

# Positive and negative effects of cellular senescence during female reproductive aging and pregnancy

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## Abstract

Cellular senescence is a phenomenon occurring when cells are no longer able to divide even after treatment with growth stimuli. Because senescent cells are typically associated with aging and age-related diseases, cellular senescence is hypothesized to contribute to the age-related decline in reproductive function. However, some data suggest that senescent cells may also be important for normal physiological functions during pregnancy. Herein, we review the positive and negative effects of cellular senescence on female reproductive aging and pregnancy. We discuss how senescent cells accelerate female reproductive aging by promoting the decline in the number of ovarian follicles and increasing complications during pregnancy. We also describe how cellular senescence plays an important role in placental and fetal development as a beneficial process, ensuring proper homeostasis during pregnancy.

## Key Words

- ▶ ovarian
- ▶ uterine
- ▶ placental
- ▶ fetal aging
- ▶ senescent cells

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## Introduction

Cells have a limited capacity to replicate. Once this limit is reached, cells are no longer able to divide, even after treatment with growth stimuli. This limit is called the Hayflick limit or cellular senescence (Hayflick 1965). Once a significant amount of proliferating cells in a tissue undergoes cellular senescence, that tissue's ability to regenerate becomes reduced (Sousa-Victor *et al.* 2014). Because lack of regenerative potential is a hallmark of tissue aging, senescent cells are often associated with aging and age-related diseases (Herbig *et al.* 2006, Baker *et al.* 2016). Indeed, the number of senescent cells increases with age in multiple tissues (Dimri *et al.* 1995, Herbig *et al.* 2006, Ressler *et al.* 2006, Jeyapalan *et al.* 2007). Cellular senescence, which is defined as the inability of cells to replicate while remaining viable and functional, should

not be confused with organismal and tissue senescence, which are defined as gradual functional deterioration of the whole organism and tissue, respectively. To minimize confusion, we reserve the term 'cellular senescence' to refer to the process in which cells undergo irreversible growth arrest but remain viable, while we use the term 'aging' to refer to deterioration in organisms or tissues due to loss of function.

Excessive cellular stress, such as telomere shortening, oncogenic activation, nuclear DNA damage and mitochondrial dysfunction, can induce cellular senescence in multiple cell types (Allsopp *et al.* 1992, Di Micco *et al.* 2006, Kang *et al.* 2015, Wiley *et al.* 2016). Hallmarks of cellular senescence include persistent elevated expression of cell cycle inhibitors cyclin

dependent kinase inhibitor 1A (CDKN1A, also known as p21<sup>WAF1</sup>) and CDKN2A (also known as p16<sup>INK4A</sup>) p16<sup>INK4A</sup>, increased activity of a lysosomal enzyme termed senescence-associated  $\beta$ -galactosidase (SA- $\beta$ -gal), presence of nuclear DNA damage foci, presence of senescence-associated heterochromatin foci (SAHFs), increased cell size, loss of nuclear High Mobility Group Box 1 (HMGB1) and decreased expression of lamin B1 (Dimri *et al.* 1995, Freund *et al.* 2012, Davalos *et al.* 2013). Senescent cells are viable and will survive in culture, as opposed to apoptotic cells, which undergo programmed cell death (Childs *et al.* 2014). Senescent cells also express several proinflammatory cytokines, growth factors and matrix metalloproteinases, collectively termed senescence-associated secretory phenotype (SASP) (Coppé *et al.* 2008). The ability of senescent cells to elicit an SASP is thought to affect tissue homeostasis and promote age-related degenerative diseases and cancers (Laberge *et al.* 2015, Liu *et al.* 2015, Xu *et al.* 2015). This is further supported by a study showing that selectively eliminating senescent cells (using CDKN2A as a marker for cellular senescence) delays the onset of age-related pathologies, such as sarcopenia, cataracts and loss of subdermal adipose tissue in BubR1-insufficient mice, a mouse model of premature aging (Baker *et al.* 2011).

Cellular senescence, particularly that caused by telomere attrition, is hypothesized to contribute to the decline in reproductive function with age. Moreover, senescent cells are associated with pregnancy complications, such as preterm births. However, cellular senescence is also implicated in maintaining proper homeostasis during pregnancy, human parturition (Behnia *et al.* 2015) and fetal development (Muñoz-Espín *et al.* 2013, Storer *et al.* 2013), suggesting that senescent cells are beneficial during early development. Here, we present evidences that senescent cells can have positive and negative effects in female reproduction and aging, depending on context. This supports a role for antagonistic pleiotropy in cellular senescence.

## Cellular senescence and ovarian aging

Ovarian aging is the process of gradual deterioration in ovaries' ability to generate gametes capable of producing healthy, viable offspring. Ovarian aging involves continuous loss of ovarian follicles until the total number of follicles falls to an amount that results in subsequent progressive decline in female fertility. As a consequence, ovarian aging also leads to a decrease in

ovaries' ability to produce enough sex steroid hormones necessary for normal physiological functions in female adults (Richardson *et al.* 1987). This decline occurs because ovarian follicular cells (granulosa and theca cells) are important sources of sex steroid hormones in women (Tureck & Strauss 1982, Voutilainen *et al.* 1986). Hence, the continuous drop in the number of follicles with age results in hormone imbalance and ovarian cycle irregularity. During perimenopause, the deteriorating amount of ovarian follicles gives rise to decreased progesterone secretion, increased estrogen production and a higher estrogen-to-progesterone ratio (Santoro *et al.* 1996, Finch 2014). During menopause, the significant loss of ovarian follicles ultimately leads to reduced levels of estrogen and progesterone production, followed by the appearance of age-related health problems, such as bone loss and hot flashes (Gold *et al.* 2006, Finkelstein *et al.* 2008, Bromberger *et al.* 2010). Moreover, aged ovaries may also contain more poor-quality oocytes, resulting in embryos that undergo developmental arrest, develop aneuploidy, fail to implant and result in miscarriages (Romeu *et al.* 1987, Navot *et al.* 1991, Templeton *et al.* 1996, Nybo Andersen *et al.* 2000, Steuerwald *et al.* 2001, Pellestor *et al.* 2003). Poor oocyte quality with advanced age is also observed in rodents (Parkening & Soderwall 1974, 1975, Fujino *et al.* 1996, Simsek-Duran *et al.* 2013).

