REVIEW ARTICLE

A review of the Evidence for Placental Ageing in Prolonged Pregnancy.

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Abstract

Prolonged pregnancy describes a pregnancy that progresses beyond 42 weeks (294 days). The aetiology of prolonged pregnancy is incompletely understood, although factors such as advanced maternal age and obesity increase the risk of prolonged pregnancy. Prolonged pregnancy is associated with an increased incidence of perinatal mortality; in particular, the incidence of stillbirth increases from 39 weeks onwards, with a significant increase beyond 41 weeks' gestation. The biological explanation for this has yet to be confirmed. Placental ageing has been proposed as a possible mechanism. As the placenta is responsible for the nutrient and energy demands of the fetus, which are considerable in late pregnancy, a decrease in its ability to function may provide an explanation for the increasing perinatal mortality rate seen in prolonged pregnancy.

Here we review evidence for ageing processes occurring in the placenta. A number of biomarkers of ageing are seen within the placenta as gestation progresses. These include evidence of increased apoptosis, senescence, autophagy, and oxidative stress in trophoblast, the primary functional cell in the placenta, in prolonged pregnancy. As these processes play a key role in the ageing process of other tissues, the accumulation of these markers here is consistent with placental ageing. In addition, there are morphological changes in trophoblast in prolonged pregnancy which suggest impaired mitochondrial and placental function. The findings summarised in this review illustrate the growing evidence of both structural and biochemical features of ageing shown in placentas of prolonged pregnancy, providing insight into underlying mechanisms which may initially be adaptation to in utero stress, but later develop to become pathological. Future work is needed to determine whether these changes impact upon placental function and whether placental biomarkers could be used as a surveillance tool in prolonged pregnancy.

Keywords: prolonged pregnancy, ageing, placenta, oxidative stress, autophagy, senescence, apoptosis.

1. Introduction

Prolonged pregnancy is defined as a pregnancy that progresses beyond 42^{+0} weeks or 294 days [1–4]. The association between maternal, fetal and neonatal complications and prolonged pregnancy has been underestimated due to a lack of biological understanding[4]. Prolonged pregnancy is relatively uncommon, it varies in incidence between 0.4%-7%, and its cause is unknown[5]. Factors which increase the likelihood of progression of gestation beyond 42 weeks include: obesity, previous prolonged pregnancy, a male fetus and genetic dispositions[6].

Prolonged pregnancies are associated with an increased incidence of pregnancy complications, particularly perinatal mortality, which includes stillbirths and early neonatal deaths. A stillbirth is defined as a

baby delivered with no signs of life irrespective of when the death occurred[4]; the threshold at which a stillbirth is defined varies between countries. In the UK, stillbirths are defined as occurring after 24 weeks' gestation. In 2017, there were 3.74 stillbirths per 1,000 births in the UK[4]. Despite this value decreasing each year, there is an increased risk of a stillbirth occurring as gestation increases after the estimated date of delivery[4]. A meta-analysis of 15 million pregnancies found that the overall prospective risk of stillbirth increased with gestational age after 39 weeks' gestation[7]. At 39 weeks' gestation, the incidence of stillbirth was 0.42 per 1,000 births which increased to 3.18 per 1,000 births at 42 weeks' gestation[4]. In the UK, early neonatal death is defined as a live-born baby at 20^{+0} weeks or later or with a birth weight of ≥400g who died before 7 completed days after birth[4]. In 2017, there were 1.12 early neonatal deaths per 1,000 live births over 24 weeks' gestation in the UK[4]. Similar to stillbirths, the risk of early neonatal death occurring as gestation progresses increases by 5% from 0.65 per 1,000 live births at 37-41 weeks to 0.68 per 1,000 live births at 42 weeks[4].

