Oxidative stress and ageing of the post-ovulatory oocyte

Tessa Lord and R John Aitken

*School of Environmental and Life Sciences, Priority Research Centre for Reproductive Biology,
University of Newcastle, Callaghan, New South Wales 2308, Australia*

Correspondence should be addressed to R J Aitken; Email: john.aitken@newcastle.edu.au

Abstract

With extended periods of time following ovulation, the metaphase II stage oocyte experiences deterioration in quality referred to as post-ovulatory oocyte ageing. Post-ovulatory ageing occurs both *in vivo* and *in vitro* and has been associated with reduced fertilization rates, poor embryo quality, post-implantation errors and abnormalities in the offspring. Although the physiological consequences of post-ovulatory oocyte ageing have largely been established, the molecular mechanisms controlling this process are not well defined. This review analyses the relationships between biochemical changes exhibited by the ageing oocyte and the symptoms associated with the ageing phenotype. We also discuss molecular events that are potentially involved in orchestrating post-ovulatory ageing with a particular focus on the role of oxidative stress. We propose that oxidative stress may act as the initiator for a cascade of events that create the aged oocyte phenotype. Specifically, oxidative stress has the capacity to cause a decline in levels of critical cell cycle factors such as maturation-promoting factor, impair calcium homeostasis, induce mitochondrial dysfunction and directly damage multiple intracellular components of the oocyte such as lipids, proteins and DNA. Finally, this review addresses current strategies for delaying post-ovulatory oocyte ageing with a particular focus on the potential use of compounds such as caffeine or selected antioxidants in the development of more refined media for the preservation of oocyte integrity during IVF procedures.

Reproduction (2013) **146** R217–R227

Introduction

Upon release from the ovary, the prophase I oocyte undergoes a process of maturation involving resumption of meiosis, breakdown of the germinal vesicle and extrusion of the first polar body (reviewed by Sun *et al.* (2009)). Following maturation, the oocyte once again enters meiotic arrest, now at metaphase II (MII). The MII stage oocyte awaits fertilization by the spermatozoon either in the oviduct of the female reproductive tract or, in an assisted reproduction setting, in *in vitro* culture media. An optimal window exists in which fertilization of this MII stage oocyte should occur; in mammalian species, this is generally within 10 h of ovulation; however, successful fertilization can still occur in the mouse for up to 15 h (Marston & Chang 1964, Tarin *et al.* 1999). In the event that no fertilization occurs within this time frame, oocytes experience a process of degradation referred to as 'post-ovulatory ageing'. It should be noted that post-ovulatory ageing is a process distinct from that of 'ovarian ageing' (reviewed by Bentov & Casper (2013)), which occurs within the ovary of females towards the end of reproductive life, as they approach the climacteric. All references to oocyte ageing within this review refer to the deterioration of MII oocytes that occurs following their release from the ovarian follicle both *in vivo* and *in vitro*.

Post-ovulatory aged oocytes exhibit numerous aberrations in their cell biology including partial cortical granule exocytosis (Szollosi 1971, Dodson *et al.* 1989, Ducibella *et al.* 1990, Xu *et al.* 1997), zona hardening (Longo 1981, Dodson *et al.* 1989, Xu *et al.* 1997), a decrease in critical cell cycle factors including maturation-promoting factor (MPF) and MAPK (Kikuchi *et al.* 2002), mitochondrial dysfunction (Chi *et al.* 1988, Takahashi *et al.* 2003, Tatone *et al.* 2011, Zhang *et al.* 2011, Lord *et al.* 2013), spindle abnormalities (Wakayama *et al.* 2004), losses of chromosomal integrity (Spielmann *et al.* 1985, Mailhes *et al.* 1998, Wakayama *et al.* 2004) and epigenetic changes (Liang *et al.* 2008; Table 1). Not surprisingly, the process of post-ovulatory ageing culminates in apoptosis (Fujino *et al.* 1996) as a result of decreases in expression of the anti-apoptotic protein BCL2 (Gordo *et al.* 2002, Ma *et al.* 2005) and the activation of caspases (Takai *et al.* 2007, Lord *et al.* 2013).