While the ovaries are considered aged by the time a woman reaches menopause at an average age of 51, the gradual deterioration process in ovarian function during aging is thought to begin early in life because the number of ovarian follicles starts to gradually decline prenatally and is not readily replaced (Wallace & Kelsey 2010), although there is a debate regarding the existence of active germ stem cells in postnatal human ovaries (White *et al.* 2012, Zhang *et al.* 2015). Nonetheless, ovarian follicles become significantly depleted with age due to continuous differentiation (i.e. maturation and ovulation) and apoptosis (i.e. follicular atresia) (McGee & Hsueh 2000). As a result, fertility in women progressively declines with age until women are no longer able to produce oocytes capable of fertilization. This decline in fertility occurs well before menopause (Dunson *et al.* 2004). Hence, it is believed that the size of the initial ovarian follicle reserves contributes to reproductive lifespan and the onset of hormone-dependent age-related diseases in women.

The role of senescent cells on aging of different tissues implicates that these cells may also promote premature aging of the ovaries. While direct evidence of this contribution to the female reproductive lifespan is limited, several studies on telomerase activity and

telomere length suggest that shortened telomeres, which can lead to replicative senescence, impair female reproduction. Indeed, oocytes from women who did not conceive vs those who conceived after *in vitro* fertilization show reduced telomere length (Keefe *et al.* 2007). Oocytes with shortened telomeres also produced fragmented embryos after fertilization (Keefe *et al.* 2005). In mice, telomerase deficiency decreases eggs' ability to fertilize with wild-type sperm, reduces fertilized eggs' ability to progress through the blastocyst stage and increases the incidence of fetal deaths, even though ovaries in telomerase-deficient mice can still produce follicles at different stages of follicular development (Lee *et al.* 1998, Liu *et al.* 2004). Because unfertilized oocytes (such as in humans and mice) have low telomerase activity (Wright *et al.* 1996, Liu *et al.* 2007), oocytes may be susceptible to telomere shortening and cellular senescence during aging.

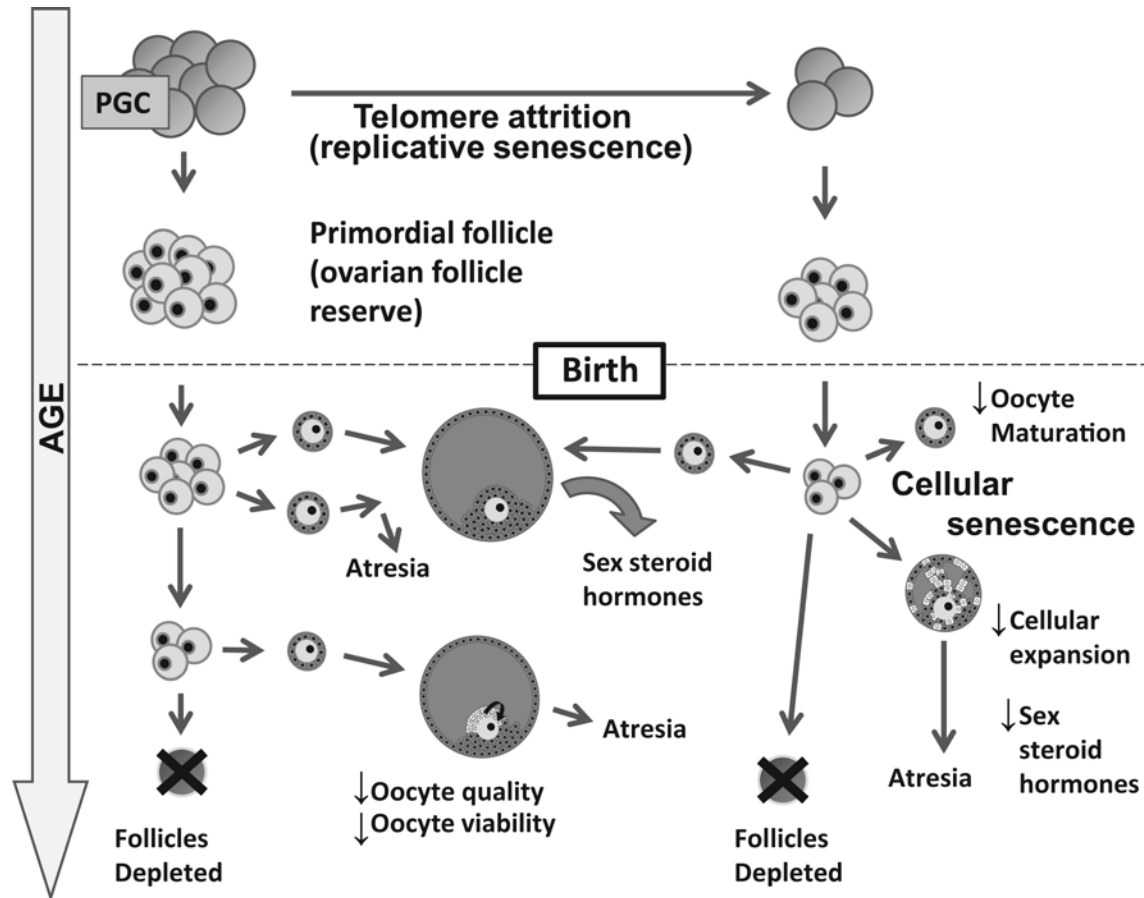
Excessive DNA double-strand breaks cause cells to undergo cellular senescence and/or apoptosis (d'Adda di Fagagna *et al.* 2003, Konishi *et al.* 2003). While the mechanism that determines how a cell is programmed to undergo cellular senescence versus apoptosis is not fully understood, it is evident that specific conditions will preferentially induce cellular senescence over apoptosis. For example, low dose of the DNA-damaging agent doxorubicin leads to cellular senescence, while high dose will induce apoptosis in rat cardiomyocytes (Spallarossa *et al.* 2009, Altieri *et al.* 2012). Low dose of H<sub>2</sub>O<sub>2</sub> also induces cellular senescence in human lung and skin fibroblasts, while high dose promotes apoptosis in these cells (Chen & Ames 1994, Chen *et al.* 2000). Some DNA-damaging agents, such as busulfan, preferentially cause human lung fibroblasts to undergo cellular senescence instead of apoptosis (Probin *et al.* 2006).

As opposed to apoptosis, cellular senescence allows cells to survive in culture, even in the presence of unrepaired DNA double-strand breaks (Chaturvedi *et al.* 1999, Marcotte *et al.* 2004, Sedelnikova *et al.* 2004). Formation of DNA double-strand breaks is part of meiotic recombination during oocyte development, but these DNA strand breaks are readily repaired by DNA repair machineries (Pittman *et al.* 1998, Baudat *et al.* 2000, 2013, Mahadevaiah *et al.* 2001, Di Giacomo *et al.* 2005). In both humans and mice, expression of DNA double-strand break repair genes, such as Breast Cancer 1 (*BRCA1*), Meiotic Recombination 11 (*MRE11*), *RAD51* recombinase (*RAD51*) and ataxia-telangiectasia mutated (*ATM*), decreases with age in oocytes and is accompanied by increasing DNA double-strand breaks (Titus *et al.* 2013). The role of DNA damage repair during

oocyte development is further demonstrated using wild-type and knockout mouse models. In the presence of excessive DNA double-strand breaks, oocytes undergo prolonged growth arrest (meiotic arrest) in culture, partly due to sustained activation of spindle assembly checkpoint (Lin *et al.* 2014, Collins *et al.* 2015). Impaired DNA double-strand break repair, such as that observed in oocytes with deficiencies in *BRCA1*, *MRE11*, *RAD51* and *ATM*, limits the number of mouse oocytes from progressing through meiosis I and prevents oocytes from maturing (Barlow *et al.* 1998, Titus *et al.* 2013).