1.1 Prolonged pregnancy and obesity

As the worldwide prevalence of obesity is increasing, many studies have investigated the association between obesity and increased risks of adverse outcomes for both mother and infant[8,9]. The risk of prolonged pregnancy is increased in obese women compared to women of normal weight. Halloran et al. found that women classed as obese (Body Mass Index (BMI) ≥30 kg/m^2) before pregnancy have an adjusted odds ratio of a 42-week pregnancy of 1.28 (95% confidence interval 1.17, 1.41) compared to women of healthy weight (BMI 18.5–24.9 kg/m^2)[10]. Additionally, Stotland et al. demonstrated women with BMI >29 kg/m^2 had a 7.2% higher chance of a pregnancy progressing to a 42-week gestation compared to 5.4% in women of normal weight[11]. Denison et al. reported that a higher maternal BMI recorded in the first trimester is associated with longer gestation; this study also found women with a high BMI had an increased risk of pregnancy complications, including stillbirth[12]. For women with a BMI >35 kg/m^2, the odds ratio for stillbirth was 3.90 (95% confidence interval (CI) 2.44–6.22) compared to women with a BMI between 20 and 25[12].

The mechanisms linking maternal obesity and prolonged pregnancy are not fully understood. As the onset of labour involves hormonal, inflammatory, and metabolic interactions between the mother, fetus and placenta, disruption of these processes could prevent initiation of labour. For example, adipokines such as TNF-alpha (TNF-α) and interleukin (IL) 6 increase and adiponectin decreases in maternal obesity, altering the balance of pro- and anti-inflammatory cytokines[13]. Another study demonstrates reduced myometrial contractility in women with diabetes (both type 1 and gestational diabetes) compared to women without diabetes[14]. These changes are similar to the reduced myometrial contractility seen in prolonged pregnancy[15]. As obesity and diabetes are closely related it is possible that further exploration of myometrial contractility could provide additional evidence for causal mechanisms of why prolonged pregnancy is seen in maternal obesity[16].

1.2 Prolonged pregnancy in advanced maternal age

Advanced maternal age (AMA), defined as a woman entering pregnancy who is ≥35 years of age, has become increasingly frequent; between 1980 and 2018 there was a 2.1% increase in births to women ≥35 years of age[17]. AMA is related to adverse pregnancy outcomes such as fetal growth restriction (FGR) and stillbirth which are associated with placental dysfunction[18]. It has been proposed that AMA is a continuing risk factor for perinatal mortality[18]. Lean et al. found that the AMA population had an increased risk of stillbirth (Odds ratio (OR) 1.75; 95%CI 1.62 to 1.89)[18]. In addition, Reddy et al. found that women over the age of 40 had the same rate of stillbirth at 39 weeks' gestation as women aged <35 years of age had at 42 weeks' gestation. At 42 weeks', women over the age of 40 were 3.3 times more likely to have a stillbirth than women <35 years of age (95% CI 2.2-4.9)[19]. This effect of maternal age persisted despite accounting for maternal co-morbidities, in combination with experimental data this suggests that placental dysfunction could mediate adverse pregnancy outcome in AMA[18,19].

1.3 Current management of prolonged pregnancy

As prolonged pregnancy is associated with significantly increased fetal and neonatal mortality and morbidity, as well as maternal morbidity, pregnancy interventions to prevent post-term pregnancies are reviewed[20–23]. The most common of these is the induction of labour at or beyond term (usually at 41-42 weeks' gestation). A meta-analysis of 30 randomised controlled trials of 12,479 women with low-risk pregnancies found that a policy of induction of labour, at or beyond term, was associated with fewer perinatal deaths (risk ratio (RR) 0.33, 95% confidence interval (CI) 0.14 to 0.78) stillbirths (RR 0.33, 95% CI 0.11 to 0.96), caesarean sections (RR 0.92, 95% CI 0.85 to 0.99), admissions to the neonatal intensive care unit (RR 0.88, 95% CI 0.77 to 1.01) and Apgar scores <7 at five minutes (RR 0.70, 95% CI 0.50 to 0.98) compared with expectant management[24]. However, the number of inductions needed to prevent one perinatal death was 426 women. In addition, there are less invasive interventions that are recommended to encourage spontaneous onset of labour[6]. One of these is membrane sweeping, which has the aim of increasing local production of prostaglandins and thus initiating labour[6]. However, there is inconsistent evidence that this reduces the incidence of caesarean section or maternal or neonatal complications[25,26].