The cellular deterioration associated with post-ovulatory ageing can strongly influence the outcome of fertilization *in vivo* and *in vitro*. This is not surprising when considering that the oocyte provides factors to the embryo that orchestrate the early events of embryogenesis (Schultz & Heyner 1992, Minami *et al.* 2007), remodel the genome (Torres-Padilla *et al.* 2006) and repair DNA damage in both male and female nuclei

Table 1 Aberrations to oocyte biology associated with mammalian post-ovulatory ageing.

Organelle	Consequences associated with ageing	<i>In vivo/in vitro</i>	Reference
Zona pellucida	Zona hardening	Both	Longo (1981), Dodson <i>et al.</i> (1989) and Xu <i>et al.</i> (1997)
Cortical granules	Partial exocytosis	Both	Szolloi (1971), Dodson <i>et al.</i> (1989), Ducibella <i>et al.</i> (1990), Xu <i>et al.</i> (1997) and Liu <i>et al.</i> (2009a, 2009b)
Spindle	Elongation, dispersal or disruption	<i>In vitro</i>	Wakayama <i>et al.</i> (2004)
Plasma membrane	Lipid peroxidation	Both	Takahashi <i>et al.</i> (2003)
ER	Depletion of Ca ²⁺ stores	Both	Takahashi <i>et al.</i> (2000)
	Decline in IP ₃ receptor/channel functionality	<i>In vitro</i>	Zhang <i>et al.</i> (2011)
Mitochondria	Increased generation of ROS	Both	Takahashi <i>et al.</i> (2003), Tatone <i>et al.</i> (2011) and Lord <i>et al.</i> (2013)
	Loss of membrane potential	<i>In vitro</i>	Zhang <i>et al.</i> (2011)
	Decreased ATP production	<i>In vitro</i>	Chi <i>et al.</i> (1988)
DNA	Epigenetic changes	Both	Liang <i>et al.</i> (2008)
	Misaligned chromosomes	Both	Wakayama <i>et al.</i> (2004)
	Premature centromere separation	Both	Spielmann <i>et al.</i> (1985) and Mailhes <i>et al.</i> (1998)

IP₃, inositol 1,4,5-trisphosphate; ROS, reactive oxygen species.

(Genesca *et al.* 1992). Unfortunately, the occurrence of post-ovulatory ageing is not uncommon. In the majority of mammalian species, mating occurs only at the time of ovulation, as determined by the onset of behavioural oestrus, or the act of mating itself triggers ovulation. The result of such mechanisms is that the delivery of spermatozoa to the female reproductive tract is relatively synchronized with oocyte release. In humans and certain primates, however, there are no visual signs of ovulation and, as a result, no mechanisms in place to ensure synchrony between intercourse and release of the oocyte. This, in turn, results in an increased likelihood that fertilization could involve an *in vivo* aged oocyte and freshly ejaculated spermatozoa. In an *in vitro* setting, oocytes employed in assisted reproduction technologies (ART) are often unavoidably subjected to extended periods of culture prior to fertilization. One circumstance in which this may occur is when 'rescue ICSI' is performed on failed-to-fertilize oocytes. In light of such considerations, it would clearly be beneficial to establish some control over the process of post-ovulatory oocyte ageing, particularly in an *in vitro* setting, where the demand for ART continues to increase exponentially (Wang *et al.* 2011).

This review explores the negative consequences associated with post-ovulatory oocyte ageing, discusses the poorly understood mechanisms that control this process and considers the current means by which post-ovulatory ageing can be prevented or delayed. Specifically, in light of recent research, we revisit the proposal that oxidative stress may act as the 'trigger' for a cascade of other events associated with oocyte ageing and hence might be an attractive approach for deterring this degenerative process in post-ovulatory oocytes.