Another potential mechanism whereby cellular senescence can accelerate ovarian aging is by limiting the proliferation of primordial germ cells (PGCs). Oocytes arise from PGCs that later develop into primordial follicles, which represent the initial ovarian reserve (Witschi 1948, Grive & Freiman 2015). Hence, cellular senescence in PGCs may result in a significant reduction in the number of primordial follicles formed during fetal development (Fig. 1). However, while this idea seems plausible, the impact of cellular senescence in establishing the initial ovarian reserve is still unknown. Furthermore, it is still unclear whether PGCs can undergo permanent cell cycle arrest because PGCs can transform into pluripotent embryonic germ (EG) cells and continue to proliferate in culture depending on the condition (Matsui *et al.* 1992, Koshimizu *et al.* 1996). Hence, future experiments are still needed to test whether cellular senescence in PGCs has an *in vivo* impact in limiting the size of the initial ovarian follicle reserve, resulting in reduced female reproductive lifespan.

While the role of replicative senescence in limiting germ cell numbers remains unclear, the impact of replicative senescence in promoting growth arrest of somatic cells is more evident. Cellular senescence is already observed in several types of somatic cells (Dimri *et al.* 1995, Noureddine *et al.* 2011, Bhat *et al.* 2012, Wu *et al.* 2013, Velarde *et al.* 2012, 2015, Demaria *et al.* 2014). Hence, it is highly likely for ovarian somatic cells (granulosa and theca cells) to also undergo cellular senescence. Short telomeres and low telomerase activity in granulosa cells are observed in women ( $\leq 37$  years old) with diminished ovarian reserve (Butts *et al.* 2009). In humans, shortened telomere length in cumulus cells, which are specialized granulosa cells, is associated with the development of poor embryos (Cheng *et al.* 2013). In aged mice, cumulus cells also facilitate apoptosis of the adjacent oocyte they enclose (Perez & Tilly 1997), partly by transferring ceramide from cumulus cells (Perez *et al.* 2005). Interestingly, ceramide is also increased in



**Figure 1**

Cellular senescence accelerates the decline in ovarian function. The number of initial primordial germ cells (PGCs) predetermines the size of the initial ovarian follicle reserve. Some of these primordial follicles further develop, but only one becomes dominant (in humans), while the remaining follicles undergo atresia. Maturation of the follicles ensures sufficient production of sex steroid hormones with each menstrual cycle. With age, oocyte quality and viability decrease partly due to impaired signaling in the oocytes and/or support cells. For example, cumulus cells may facilitate apoptosis of the oocyte that they enclose, as depicted by a small black arrow. The total number of ovarian follicles eventually becomes depleted, causing a woman to become infertile. Telomere attrition in PGCs, which leads to replicative senescence, reduces the size of the initial follicle reserve, resulting in a faster decline in ovarian follicle numbers and sex steroid hormone production. Cellular senescence in oocytes inhibits meiotic progression and oocyte maturation. Cellular senescence in support cells limits cellular expansion in these cells, causing failure in the ability of the growing follicles (including the dominant follicle) to fully mature. Inhibiting the ability of follicles to reach preovulatory stage will trigger atresia to immature follicles, resulting in decreased fertility per menstrual cycle, which also results in impaired sex steroid hormone production.

senescent fibroblasts (Venable *et al.* 1995). Ceramide inhibits DNA replication and induces expression of the senescence marker  $\beta$ -galactosidase (Mouton & Venable 2000). This suggests that increased ceramide production in cumulus cells during old age may be associated with cellular senescence in these cells.

Support cells surrounding the oocyte play important roles in egg development. During folliculogenesis, one or a few ovarian follicles become dominant and go through ovulation, while others fail to reach preovulatory stage, trigger apoptosis and succumb to atresia (Block 1951, Yuan & Giudice 1997). Because support cells are important for selecting follicles, which will either become

dominant or undergo atresia (Matsuda *et al.* 2012), lack of proliferation and cellular expansion in support cells due to replicative senescence may suppress the ability of growing follicles to advance to the preovulatory stage, making all of these immature follicles destined for apoptosis instead (Fig. 1). In humans, chemotherapy (doxorubicin), which induces DNA double-strand breaks and cellular senescence in normal cells, also promotes a majority of oocytes (34.7%) and granulosa cells (12.1%) to undergo apoptotic death, reminiscent of premature ovarian aging (Soleimani *et al.* 2011). In other species, such as bovine and rodents, decreased telomerase activity in granulosa cells is associated with an increased

rate of apoptosis and follicular atresia (Lavranos *et al.* 1999, Yamagata *et al.* 2002). Proteomic analysis of aged vs immature rat primordial follicles also shows decreased expression of proteins associated with DNA repair (Govindaraj & Rao 2015).

Granulosa and theca cells of the ovarian follicles are also important sources of estrogen and progesterone in female physiology. These cells orchestrate a series of enzymatic processes to produce estrogen and progesterone during the menstrual cycle. Granulosa cells synthesize estrogen through aromatization of theca-derived androgens, while luteinized follicular cells produce increased amounts of progesterone (Tureck & Strauss 1982, Voutilainen *et al.* 1986). Permanent growth arrest in granulosa and theca cells may inhibit ovarian follicles' ability to mature; as a result, immature follicles undergo atresia (Fig. 1). If this were to happen, we speculate that ovarian follicles may deplete faster due to continuous ovulation and follicular atresia, which causes the individual to reach menopause at an earlier age. Whether cellular senescence in support cells causes females to manifest distinct perimenopausal symptoms before menopause is not yet known. Nevertheless, an increased number of nondividing senescent follicular cells may lower female reproductive capacity and sex steroid hormone production and negatively impact normal female physiology.