Another common intervention for prolonged pregnancy is antepartum fetal surveillance. This has become common practice in women who reach 42 weeks' gestation and opt to continue with their pregnancy, due to ethical and medico-legal considerations[27]. The assessment of amniotic fluid volume appears to be an important antepartum fetal surveillance tool, as oligohydramnios can indicate fetal compromise[28]. Adverse pregnancy outcomes such as admission to the neonatal unit are more common when oligohydramnios is present[29]. Oligohydramnios could be the consequence of feto-placental insufficiency and can also pre-dispose to umbilical cord compression, which can lead to fetal hypoxemia or meconium aspiration[29]. Thus, frequent amniotic fluid screening in post-term pregnancies is suggested[30]. However, there is no evidence to suggest that additional

antenatal surveillance decreases perinatal mortality[31].

Despite the risks of prolonged pregnancy being established, there is little biological understanding behind the cause(s) of perinatal mortality in prolonged pregnancies. In addition, investigations for prolonged pregnancy are unable to predict the risk of perinatal mortality. A better understanding of the underpinning biology of prolonged pregnancy could improve the development of tests to identify fetal or placental compromise allowing focussed intervention in women most likely to benefit.

One potential explanation for the increased perinatal mortality and morbidity in prolonged pregnancy is placental ageing and the consequences of this for meeting the nutrient or energy demands of the fetus, which are considerable in late pregnancy[32]. However, little of this ageing theory has been explored. In this review, we focus on structural and biochemical markers of ageing, to place this in context we briefly describe placental development at different stages of pregnancy. We then review the structural and biochemical markers of ageing found in the placenta and the evidence that links placental ageing to placental pathology and pregnancy complications.

2. Placental structure

The placenta is a specialised organ that changes during gestation to support the growth of the fetus[33]. The placenta sits at the interface between mother and baby, it is primarily a fetal organ, although its structures comprise fetally and maternally derived tissue and cells. In a mature placenta, the fetal facing aspect is known as the chorionic plate, which contains the fetal blood vessels which branch from the umbilical vessels[33]. The maternal aspect of a mature placenta is the basal plate which is in direct contact with the maternal decidua. In between these two regions is intervillous space which contains the main functional units of the placenta; branched villous structures[33]. In the first trimester, the villi begin as stem villi which undergo sequential branching that continues throughout pregnancy to form terminal villi at around 20 weeks' gestation[34]. These villi are regarded as the functional units of the placenta and their highly branched morphology provides a large surface area to maximise transfer between the maternal and fetal circulations[35].

The placental villi are covered with a multinucleated syncytium. Initially, there are four different layers between maternal and fetal circulations: the maternal facing syncytiotrophoblast, a layer of cytotrophoblast cells, connective tissue of the villus and the endothelium lining the fetal capillaries (Figure 1)[33]. The syncytiotrophoblast is maintained by proliferation, differentiation, and fusion of underlying villous cytotrophoblast; nuclei within the syncytiotrophoblast then exhibit signs of chromatin condensation and are gathered in syncytial nuclear aggregates. As pregnancy progresses, these layers change due to changing in-utero environment. Initially, the placenta develops in a hypoxic environment[23], but towards the end of the first-trimester maternal blood begins to flow from maternal spiral arteries into the intervillous space[36]. During the second trimester at around 20 weeks' gestation, the cytotrophoblast layer reduces and becomes discontinuous[37]. Subsequently, in the majority of the chorionic villi, three layers remain but some areas become extremely thin (termed vasculosyncytial membranes) allowing the syncytiotrophoblast to come

much closer to the fetal endothelium, such as demonstrated in Figure 1[33,38].