Clinical implications of oocyte ageing

The clinical implications of oocyte ageing include a decreased capacity for fertilization both *in vivo* (Marston & Chang 1964) and *in vitro* (Ben-Rafael *et al.* 1986, Badenas *et al.* 1989), production of poor-quality embryos (Yanagida *et al.* 1998, Lord *et al.* 2013), an increased likelihood of early pregnancy loss (Wilcox *et al.* 1998) and abnormalities in offspring (Tarin *et al.* 1999). The attributes of aged oocytes responsible for these adverse outcomes are discussed below.

Decreased fertilization rate

A decrease in fertilization rate has been associated with aged oocytes in many species including the humans (Lash & Whittaker 1974), mice (Marston & Chang 1964, Liu *et al.* 2009b), cows (Chian *et al.* 1992) and pigs (Kikuchi *et al.* 2000). This phenomenon can be attributed to multiple biochemical and functional alterations to the oocyte that accumulate with post-ovulatory age. First, aged murine oocytes are known to experience premature cortical granule exocytosis (Szolloi 1971, Dodson *et al.* 1989, Ducibella *et al.* 1990, Xu *et al.* 1997) and zona hardening (Longo 1981, Dodson *et al.* 1989, Xu *et al.* 1997); changes that would obviously impose limitations on the ability of the zona pellucida to interact with a spermatozoon and initiate the acrosome reaction. Additionally, as levels of lipid peroxidation are known to be elevated within post-ovulatory aged oocytes (Takahashi *et al.* 2003; Table 1), the fluidity of the oolemma may be decreased, reducing the likelihood of sperm–oolemma fusion and thereby fertilization.

In the event that sperm–zona interaction and sperm–oolemma fusion progress unimpaired, the abilities of the

post-ovulatory aged oocyte to exhibit normal activation and initiate embryo development following fertilization are nonetheless compromised. Upon fertilization, inositol 1,4,5-trisphosphate (IP₃) receptors located on the oocyte's endoplasmic reticulum (ER) initiate calcium (Ca²⁺) oscillations in response to IP₃ produced by the sperm-derived factor PLCζ1 (Yoon *et al.* 2008, Kuroda 2010). However, post-ovulatory aged oocytes experience a decline in IP₃ receptor/channel functionality (Zhang *et al.* 2011), as well as suppressed ER Ca²⁺-ATPase activity, and exhibit a consequential depletion of Ca²⁺ stores that reside within the ER (Takahashi *et al.* 2000). As a result of this impaired Ca²⁺ homeostasis, aged oocytes exhibit abnormal Ca²⁺ oscillations at fertilization that have significantly higher frequency and lower amplitude than those of fertilized fresh oocytes (Igarashi *et al.* 1997, Takahashi *et al.* 2003). These abnormal Ca²⁺ oscillations are purportedly associated with the onset of apoptosis following fertilization, rather than entry into a developmental pathway (Gordo *et al.* 2000, 2002).

Poor embryo quality

While the fertilization potential of post-ovulatory aged oocytes is obviously compromised, circumstances exist in which fertilization does still occur, particularly in clinical practice when ICSI is used to bypass the physiological mechanisms that would normally prevent the association of defective gametes. Not surprisingly, embryos originating from post-ovulatory aged oocytes are generally of poor quality and exhibit delayed or impaired development during subsequent embryogenesis (Yanagida *et al.* 1998, Lord *et al.* 2013).

The poor quality of these embryos is highlighted when we assess their capacity to implant in the endometrium and carry a pregnancy to term. With extensive periods of post-ovulatory ageing (>24 h *in vitro*), ICSI-generated embryos reportedly lose their capability for uterine implantation following embryo transfer (Chen *et al.* 1995). Despite this, oocytes that are subjected to shorter periods of post-ovulatory ageing can still produce embryos capable of implantation, but are prone to post-implantation errors. A clinical study assessing the effects of *in vivo* post-ovulatory oocyte ageing found a significant correlation between early pregnancy loss and the likelihood that delayed fertilization had occurred (Wilcox *et al.* 1998). These findings have also been reported in other mammalian species such as the mouse (Marston & Chang 1964).