### Cellular senescence and uterine aging

In contrast to the ample evidence of aging's negative effects in the ovaries, the impact of age on uterine function is less clear. Several studies on human embryo transfers show that the age of female recipients does not affect implantation rates (Serhal & Craft 1989, Navot *et al.* 1991, Sauer *et al.* 1992, Abdalla *et al.* 1997), suggesting that the uterus is able to support pregnancy even at older ages. However, fetal health conditions originating in the perinatal period, particularly stillbirths, are elevated with increasing maternal age (Fretts *et al.* 1995, Huang *et al.* 2008, Flenady *et al.* 2011). This correlation is also evident in mice and rabbits in which there is increased embryo mortality when blastocysts are transferred from young animals to the uteri of older recipients (Maurer & Foote 1971, Gosden & Fowler 1979). While the underlying cause for the age-dependent effect on fetal deaths is still unclear, it is hypothesized that impaired decidualization and uterine–embryo crosstalk are partly responsible for the perinatal mortality. Indeed, uteri of aged rodents showed impaired induction of a decidual response relative to

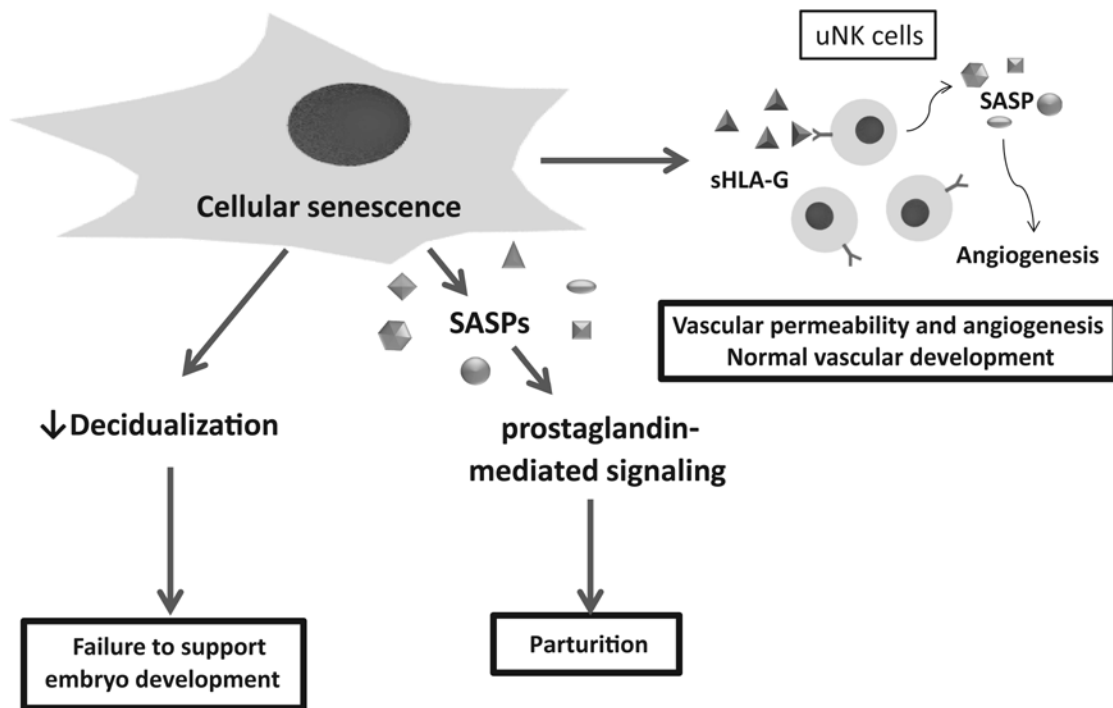
uteri of younger animals (Parkening & Soderwall 1973, Shapiro & Talbert 1974, Holinka & Finch 1977, Ohta 1987).

In addition to impaired decidualization, intra-partum complications, such as long duration of labor and elevated risk of operative vaginal birth and emergency Cesarean deliveries, also increase with age (Smith *et al.* 2008), which suggests that parturition is significantly compromised at older ages. Because the myometrium plays a direct role in uterine contractions, myometrial function may also be impaired with age. Indeed, myometrium from older women have reduced spontaneous activity and increased likelihood of multiphasic spontaneous myometrial contractions relative to younger women (Smith *et al.* 2008).

Senescent cells are observed in the uterus. A study on uterine fibroids reports that tissues from older patients ( $\geq 45$  years old) have increased cellular senescence relative to those from younger patients ( $< 45$  years old) (Laser *et al.* 2010). In mice, aged uteri are associated with the downregulation of several genes related to cell proliferation and stem cell renewal (Simmen *et al.* 2015), suggesting the presence of cells with impaired proliferation during uterine aging. The impact of cellular senescence on uterine function is further implied from studies on telomerase activity. In telomerase RNA (mTR) null mice, telomerase deficiency in the uterus impairs its ability to support wild-type embryos up to full term (Lee *et al.* 1998). These mice have reduced myometrial thickness, atrophic smooth muscle cells and slight reduction in uterine size (Lee *et al.* 1998). Hence, the presence of shortened telomeres, presumably leading to cellular senescence, may negatively affect uterine environment conducive for successful pregnancy.

Cellular senescence in the uterus is thought to decrease decidualization response and promote preterm births during pregnancy (Fig. 2). Indeed, cellular senescence in human endometrial stromal cells is accompanied by impaired decidualization as a consequence of inhibiting neddylation (Liao *et al.* 2015) or suppressing the cAMP mediator, Rap guanine nucleotide exchange factor 3 (RAPGEF3) (Kusama *et al.* 2014), resulting in a phenotype reminiscent of that observed with advanced age. In mice, persistently high levels of senescent cells in uteri by tissue-specific deletion of the tumor protein p53 (*Trp53*) gene in progesterone receptor-expressing cells also reduced decidual growth without altering the number of implantation sites (Hirota *et al.* 2010). Further proteomic analysis on these mice showed downregulated expression of a cluster of antioxidant enzymes in *Trp53*-deficient decidua (Burnum *et al.* 2012),





**Figure 2**

Cellular senescence accelerates the decline in uterine function. Senescent cells have been associated with impaired decidualization, which causes failure of the uterus to support embryo development. Senescent uterine cells also produce SASP, which activates prostaglandin-mediated signaling pathway and promote parturition. Premature activation of this pathway may be detrimental to pregnancy and can cause adverse pregnancy outcomes, such as spontaneous preterm birth and pPROM. In contrast to uterine cells, cellular senescence in uterine NK cells promotes vascular permeability and angiogenesis during pregnancy.

implicating the involvement of oxidative stress with increased presence of senescent cells in the uterus. Moreover, these tissue-specific *Trp53*-deficient mice have increased the incidence of preterm birth and neonatal deaths, partly due to the activation of a prostaglandin-mediated pathway (Hirota *et al.* 2010). Low doses of rapamycin, an inhibitor of mTORC1 signaling, can prevent preterm birth in these mice (Hirota *et al.* 2011). mTOR is an important positive regulator of the SASP, and rapamycin can inhibit expression of SASP factors (Lberge *et al.* 2015). Hence, we speculate that SASP produced by senescent cells in the uterus contributes to preterm birth and neonatal deaths, supporting the involvement of senescent cells in uterine function. However, while senescent cells are implicated in the uterus, the physiological and pathophysiological factors that promote and establish cellular senescence in the uterus during aging remain elusive. For example, it remains unknown whether reduced levels of sex steroid hormones during menopause can also promote cellular senescence in the uterus, in addition to creating a state of quiescence.