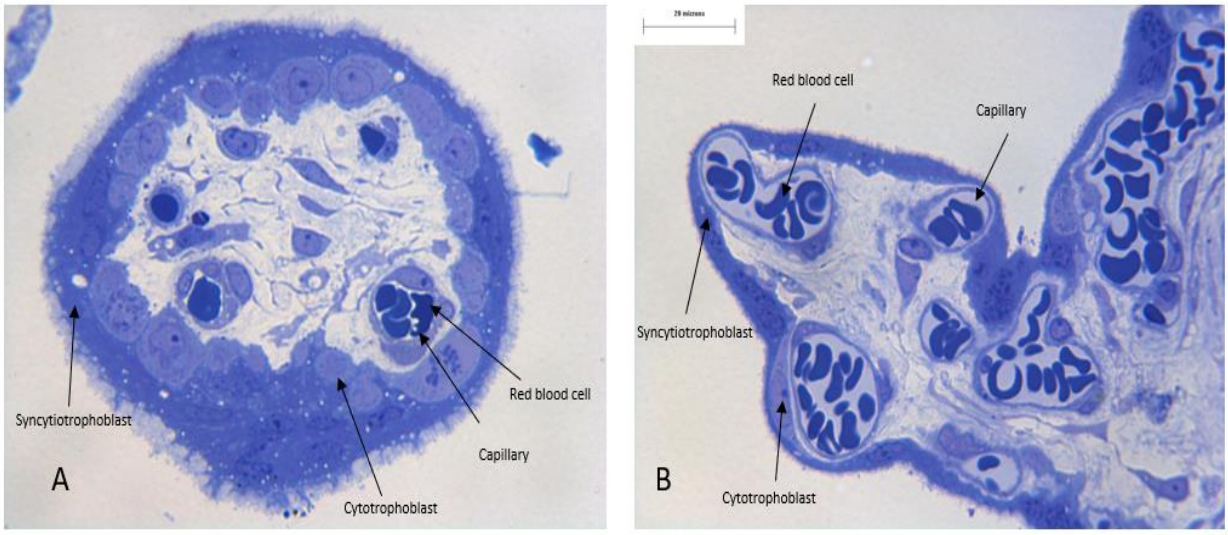

Figure 1: Transmission electron microscopy of placental villous structure stained with toluidine blue. *A) First trimester villus, B) Third trimester villus. Note the extending capillaries and thinning syncytium in the third trimester tissue to maximise exchange between maternal and fetal circulation. Image by Dr C. Jones.*

3. Placental function

The syncytiotrophoblast is responsible for gas and nutrient exchange between maternal and fetal circulations. Maternal blood enters the intervillous space via spiral arteries, it then bathes the villi and drains through endometrial veins[38]. At the same time, the oxygen-deficient fetal blood enters the chorionic arteries within the chorionic villi via the umbilical arteries, then oxygenated blood returns to the fetus via the umbilical vein[38]. Fetal growth is directly related to maternal nutrient availability and the placenta's ability to transport nutrients[39]. As there are four layers in the placental villi through which substrates, gases and water from maternal circulation must cross to reach the fetus, transport molecules, electrochemical gradients and diffusion channels are of high importance[39]. Different nutrients utilise various transport mechanisms. Small molecules such as oxygen and carbon dioxide diffuse. Glucose transporters allow facilitated diffusion down a concentration gradient within the placenta[40]; Glucose transporter 1 (GLUT1) is the primary glucose transporter in humans with GLUT3 and GLUT4 most highly expressed in first trimester placentas and decreasing in the second and third trimesters[41,42].

Amino acids are essential for the growth of fetal tissues. Most amino acids are found in higher concentrations in fetal blood compared to the maternal circulation, which suggests that amino acids are actively transported across the syncytiotrophoblast, which relies on cellular energy generated by

mitochondria[43,44]. There are several transport systems depending upon the type of amino acid. System A, which has been well-studied, is a sodium-dependent transport system which transports small neutral amino acids[44]. As the placenta develops in the third trimester, it is found to expresses system A isoforms: SNAT1, SNAT2 and SNAT4[45].

The placenta has important endocrine functions, which alter the maternal environment, which changes across gestation. Human chorionic gonadotropin (hCG) is at its highest levels in the first trimester; it plays a role in the differentiation of cytotrophoblast into syncytiotrophoblast[46]. hCG also promotes angiogenesis in specific areas such as the uterine endothelium, where it binds to its receptor and can cause an increase in proliferation of uterine natural killer cells at the maternal-fetal interface[47]. The syncytiotrophoblast also produces progesterone in the mitochondria[46], which is converted into fetal cortisol and oestrogen, which has been linked to regulating vasculoendothelial growth factor production and angiogenesis[48]. Human placental lactogen (hPL) is another hormone that is produced by the syncytiotrophoblast[49,50]. hPL increases during gestation and it is released into both maternal and fetal circulations[51]. hPL production mirrors placental mass, specifically the syncytiotrophoblast mass[52].

