Zheng W, Shu X-O, Long J. Evaluating GWAS-identified SNPs for age at natural menopause among Chinese women. *PLoS One* 2013;**8**:e58766.

Smith KR, Gagnon A, Cawthon RM, Mineau GP, Mazan R, Desjardins B. Familial aggregation of survival and late female reproduction. *J Gerontol A Biol Sci Med Sci* 2009;**64**:740–744.

Smith KR, Hanson HA, Hollingshaus MS. BRCA1 and BRCA2 mutations and female fertility. *Curr Opin Obstet Gynecol* 2013;**25**:207–213.

Smith KR, Hanson HA, Mineau GP, Buys SS. Effects of BRCA1 and BRCA2 mutations on female fertility. *Proc Biol Sci* 2012;**279**:1389–1395.

Snopkowski K, Moya C, Sear R. A test of the intergenerational conflict model in Indonesia shows no evidence of earlier menopause in female-dispersing groups. *Proc Biol Sci* 2014;**281**:20140580.

Stearns SC. Trade-offs in life-history evolution. *Funct Ecol* 1989;**3**:259.

Steiner AZ, D'Aloisio AA, DeRoo LA, Sandler DP, Baird DD. Association of intrauterine and early-life exposures with age at menopause in the Sister Study. *Am J Epidemiol* 2010;**172**:140–148.

Steuerwald NM, Bermúdez MG, Wells D, Munné S, Cohen J. Maternal age-related differential global expression profiles observed in human oocytes. *Reprod Biomed Online* 2007;**14**:700–708.

Stolk L, Perry JRB, Chasman DI, He C, Mangino M, Sulem P, Barbalic M, Broer L, Byrne EM, Ernst F *et al*. Meta-analyses identify 13 loci associated with age at menopause and highlight DNA repair and immune pathways. *Nat Genet* 2012; **44**:260–268.

Strohsnitter WC, Hatch EE, Hyer M, Troisi R, Kaufman RH, Robboy SJ, Palmer JR, Titus-Ernstoff L, Anderson D, Hoover RN *et al*. The association between in utero cigarette smoke exposure and age at menopause. *Am J Epidemiol* 2008; **167**:727–733.

Sun Q, Fuchimoto D, Nagai T. Regulatory roles of ubiquitin–proteasome pathway in pig oocyte meiotic maturation and fertilization. *Theriogenology* 2004;**62**: 245–255.

Sun Y-C, Sun X-F, Dyce PW, Shen W, Chen H. The role of germ cell loss during primordial follicle assembly: a review of current advances. *Int J Biol Sci* 2017;**13**: 449–457.

Takemoto Y, Ota E, Yoneoka D, Mori R, Takeda S. Japanese secular trends in birthweight and the prevalence of low birthweight infants during the last three decades: a population-based study. *Sci Rep* 2016;**6**:31396.

Tanskanen AO, Danielsbacka M *Intergenerational Family relations. An Evolutionary Social Science Approach*. London: Routledge, 2018.

Tarnawa ED, Baker MD, Aloisio GM, Carr BR, Castrillon DH. Gonadal expression of Foxo1, but not Foxo3, is conserved in diverse mammalian species. *Biol Reprod* 2013;**88**:103.

Tawfik H, Kline J, Jacobson J, Tehranifar P, Protacio A, Flom JD, Cirillo P, Cohn BA, Terry MB. Life course exposure to smoke and early menopause and menopausal transition. *Menopause* 2015;**22**:1076–1083.

Ting AY, Zelinski MB. Characterization of FOXO1, 3 and 4 transcription factors in ovaries of fetal, prepubertal and adult rhesus macaques. *Biol Reprod* 2017;**96**: 1052–1059.

Tom SE, Cooper R, Kuh D, Guralnik JM, Hardy R, Power C. Fetal environment and early age at natural menopause in a British birth cohort study. *Hum Reprod* 2010; **25**:791–798.

Treloar SA, Sadrzadeh S, Do KA, Martin NG, Lambalk CB. Birth weight and age at menopause in Australian female twin pairs: exploration of the fetal origin hypothesis. *Hum Reprod* 2000;**15**:55–59.

Truman AM, Tilly JL, Woods DC. Ovarian regeneration: the potential for stem cell contribution in the postnatal ovary to sustained endocrine function. *Mol Cell Endocrinol* 2017;**445**:74–84.

Tsutsumi M, Fujiwara R, Nishizawa H, Ito M, Kogo H, Inagaki H, Ohye T, Kato T, Fujii T, Kurahashi H. Age-related decrease of meiotic cohesins in human oocytes. Shi Q (ed). *PLoS One* 2014;**9**:e96710.

Tšuiko O, Catteeuw M, Zamani Esteki M, Destouni A, Bogado Pascottini O, Besenfelder U, Havlicek V, Smits K, Kurg A, Salumets A *et al*. Genome stability of bovine in vivo-conceived cleavage-stage embryos is higher compared to in vitro-produced embryos. *Hum Reprod* 2017;**32**:2348–2357.

Ulloa-Aguirre A, Zariñán T, Pasapera AM, Casas-González P, Dias JA. Multiple facets of follicle-stimulating hormone receptor function. *Endocrine* 2007;**32**: 251–263.