In contrast to uterine cells, a surprising beneficial role of senescent cells is found in uterine immune cells during pregnancy (Fig. 2). Natural killer (NK) cells are abundant in the uterus, where they are thought to play important roles for embryo implantation and pregnancy maintenance (Bulmer & Lash 2015). Several factors, such as soluble HLA-G (sHLA-G), a major histocompatibility complex molecule secreted by fetal trophoblasts during early pregnancy, can activate NK cells (Rajagopalan & Long 1999, Rajagopalan *et al.* 2006). Interestingly, sHLA-G, through its receptor KIR2DL4 (CD158d), can induce a DNA damage response, promote cellular senescence and trigger an SASP-like secretion in uterine NK (uNK) cells (Rajagopalan & Long 2012). This KIR2DL4-induced SASP-like expression in senescent uNK cells is capable of inducing vascular permeability and angiogenesis (Rajagopalan & Long 2012). Hence, cellular senescence in uNK cells may be a part of normal vascular development, which is necessary in establishing and maintaining healthy pregnancy. On the other hand, it remains unknown whether senescent uNK cells play a role in uterine function during aging.

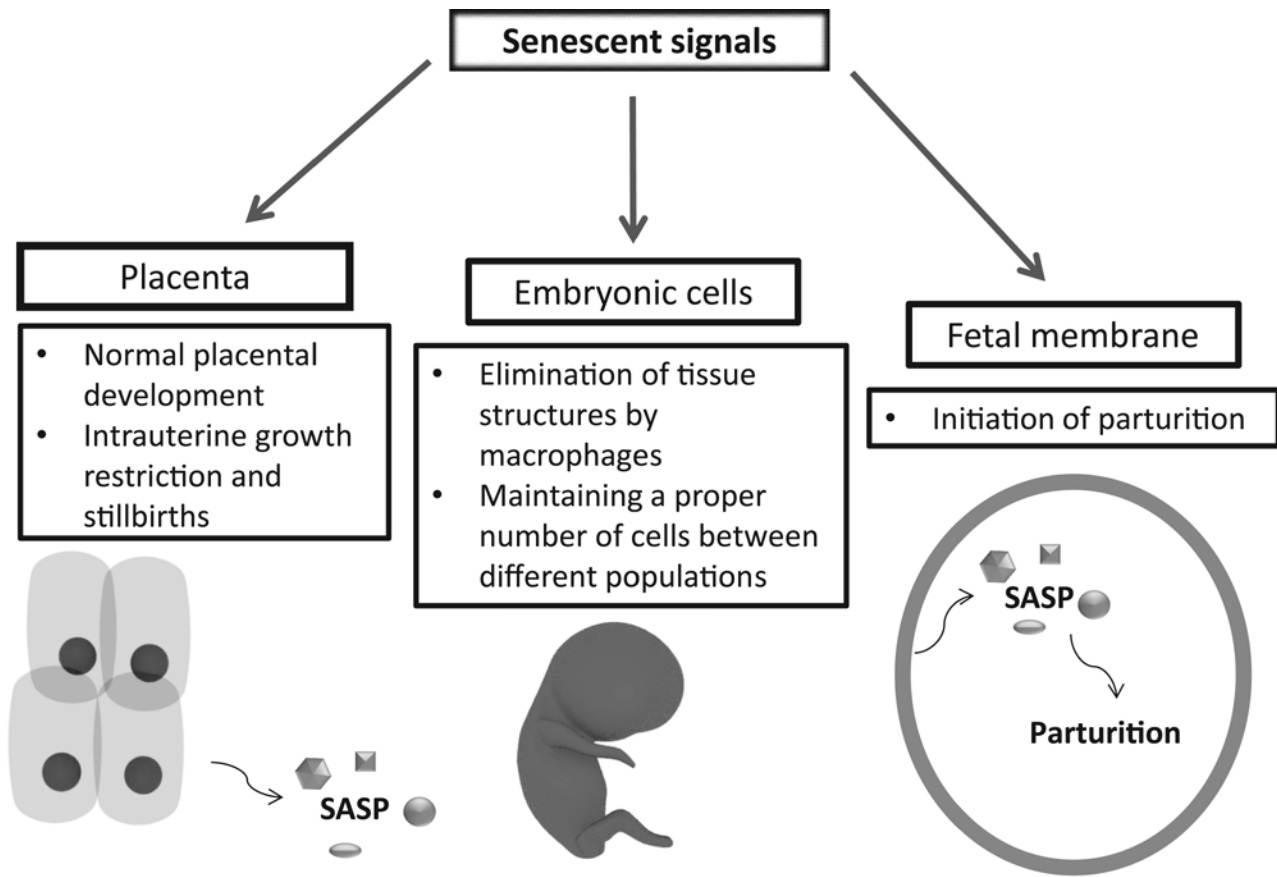
## Cellular senescence, placental aging and fetal membrane aging

Placental weight increases as the age of the mother increases (Haavaldsen *et al.* 2011). This increase also occurs in rats; the placental weight of older rats (9–12 months) is 40–70% higher than younger (3–5 months) animals (Rahima & Bruce 1987). However, whether this age-dependent change in placental size contributes to its function remains unclear. One possible hypothesis is that placental enlargement may compensate for accumulative cellular damage. Consistent with this idea, large placentas with low fetal birthweights are associated with increased risk of various adult-onset diseases, including cardiovascular diseases (Eriksson *et al.* 2011), suggesting that age-related placental topographic changes may preprogram the fetus for a shorter healthspan during adulthood. Unfortunately, studies on the relationship of age and placental function are limited. Some studies even suggest that age does not influence placental development, as reflected by a similar odds ratio of abnormal placentation in both older (>49 years old) and younger (<43 years old) women who underwent assisted reproductive technology (Kort *et al.* 2012). Hence, the impact of aging on placental function, as well as the contribution of placenta in aged individuals to adult health span of succeeding progenies, is an important avenue to study.