The decline in embryo quality associated with post-ovulatory aged oocytes is presumably attributed to multiple factors. First, cytoplasmic ageing is likely to greatly hinder the capacity of the oocyte to support and orchestrate embryo development, particularly when changes in both the protein and mRNA composition of the oocyte are demonstrable with prolonged ovulation

to fertilization latency (Tarin *et al.* 2000, Hamatani *et al.* 2004). Additionally, aged oocytes are likely to display a corresponding increase in the proportion of non-viable embryos following fertilization as a result of chromosomal abnormalities. Post-ovulatory aged oocytes purportedly exhibit significantly higher instances of chromosome misalignment, potentially as a consequence of meiotic spindles that are elongated, dispersed or disrupted (Wakayama *et al.* 2004). These characteristics increase the likelihood for erroneous chromosome separation and hence aneuploidy (Mailhes *et al.* 1998). Further to this, in certain species such as the bovine, advancing oocyte age is also associated with a significantly increased susceptibility to polyspermy (Chian *et al.* 1992), which again results in the generation of embryos that are essentially non-viable.

Abnormalities in offspring

Despite the obstacles associated with fertilization of post-ovulatory aged oocytes and subsequent embryo development and implantation, birth of live offspring originating from these gametes does still occur. It was originally thought that aged oocytes that manage to support embryo development to the blastocyst stage were no less capable of producing healthy offspring than their fresh oocyte counterparts (Wilcox *et al.* 1998). However, more recent research has demonstrated a significant decline in live birth rates and an elevated instance of abnormalities in offspring (Tarin *et al.* 1999).

Tarin *et al.* (1999, 2002) demonstrated that offspring originating from *in vivo* aged oocytes exhibited growth retardation, delayed development, heightened emotional state, decreased reproductive fitness and importantly decreased longevity. Although the specific factors within the post-ovulatory aged oocyte that so prominently affect offspring integrity have not been determined, it has been hypothesized that these abnormalities may stem from the transference of a subpopulation of dysfunctional mitochondria (Tarin *et al.* 2002). Impaired mitochondrial function has indeed been linked with diseases such as schizophrenia (Whatley *et al.* 1996), Alzheimer's disease (Hutchin & Cortopassi 1995, Budd & Nicholls 1998) and a decreased lifespan (Sont & Vandenbroucke 1993).

An alternative explanation for the abnormalities observed in the offspring of *in vivo* and *in vitro* aged oocytes may lay with an altered epigenetic profile, which would essentially affect gene expression in the embryo. Both the male and female genomes experience significant demethylation events following fertilization (Mayer *et al.* 2000) and post-ovulatory oocyte ageing has been shown to significantly alter the methylation pattern of imprinted genes in both the oocyte and the developing placenta (Liang *et al.* 2008, 2011).

Factors affecting oocyte ageing and apoptosis

Post-ovulatory oocyte ageing is clearly a prominent issue affecting fecundity; however, the molecular mechanisms that control this process are not well elucidated. It is currently undetermined as to whether a single event acts to trigger a cascade of other factors, or if several biochemical and functional changes occur separately to create an 'aged', degenerating MII oocyte. Below we assess factors that are known to influence the onset of post-ovulatory ageing and specifically highlight the potential role of oxidative stress as the 'trigger' for ageing and apoptosis of the oocyte both *in vitro* and *in vivo*.