Vabre P, Gatimel N, Moreau J, Gayrard V, Picard-Hagen N, Parinaud J, Leandri RD. Environmental pollutants, a possible etiology for premature ovarian insufficiency: a narrative review of animal and human data. *Environ Health* 2017;**16**:37.

van Disseldorp J, Franke L, Eijkemans R, Broekmans F, Macklon N, Wijmenga C, Fauser B. Genome-wide analysis shows no genomic predictors of ovarian response to stimulation by exogenous FSH for IVF. *Reprod Biomed Online* 2011; **22**:382–388.

Vanneste E, Voet T, Le Caignec C, Ampe M, Konings P, Melotte C, Debrock S, Amyere M, Vikkula M, Schuit F *et al*. Chromosome instability is common in human cleavage-stage embryos. *Nat Med* 2009;**15**:577–583.

Wallace WHB, Kelsey TW. Human ovarian reserve from conception to the menopause. Vitzthum VJ (ed). *PLoS One* 2010;**5**:e8772.

Wang S, Hassold T, Hunt P, White MA, Zickler D, Kleckner N, Zhang L. Inefficient crossover maturation underlies elevated aneuploidy in human female meiosis. *Cell* 2017b;**168**:977–989.e17.

Wang N, Satirapod C, Ohguchi Y, Park E-S, Woods DC, Tilly JL. Genetic studies in mice directly link oocytes produced during adulthood to ovarian function and natural fertility. *Sci Rep* 2017a;**7**:10011.

Webster A, Schuh M. Mechanisms of aneuploidy in human eggs. *Trends Cell Biol* 2017;**27**:55–68.

Weenen C, Laven JSE, Von Bergh ARM, Cranfield M, Groome NP, Visser JA, Kramer P, Fauser BCJM, Themmen APN. Anti-Müllerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment. *Mol Hum Reprod* 2004;**10**:77–83

Wells JCK, Nesse RM, Sear R, Johnstone RA, Stearns SC. Evolutionary public health: introducing the concept. *Lancet* 2017;**390**:500–509.

Wijayarathna R, de Kretser DM. Activins in reproductive biology and beyond. *Hum Reprod Update* 2016;**22**:342–357.

Williams GC. Pleiotropy, natural selection, and the evolution of senescence. *Evolution (N Y)* 1957;**11**:398–411.

Wood J, O'Connor K, Holman DJ, Brindle E. The evolution of menopause by antagonistic pleiotropy. *Homo* 2000;**51**:S149.

Wood M, Rajkovic A. Genomic markers of ovarian reserve. *Semin Reprod Med* 2013;**31**:399–415.

Wortzman GB, Evans JP. Membrane and cortical abnormalities in post-ovulatory aged eggs: analysis of fertilizability and establishment of the membrane block to polyspermy. *Mol Hum Reprod* 2005;**11**:1–9.

Xie W, Wang H, Wu J. Similar morphological and molecular signatures shared by female and male germline stem cells. *Sci Rep* 2014;**4**:5580.

Xiong J, Lu Z, Wu M, Zhang J, Cheng J, Luo A, Shen W, Fang L, Zhou S, Wang S. Intraovarian Transplantation of Female Germline Stem Cells Rescue Ovarian Function in Chemotherapy-Injured Ovaries. Tian X (Cindy) (ed). *PLoS One* 2015;**10**:e0139824.

Yu C, Ji S-Y, Sha Q-Q, Sun Q-Y, Fan H-Y. CRL4-DCAF1 ubiquitin E3 ligase directs protein phosphatase 2A degradation to control oocyte meiotic maturation. *Nat Commun* 2015;**6**:8017.

Zhang H, Liu K. Cellular and molecular regulation of the activation of mammalian primordial follicles: somatic cells initiate follicle activation in adulthood. *Hum Reprod Update* 2015;**21**:779–786.

Zhang H, Risal S, Gorre N, Busayavalasa K, Li X, Shen Y, Bosbach B, Brännström M, Liu K. Somatic cells initiate primordial follicle activation and govern the development of dormant oocytes in mice. *Curr Biol* 2014;**24**:2501–2508.

Zhang X, Wu XQ, Lu S, Guo YL, Ma X. Deficit of mitochondria-derived ATP during oxidative stress impairs mouse MII oocyte spindles. *Cell Res* 2006;**16**:841–850.

Zhang Y, Yang Z, Yang Y, Wang S, Shi L, Xie W, Sun K, Zou K, Wang L, Xiong J *et al*. Production of transgenic mice by random recombination of targeted genes in female germline stem cells. *J Mol Cell Biol* 2011;**3**:132–141.

Zheng J, Erzurumluoglu AM, Elsworth BL, Kemp JP, Howe L, Haycock PC, Hemani G, Tansey K, Laurin C, Pourcain B St. *et al*. LD hub: a centralized database and web interface to perform LD score regression that maximizes the potential of summary level GWAS data for SNP heritability and genetic correlation analysis. *Bioinformatics* 2017;**33**:272–279.

Zhou L, Wang L, Kang JX, Xie W, Li X, Wu C, Xu B, Wu J. Production of fat-1 transgenic rats using a post-natal female germline stem cell line. *Mol Hum Reprod* 2014;**20**:271–281.

Zou K, Yuan Z, Yang Z, Luo H, Sun K, Zhou L, Xiang J, Shi L, Yu Q, Zhang Y *et al*. Production of offspring from a germline stem cell line derived from neonatal ovaries. *Nat Cell Biol* 2009;**11**:631–636.