Another proposed concept is the idea that placentas undergo a transformation process during normal pregnancy, leading to tissue aging and cell death. Placental aging was thought to occur because placentas undergo considerable histological changes throughout normal gestation (Kosanke *et al.* 1993). Moreover, prolonged pregnancy (beyond 42nd week of gestation) was hypothesized to increase perinatal mortality as a consequence of a placenta reaching its aged state, termed placental insufficiency (Vorherr 1975). Extensive early placental calcification was proposed to be associated with adverse pregnancy outcome and was used as a clinical sign of placental aging (Tindall & Scott 1965, Hassler 1969). Previously, X-rays were used to identify increased placental calcification, which is associated with the term human pregnancies, corresponding to the accumulation of mineral deposits, especially around the vessels and in the allantois (Tindall & Scott 1965). In later years, observation of calcification in the basal and chorionic plates by a sonographic placental grading system was postulated as an indirect measurement of placental age and maturity (Grannum *et al.* 1979). Grannum's placental

grading system was extensively used by clinicians to predict complications in high-risk pregnancy (Kazzi *et al.* 1983, Patterson *et al.* 1983, Vosmar *et al.* 1989, Chitlange *et al.* 1990, McKenna *et al.* 2005, Chen *et al.* 2011, 2012). Placental calcification was used to reflect aging, as well as underlying placental dysfunction, especially when it occurred in earlier stages of pregnancy (Chen *et al.* 2011). However, heterogeneities in diagnosing adverse pregnancy outcomes based on Grannum's criteria created more controversy rather than providing a strategy to detect placental-associated pathologies and adverse pregnancy outcomes. Moreover, there is no evidence that aging causes placental calcification. Hence, placental aging may not be an accurate terminology, because a full-term placenta does not necessarily have reduced tissue function, impaired homeostasis and decreased tissue repair (Boyd 1984, Jackson *et al.* 1992). A systematic review (Poletini *et al.* 2015a) reports that although placental aging has been mentioned in the literature since the early 1970s as a natural physiologic process depicting the life of placenta, there are no reports on mechanistic progression of placental aging, which is partly attributed to impracticality of longitudinal sampling of human placenta. As a result, examinations of placental specimens are always confounded with multiple unknown factors. Hence, the cause–effect relationship is hard to interpret.

Cellular senescence is implicated in normal placental development (Fig. 3). Decreased telomerase activity, which can lead to replicative senescence, seems to be associated with placental maturation (Kyo *et al.* 1997). Moreover, markers of cellular senescence are observed in human syncytiotrophoblasts after fusion of cytotrophoblasts (Chuprin *et al.* 2013). After fusion, these senescent cells also secrete SASP components, such as interleukin 6 (IL6), IL8, chemokine C-X-C motif ligand 1 (CXCL1) and chemokine C-C motif ligand 5 (CCL5) (Chuprin *et al.* 2013). Because senescent cells are irreversibly growth-arrested, induction of cellular senescence in syncytiotrophoblasts is hypothesized to maintain cell cycle arrest and support cell viability in the postmitotic syncytium. However, it is still unclear whether senescent placental cells are overall beneficial or detrimental to pregnancy. One study links telomere shortening and increased cellular senescence in placenta to intrauterine growth restriction (Davy *et al.* 2009), a condition which increases infant mortality and adult morbidity, such as cardiovascular disease, obesity and diabetes (Hales *et al.* 1991, Barker *et al.* 1993, Osmond *et al.* 1993, McCance *et al.* 1994, Valdez *et al.* 1994). Hence, the role of senescent placental cells in maintaining pregnancy needs further

**Figure 3**

Cellular senescence maintains proper homeostasis during pregnancy by ensuring normal placental, fetal and fetal membrane development. Cellular senescence contributes to the complex interplay of chemical signals during pregnancy. In the placenta, cellular senescence ensures normal placental development, but may also be associated with intrauterine growth restriction and stillbirth. In the embryo, cellular senescence facilitates elimination of tissue structures by macrophages and maintains a proper number of cells between different populations. In the fetal membrane, senescent cells initiate parturition.

investigation. Nonetheless, these reports provide evidence for the presence of senescent cells in the placenta.

The placenta grows along with the fetus throughout pregnancy, especially in the first half, to support growth and development of the fetus. Placental (trophoblast) cells divide and become highly invasive by 12th week of gestation (Caniggia *et al.* 2000). During pregnancy, amnion and chorion develop independently until week 14–15, fuse around 15–16 weeks, and form the intrauterine cavity surrounding the fetus. Fetal membranes are an independent entity between the mother and the fetus with unique function that is different than the placenta and should not be considered as an extension of the placenta. They provide unique functions to the growing fetus and for the maintenance of pregnancy. Like placenta, fetal membranes act as mechanical and immune barriers and protect the fetus against infectious agents and noxious environmental insults (Kjaergaard *et al.* 1999).

Membranes also regulate several endocrine signals (Li & Challis 2005, Menon *et al.* 2008, Kumar *et al.* 2015, Meng *et al.* 2016). Although fetal membranes and the placenta are both fetal in origin, are attached to one another and develop together with the fetus, placenta and fetal membranes have distinct cellularity and physiology. Placentas are highly vascularized and are the major supply route for the growing fetus, while fetal membranes are avascular and surround the fetus to serve as the main mechanical barrier (Pijnenborg *et al.* 1980, Meekins *et al.* 1997, König *et al.* 2012). The endocrine and uterotonic functions played by placenta and fetal membranes and the biochemical markers that they produce are also distinct (Kacerovsky *et al.* 2014, Menon *et al.* 2015). Moreover, while the placenta retains the functional capacity to supply materials to the fetus and ensures fetal survival until it is delivered, fetal membranes are readily ruptured before parturition (El Khwad *et al.* 2005, 2006, Pandey *et al.* 2007,



Strohl *et al.* 2010). Hence, fetal membranes are anatomically and functionally distinct from placenta.

Telomere length reduction in preterm fetal membranes suggests that preterm birth has similar features to premature aging and age-related pathologies (Menon *et al.* 2012). Consistent with this idea, cellular senescence in fetal membranes is implicated in pregnancy complications, such as preterm premature rupture of the membranes (pPROM) (Menon *et al.* 2014a). In relation to pregnancies with preterm birth without rupture of membranes (PTB with no ROM), pPROM pregnancies also have elevated amniotic fluid (AF) inflammatory markers (Athayde *et al.* 1998, 1999), high salivary collagenolytic activity (a surrogate for lower uterine segment activity) (Menon *et al.* 2006), elevated AF F2-Isoprostane concentrations (a marker for oxidative stress) (Menon *et al.* 2011a), shortened fetal leukocyte and placental DNA telomere length (a marker of cellular senescence) (Menon *et al.* 2012), and distinct single nucleotide polymorphisms (SNPs) associated with common inflammatory pathway genes (Wang *et al.* 2006, Romero *et al.* 2010a,b). Hence, cellular senescence in fetal membranes is suggested to promote pregnancy complications. Indeed, increasing the number of senescent cells in fetal membranes, such as those induced by cigarette smoke extract, is thought to cause PTB (Menon *et al.* 2013).