The placenta also plays a key role in immunity. Human placentas contain immunoglobulin (Ig) G, A and M[53,54]. These different immunoglobulins have important roles throughout pregnancy and in development of fetal immunity[55]. IgG in maternal circulation crosses the syncytiotrophoblast to provide some protection to the fetus, through binding to the neonatal Fc receptor in the third trimester[56,57].

4. Placental changes in prolonged pregnancy

As gestation progresses the placental tissue ages in similar ways to that observed in other human tissues outside of pregnancy. Some of these ageing processes could account for the increased risk of adverse pregnancy outcomes in prolonged pregnancies.

4.1 Morphology of the placenta in prolonged pregnancy

Ultrastructural studies of the placenta can provide information on cellular structures[58]. Jones and Fox (1978) investigated 19 placentas from prolonged pregnancies. Several morphological abnormalities were observed including prominent cytotrophoblast cells. Mitochondria were decreased in number and size and had angular cristae in prolonged pregnancy. Mitochondria are cellular organelles providing energy for normal cell function and survival in the face of external stressors. Mitochondria generate cellular energy in the form of ATP, are the site of steroid hormone synthesis, produce and detoxify reactive oxygen species (ROS), and control the onset of apoptosis. Alterations to the number and function of mitochondria could, therefore, have consequences for cellular function and survival. Golgi bodies were sparse in post-term placentas and endoplasmic reticulum had narrow cisternae in these cases[58]. Also, the syncytiotrophoblast surface frequently had irregular protrusions and the microvilli had irregular shapes or swollen tips[58]. Furthermore, in many areas, there was a reduction in microvilli seen which has been associated with syncytial necrosis[58]. These observed morphological abnormalities seen in post-term placentas suggest a possible decline in trophoblast function apparent in

the late stages of normal gestation. In particular, the changes in mitochondria would alter metabolic capacity, and in Golgi/ER would alter the release of placental hormones.

4.2 Syncytial nuclear aggregates

Within the syncytiotrophoblast, nuclei can cluster to form structures known as syncytial nuclear aggregates (SNAs)[59]. SNAs accumulate throughout pregnancy but have been especially noted in prolonged pregnancies[58]. Syncytial knots, a subtype of SNAs, are increasingly frequent as gestation increases and are often thought to represent the placenta ageing[60,61]. Nuclei within knots have specific morphology defined by dense heterochromatin thought to be evidence of epigenetic changes associated with oxidative damage[62]. Syncytial knots are uncommon before 32 weeks of gestation. As gestation progresses their frequency increases, being seen in 10-30% of villi in normal term placentas[63]. Syncytial knots are strongly associated with post maturity, with a sudden increase in their frequency at 42 weeks' gestation[64,65]. Syncytial knots are thought to represent an ageing status due to senescence[66]. Jones and Fox concluded that increased formation of syncytial knots in term placentas is a way of discarding unwanted aged nuclei to prevent them from hindering the formation of vasculosyncytial membranes.

4.3 Oxidative stress

Oxidative stress is a contributing factor in the ageing process. Oxidative stress is described as an imbalance in the generation of reactive oxygen species (ROS) and the effectiveness of antioxidant defences[67]. ROS induce cellular oxidative damage by interacting with DNA and intracellular molecules such as proteins and membrane lipids, leading to cellular dysfunction[68]. Pregnancy is regarded as a state of oxidative stress due to the increased metabolic demand of the growing fetus[69]. In prolonged pregnancy, the accumulation of oxidative damage to lipids, proteins and DNA in placental tissue may induce a form of advanced ageing[70]. Increasing oxidative stress can alter the intrauterine environment and result in irreversible changes to placental tissue[71,72].

8-hydroxy-2'-deoxyguanosine (8-OHdG) is a common biomarker used to measure DNA/RNA oxidation. The intensity of nuclear 8-OHdG staining was increased in late-term and stillbirth placentas compared to 37- to 39-week placentas[32]. Londero et al. also demonstrated that as gestational age increased there was a parallel increase in 8-OHdG in nuclei[73]. Lipid peroxidation can also result from free radical damage to produce 4-Hydroxynonenal (4-HNE). Maiti et al. found a significant increase in 4-HNE staining in syncytiotrophoblast in late-term placentas and those associated with stillbirths, although there is an inherent difficulty in knowing if this happened before or after fetal demise. In addition, aldehyde oxidase I (AOX1), which oxidizes a range of aldehydes including 4HNE, co-localised with 4-HNE positive particles in late-term placentas. Term placental explant culture by Maiti et al. showed serum deprivation resulted in increased 4HNE production at 24 hours post-incubation, which was successfully inhibited with an AOX1 inhibitor[32]. Furthermore, late-term and stillbirth associated placentas expressed significantly higher mRNA for AOX1 than 37-39 week control placentas[32]. Thus, supporting the idea that AOX1 has a key role in oxidative damage that is seen in late-term placentas[32].