Human fetal membrane cells are capable of proliferating, but they enter a cellular senescence, growth arrest phase, before normal labor (Behnia *et al.* 2015). In mouse fetal membranes, senescent cells also progressively increase throughout gestation and peaks at term (Bonney *et al.* 2016), suggesting that cellular senescence in human and mouse fetal membranes may play a role in initiating parturition (Fig. 3). Oxidative stress peaks at term and is thought to accelerate senescence of human fetal membranes (Menon *et al.* 2014a). The third trimester of pregnancy is a state of hypoxic environment, but metabolism increases as fetal maturity is completed, enhancing the release of reactive oxygen species (ROS) (Diamant *et al.* 1980, Many & Roberts 1997). DNA bases, especially guanine nucleotides, are vulnerable targets for ROS (Kasai & Nishimura 1986, Kasai *et al.* 1986). Increased oxidative stress-induced DNA damage, predominated by accumulation of oxidized guanine bases (8-oxoguanine), in fetal membranes causes guanine-rich telomere attrition and activation of cellular senescence (Menon *et al.* 2014b). This reduction in telomeres and lack of base excision repair of guanine bases are correlated with reduced expression of the base excision repair gene 8-oxoguanine DNA glycosylase (OGG1) in human fetal membranes

(Menon *et al.* 2014b). Massive influx of oxidative stress before initiation of labor creates irreversible damage to intracellular compartments (Cindrova-Davies *et al.* 2007, Khan *et al.* 2010). Oxidative stress-induced damage in fetal membrane cells may promote cellular senescence and SASP secretion. Hence, DNA damage-induced inflammation and SASP factors secreted from senescent fetal membranes are suggested to promote signals of parturition at term (Behnia *et al.* 2015).

In addition, a gradual decrease in telomere length is also thought to promote the senescent phenotype in fetal membranes, as indicated by morphologic (enlargement of organelles) and biochemical alterations (activation of MAPK14, increase in SA- $\beta$ -gal activity and development of the SASP) (Menon *et al.* 2014a). This decrease in telomere length can be considered as a sign of replicative senescence, eventually resulting in the secretion of SASP factors, which are thought to initiate parturition during normal labor (Behnia *et al.* 2015). Moreover, telomere mimetics (TTAGGG<sub>(2)</sub> repeat sequences), which induce cellular senescence in multiple cancer cells (Klungland *et al.* 1999, Yaar *et al.* 2007, Sarkar & Faller 2011, Rankin *et al.* 2012, Pitman *et al.* 2013, Puri *et al.* 2014), can also cause amnion cell oxidative stress, MAPK14 activation and cellular senescence in fetal membranes (Polettini *et al.* 2015b). Telomere fragments also increased the expression of SASP factors IL6 and IL8 from amnion epithelial cells, suggesting a mechanism of sterile inflammation in the intrauterine cavity. These results were further supported by *in vivo* experiments, wherein injection of T-oligos into the uteri of pregnant CD1 mice on day 14 of gestation led to increased oxidative stress in the amniotic sac and placenta, increased MAPK14 activation and SA- $\beta$ -gal activity in murine amniotic sac and higher IL8 levels in amniotic fluid compared with saline-treated controls (Polettini *et al.* 2015b). These data indicate that telomere shortening can promote cellular senescence in fetal membranes and trigger an inflammatory cytokine signature to activate uterotonins and promote parturition at term, which is consistent with the idea that telomere shortening in fetal membranes can act as a biological clock to facilitate proper timing of parturition in humans and mice (Bonney *et al.* 2016, Menon *et al.* 2016).

Fetal hypothalamic–pituitary–adrenal (HPA) axis during parturition is well documented, especially in animal models (e.g. sheep). Corticotropin-releasing hormone (CRH) as a biomarker of term and preterm parturition is also reported in humans (Menon *et al.* 2011b). In animal models, parturition is either fetal-driven (e.g. fetal-HPA and other fetal-derived endocrine factors) and/or

maternal-driven (e.g. maternal-dependent progesterone withdrawal). However, in humans, the exact initiator of parturition is still unclear. Endocrine signals like CRH alone are insufficient to produce changes in myometrium. Hence, we propose that premature telomere shortening in human fetal membranes may also trigger preterm labor or premature rupture of the membranes (Menon *et al.* 2014a, Dutta *et al.* 2016), providing an additional mechanism to induce parturition in humans.

## Cellular senescence and fetal development

The presence of senescent cells in normal developing placenta and fetal membranes suggests that cellular senescence has important functions during pregnancy. In mice, the positive contribution of senescent cells is also observed during normal embryogenesis. During embryo development (E9.5–E14.5), senescent cells are temporally present in the mouse endolymphatic sacs near the ear canals, the posterior cranium that corresponds to the closing neural tube, the otic vesicle, the brain vesicles, the fusing sternum midline, the gut endoderm, the apical ectodermal ridge (AER) of the limbs, the bottom of the regressing interdigital webs, the vibrissae area below the eye, the mesonephric tubules and the vertebral osteoblasts and chondroblasts of the cervical region (Muñoz-Espín *et al.* 2013, Storer *et al.* 2013). While the function of senescent cells in these fetal tissues remains largely unclear, the importance of senescent cells in the fetus has been implicated in normal embryo development.

During sexual differentiation in females, lack of androgen and Müllerian inhibiting substance results in the regression of the Wolffian duct (mesonephric tubules) and the development of the Müllerian duct, respectively (Behringer *et al.* 1994, Welsh *et al.* 2009). In addition, senescent cells in the mesonephric tubules are also thought to play a role in ensuring a properly timed regression of the tubules during fetal development through the clearance of senescent cells by infiltrating macrophages (Muñoz-Espín *et al.* 2013), which results in a widespread apoptosis as a means to compensate for the delayed tubule regression. However, despite this compensatory mechanism, absence of cellular senescence in mesonephric tubules of *Cdkn1a*-null mice during female development is associated with the increased incidence of dorsoventral vaginal septum (Muñoz-Espín *et al.* 2013), a congenital condition where the vagina is divided. This association suggests that delayed involution of the mesonephric tubules may somehow cause improper

fusion of the lower parts of the Müllerian ducts with the urogenital sinus, resulting in the formation of vaginal septum. This increased incidence of septate vagina in *Cdkn1a*-null mice also leads to reduced litter size (Muñoz-Espín *et al.* 2013).

Aside from regression of mesonephric tubules, senescent cells are also implicated in eliminating interdigital cells of the AER. Senescent cells in AER are associated with increased apoptosis and macrophage infiltration (Storer *et al.* 2013). Absence of senescent cells in AER by inhibiting phosphorylation of ERK or by inhibition of CDKN1A signaling results in patterning defect of the limbs (Storer *et al.* 2013).

In addition to the role of senescent cells in eliminating tissue structures through a macrophage-mediated process, senescent cells also maintain a proper distribution of cell population during mouse fetal development (Fig. 3). Cellular senescence in the endolymphatic sac is thought to promote growth arrest in the majority of cells present at E14.5 to allow colonization of a small number of pendrin-positive cells (Muñoz-Espín *et al.* 2013). Absence of cellular senescence triggers apoptosis and macrophage infiltration to correct the imbalance in cell population during mouse fetal development (Muñoz-Espín *et al.* 2013). Because this mechanism seems to completely compensate for the lack of cellular senescence response, the significance of cellular senescence in endolymphatic sac needs further investigation.

It is important to note that cellular senescence during mouse fetal development is different from senescent fibroblasts induced by DNA damage, repetitive culture, oncogene activation or tumor suppressor activation. Cellular senescence in mouse embryogenesis is dependent on CDKN1A, but this activation is independent of TRP53, because TRP53 expression is low in fetal tissues and *Trp53*-null mice show similar presence of senescent cells (Muñoz-Espín *et al.* 2013, Storer *et al.* 2013). This is in contrast to the requirement of TRP53 in senescent cells of other somatic tissues, including placenta (Chuprin *et al.* 2013). Senescent cells during mouse fetal development do not have increased DNA damage and do not express high levels of CDKN2A, but instead have increased expression of other cell cycle inhibitors, CDKN1B (also known as p27) and CDKN2B (also known as p15) (Muñoz-Espín *et al.* 2013, Storer *et al.* 2013).

While senescent cells during mouse fetal development differ in their gene expression profile compared with the classic view of cellular senescence, these cells still share a set of commonly regulated genes that are involved in TGFB/SMAD, PI3K/FOXO and SASP regulation

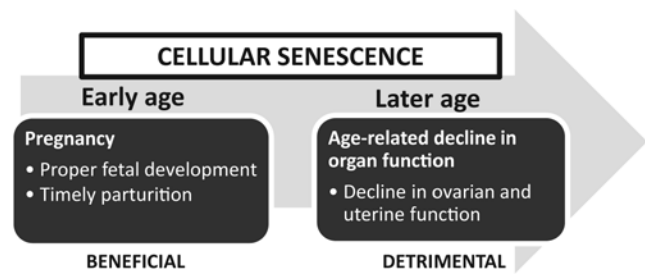
(Muñoz-Espín *et al.* 2013, Storer *et al.* 2013). Activation of TGF $\beta$  signaling, by upregulating SMAD phosphorylation and *Cdkn1a* gene expression, is associated with increased cellular senescence, while inhibition of PI3K and reduced phosphorylation of FOXO1/3, which is a downstream target of PI3K, are correlated with increased cellular senescence in mesonephric tubules and endolymphatic sac during fetal development (Muñoz-Espín *et al.* 2013). Genes associated in the regulation of cellular senescence and the SASP, such as CCAAT/enhancer-binding protein beta (*Cebpb*), insulin-like growth factor-binding protein 5 (*Igfbp5*), wingless-type MMTV integration site family, member 5A (*Wnt5a*), colony-stimulating factor 1 (*Csf1*) and *Cd44*, are comparably increased in developmentally senescent AER and oncogene-induced senescent fibroblast (Storer *et al.* 2013).

The role of cellular senescence in fetal development is observed in mouse models. However, there is still no evidence indicating the existence of senescent cells during human embryogenesis. Studies are needed to determine whether cellular senescence also plays a role in human fetal development or whether this phenomenon is restricted to mice.

### Antagonistic pleiotropic roles of senescent cells

Senescent cells have positive and negative effects on reproduction. They contribute to female reproductive aging by accelerating the decline in ovarian and uterine function. In the ovaries, senescent cells limit the number of ovarian follicle reserves, decrease the proliferation of granulosa and theca cells and diminish the amount of sex steroid hormone production. In the uterus, senescent cells reduce decidual growth and promote preterm labor. However, senescent cells also maintain proper homeostasis during pregnancy by ensuring normal placental and fetal development. In the placenta, senescent cells are present in syncytiotrophoblasts where they are thought to maintain cell cycle arrest and support cell viability in the postmitotic syncytium. In the fetus, senescent cells facilitate in eliminating tissue structures and in limiting overpopulation of specific cell types. Hence, the positive and negative impact of senescent cells on female reproduction depends on context.

The pleiotropic contributions of senescent cells to reproduction and pregnancy seem very contradictory. Why would nature retain cellular senescence if this process is also deleterious to the organism's ability to



**Figure 4**

Pleiotropic contributions of senescent cells during aging. Beneficial effects of cellular senescence manifest early in life to facilitate proper fetal development and timely parturition. However, detrimental effects of cellular senescence later in life appear to accelerate the decline in ovarian and uterine function.

reproduce? One possible hypothesis is that the positive effects of senescent cells may outweigh the negative effects. The beneficial roles of senescent cells in ensuring proper homeostasis during pregnancy may be more important than the detrimental effects of these cells on ovarian and uterine aging. Genes controlling the process of cellular senescence may have been selected for during evolution because their positive effects manifest early in life to facilitate proper fetal development and timely parturition, while its negative effects only become apparent later in life when the function of the ovaries and the uterus begins to decline (Fig. 4). This is consistent with the concept of antagonistic pleiotropy theory of aging, originally proposed by George Williams (1957). This theory stipulates that one gene can be both beneficial and detrimental to an organism's fitness, and that this fitness tradeoff is overall beneficial for the species' survival. Current data on senescent cells suggest that antagonistic pleiotropy may also apply to genes involved in cellular senescence. Further investigations on the effects of cellular senescence on female reproductive aging and pregnancy can help address this hypothesis.

### Conclusion

Cellular senescence may be both beneficial and detrimental to reproduction. On one hand, cellular senescence contributes to the progressive decline in female reproductive capacity by promoting a decrease in the number of ovarian follicles and increasing complications during pregnancy. On the other hand, cellular senescence may be important for proper homeostasis during pregnancy by regulating normal placental and

fetal development. The pleiotropic contribution of senescent cells to female reproductive function and life span implicates the importance of understanding the complexity of cellular senescence in specific context of reproduction. It will be helpful to further investigate the impact of senescent cells on reproduction using mouse models that allow specific elimination of senescent cells *in vivo* (Baker *et al.* 2011, Demaria *et al.* 2014).

#### Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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#### Author contribution statement

R M and M C V wrote the review.

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