4.4 Autophagy

Autophagy is the catabolic mechanism that involves the digestion of damaged or dysfunctional cellular components through fusion with lysosomes[74,75]. Autophagy is an important mechanism that helps to maintain cellular homeostasis during proliferation and differentiation, as well as cell survival during a period of nutrient starvation or reduced metabolism. The autophagy pathway can be activated by mTOR (target of rapamycin) in mammalian cells[76]. The activity of mTOR is reduced during stressful situations, having a downstream effect on ATG protein kinases[70]. The formation of an autophagosome relies on kinase complexes to cleave LC3B to LC3B-II which is central to the autophagy pathway and bind to the outer membrane[77]. Once the autophagosome membrane is formed LAMP2 is needed for lysosome fusion to create the autophagolysosome[78]. Thus, markers LC3B-II and LAMP2 are used to assess autophagosome formation[79]. Maiti et al. examined LAMP2 expression in early term, late-term and stillbirth and found that lysosomes change position in the syncytiotrophoblast[32], initially in the early-term syncytiotrophoblast LAMP2 expression was seen on the apical surface whereas in late-term and stillbirth associated syncytiotrophoblast expression was relocated to the perinuclear and basal surface[32]. These findings closely resemble previous recordings of lysosomal positioning in cells under nutritional stress[80]. Hung et al. found that significantly higher levels of LC3B-II were found in villous tissue from early and mid-gestation than late-gestation tissue[81]. Furthermore, Maiti et al. demonstrated an increase in autophagosome size in both late-term and stillbirth associated placentas compared to controls[32]. Increased size is seen when the autophagosome function is inhibited and there is a failure of fusion with lysosomes, leading to failure of autophagy[82,83]. Without autophagic function accumulation of abnormal proteins can occur resulting in deterioration in the function of the syncytiotrophoblast[32].

4.5 Senescence

A key feature of the ageing process is a loss of function at cellular, tissue and organ level[84]. This results in a reduced ability to adapt to stress, increasing vulnerability to disease processes[84]. In mitotic tissues, the accumulation of senescent cells is thought to be a causal factor for ageing[85]. Cellular senescence is a state of irreversible arrest of proliferation. It is triggered by an abundance of intrinsic and extrinsic stimuli or stressors including oxidative stress, DNA damage, nucleolar stress, epigenetic stress, telomere damage, chronic mitogen signalling, and oncogene activation/inactivation[86]. Once in a senescent state, cells become resistant to apoptosis resulting in an accumulation of senescent cells within tissues[87]. It is this accumulation of senescent cells within tissues that contributes to ageing and produces ageing phenotypes via reduced tissue renewal and repair, damaging structural components, altering metabolic functions, and altering the behaviour of neighbouring cells and the extracellular environment[85].

Senescence stressors ultimately activate the p53 and/or $p16^{Ink4a}$ pathways. When activated, p53 inhibits proliferation by activating transcription factor p21[86]. p21 along with p16 then act to keep retinoblastoma tumour suppressor protein (Rb) in a hypophosphorylated and active state[88]. Rb in this state suppresses the transcription factor E2F which prevents

expression of genes that regulate the progression of the cell cycle from the G1/S phase[88]. Chuprin et al. demonstrated that the human syncytiotrophoblast exhibit characteristics and activation of the molecular pathways of cellular senescence via immunohistochemically staining of third-trimester placentas for senescence markers[89]. They found that the syncytiotrophoblast exhibited specific staining for the CDK inhibitors p16 and p21, indicating that both central pathways of senescence are activated in these cells[89]. Davies et al. detected significant increases in senescence markers p21 and p16 in homogenates of healthy placentas with advancing gestational age[90]. They also quantified the number of p21 positive syncytiotrophoblast nuclei in a collection of placentas that were delivered 7-20 days following their predicted due date[90]. A significant increase in the number p21 positive nuclei was seen in the placentas from prolonged pregnancies compared to term controls[89,90]. Similarly, Torricelli et al. found p21 mRNA levels to be higher in placentas from prolonged pregnancies[91].

Telomeres are repetitive DNA regions located at the end of linear chromosomes and are essential in maintaining chromosomal stability and cell survival[84]. They consist of tandem arrays of the nucleotide sequence TTAGGG and are typically 10-15 kb long[92]. Telomeres protect DNA ends from double-stranded breaks, end to end fusion and degradation[85]. Telomeres progressively shorten every time a cell divides due to an inability to replicate telomeric DNA[85]. When a telomere becomes critically short, their protective structure is disfigured[93], and the end of the nuclei which telomeres protect can then be recognised as double-strand damage regions (DDR)[94]. This results in the exposure of DNA ends to damage, which leads to the activation of senescence[93]. DNA strand damage activates the ataxia telangiectasia and Rad3-related (ATR) and ataxia telangiectasia-mutated (ATM) pathways, resulting in arrested cell division and induction of cellular senescence, and promotion of cell death[94]. As telomeres shorten and proliferation increases, cells senesce or enter a phase of growth retardation, known as Hayflick's limit, which culminates in cell death[95].

Placental ageing is associated with shortened telomeres; the enzyme telomerase which regulates telomere length is significantly downregulated as pregnancy progresses[96]. Maiti et al. showed by real-time PCR that telomere length decreased in placentas with a gestational age of 41 weeks compared to those at 38 weeks of gestation[97]. Importantly, the rate of telomerase activity was low in cases of fetal death, regardless of the gestation in which they occurred[96]. These findings suggest that as gestation advances, telomerase activity decreases resulting in telomere shortening, thus activating placental senescence, which is associated with ageing of the placenta[96,97].

4.6 Apoptosis

Apoptosis is a cellular mechanism that leads to self-destruction of the cell. It is essential for cellular survival and development[98]. It is sometimes referred to as programmed cell death. The initiation of apoptosis is dependent upon the activation of a series of proteases, known as caspases, through either the intrinsic or extrinsic pathway[99]. The intrinsic pathway is a mitochondrial response to cellular stress, commonly DNA damage, culminating in activation of apoptotic proteins such as Bcl-2 family proteins. The extrinsic pathway functions by the death

receptor pathway. If the two pathways crossover, it can amplify the apoptotic response[100,101].

Apoptosis is increased in placentas from prolonged pregnancies compared to those from term, principally detected within trophoblast and stromal tissue[102,103]. The Bcl-2 protein family have an important role in apoptosis[104]. This protein family includes Bcl-2, an anti-apoptotic protein and Bax, a pro-apoptotic protein; the ratio of these molecules influences the rate of apoptosis[105]. As gestation advances, there is an increase in apoptosis which is closely linked to Bcl-2 and Bax expression[106,107]. Daher et al., when comparing pre- and post-term placentas, demonstrated that Bcl-2 and Bax were higher in post-term placentas[108]. The post-term placentas had a higher Bax/Bcl-2 ratio and further studies showed an increase in Bax/Bcl-2 mRNA in prolonged pregnancy, which can cause increased susceptibility to apoptotic stimuli in the placenta[91,109].

5. Conclusions

High-grade evidence from meta-analyses demonstrates that prolonged pregnancy is associated with fetal, neonatal and maternal complications including perinatal mortality and increased frequency of caesarean section, and that this can be reduced by a policy of induction of labour /before 42 weeks' gestation. However, the number of interventions needed to prevent one perinatal death is high at 426. Improved understanding of the underlying pathobiology could better define the association observed between prolonged pregnancy and adverse outcomes and allow identification of prolonged pregnancies at highest risk of adverse outcome.

The findings summarised here demonstrate growing evidence of structural and biochemical features of ageing at play in placentas from prolonged pregnancies (Figure 2). This evidence provides insight into placental ageing and mechanisms which may initially allow the placenta to adapt to in utero stress but later become pathological. In prolonged pregnancy, we propose that the structural changes observed in mitochondria would reduce their efficiency leading to accumulation of ROS which cause DNA and lipid damage, as evidenced through increased 8-OHdG and 4-HNE staining. The cellular response of autophagy and senescence is initially increased, but when the cell becomes excessively damaged there is apoptosis. Given the close relationship between placental structure, cell processes and placental function these changes would be expected to lead to a reduction in placenta function. However, we have not been able to identify any studies which have examined this link.

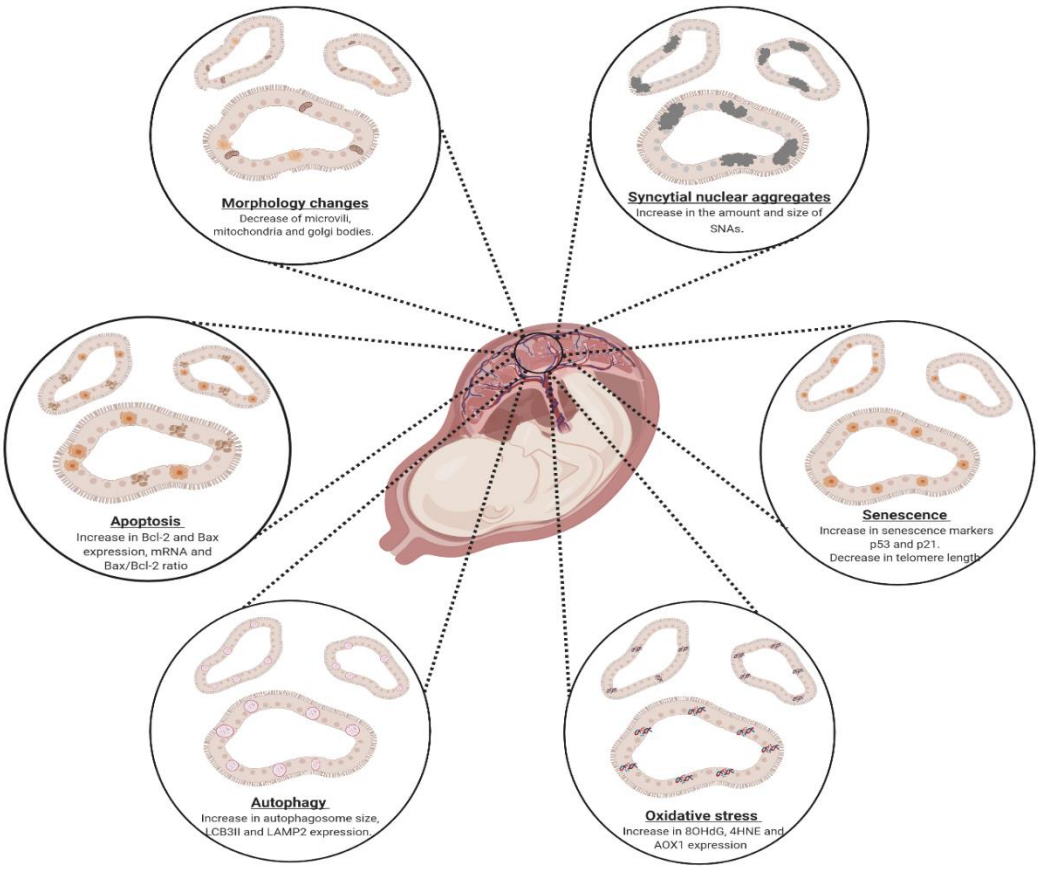

Figure 2: Evidence of biomarkers of ageing observed in prolonged pregnancy placentas.

Further research of mitochondrial function, nutrient transport and endocrine function is needed to determine whether there is a loss of placental function in prolonged pregnancy. If such changes are observed, this further strengthens the argument for a policy of induction of labour before 42 weeks' gestation. Identification of placental dysfunction in prolonged pregnancy may also advance identification of placental biomarkers so that intervention could be targeted to women at greatest risk of complications. Finally, a better understanding of whether placental ageing occurs will also aid understanding of pregnancies complicated by maternal obesity or maternal age ≥40 years of age, as these are also associated with increased risk of preeclampsia, FGR and stillbirth in late pregnancy.

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