Cumulus cells

In the presence of cumulus cells, MII stage oocytes purportedly 'age' more rapidly. *In vivo* aged oocytes and *in vitro* aged oocytes enclosed within the cumulus cell complex experience increased rates of spontaneous activation and fragmentation (Miao *et al.* 2005, Wu *et al.* 2011), accelerated decline of MPF/MAPK (Miao *et al.* 2005) and decreased levels of blastocyst formation (Wu *et al.* 2011) when compared with denuded oocytes aged *in vitro*. Qiao *et al.* (2007) reported that the addition of cumulus cells to culture medium containing denuded oocytes caused an acceleration of post-ovulatory ageing on par with that of oocytes enclosed in a cumulus–oocyte complex, while other research groups have demonstrated that blocking gap junctions within the cumulus–oocyte complex does not prevent accelerated ageing (Wu *et al.* 2011). Following these observations, it has been hypothesized that cumulus cells secrete soluble/paracrine factor(s) that promote post-ovulatory ageing, potentially an event that coincides with the entry of the cumulus cells into apoptosis (Wu *et al.* 2011). Additionally, the accelerated depletion of the oocyte metabolite pyruvate from culture medium by cumulus cells (Downs *et al.* 2002) appears to directly affect oocyte metabolism and hence post-ovulatory ageing, potentially by causing downstream inhibition of protein synthesis within the oocyte and upsetting redox equilibrium (Liu *et al.* 2009b, Li *et al.* 2011).

An alternative explanation for cumulus cell-associated acceleration of post-ovulatory ageing has been detailed in publications by Perez *et al.* (2005) and Kujjo & Perez (2012). These researchers propose that the bioactive sphingolipid, ceramide, generated by the cumulus cells may be responsible for mitochondrial dysfunction and subsequent ageing and apoptosis in post-ovulatory oocytes. This is attributed to an increased sensitivity of aged oocytes to cytosolic ceramide spikes and the ability of ceramide to act as a pro-apoptotic factor upstream of Bax.

Although the presence of cumulus cells clearly exacerbates degeneration in ageing post-ovulatory oocytes, additional mechanisms must be at play in controlling this process, as oocytes that have been

denuded of their cumulus cells also experience ageing and apoptosis (Miao *et al.* 2005, Lord *et al.* 2013).

MPF and MAPK

The critical cell cycle factors MPF and MAPK control meiotic resumption in germinal vesicle stage oocytes and then act to maintain cell cycle arrest once oocytes have reached the MII phase. A gradual decline in the concentration of these factors within the MII oocyte has been detected with post-ovulatory age and has been associated with increased levels of parthenogenetic activation and fragmentation (Kikuchi *et al.* 2000). The mechanism by which MPF specifically becomes degraded with post-ovulatory age has been studied extensively – particularly as a potential target for reversing the ageing process (to be discussed later in this review). MPF comprises two molecules: the catalytic subunit p34^{cdc2} and the regulatory subunit cyclin B. In the active form of MPF, these two subunits are coupled and T-161 of the catalytic subunit is phosphorylated. Following fertilization, MPF is inactivated by the dephosphorylation of T-161 and degradation of the decoupled cyclin B. Interestingly, inactivation of MPF occurs via an alternate pathway in the ageing post-ovulatory oocyte. Under these circumstances, the inactive compound 'pre-MPF' is formed, in which the two subunits are still bound but phosphorylation of T-14 and/or T-15 of p34^{cdc2} occurs (Kikuchi *et al.* 2002). The accumulation of pre-MPF is clearly a factor controlling at least some aspects of post-ovulatory ageing as acceleration of pre-MPF formation using the tyrosine phosphatase inhibitor, vanadate, has been shown to increase susceptibility to parthenogenetic activation and fragmentation, while maintenance of active MPF levels within the oocyte using caffeine decreases levels of parthenogenesis and fragmentation (Kikuchi *et al.* 2002), and extends the window for fertilization (Ono *et al.* 2011).

Mitochondrial dysfunction

Mitochondrial dysfunction appears to be another potent mediator of the post-ovulatory oocyte ageing process. Operational mitochondria are crucial to normal oocyte function, with these organelles representing the primary source of ATP production within both oocytes and early embryos (Dumollard *et al.* 2007). The functionality of the mitochondria does, however, become compromised with extended periods of time following ovulation, a factor that is thought to heavily influence oocyte quality. Diminished mitochondrial integrity in the *in vitro* aged oocyte has been demonstrated by a loss of mitochondrial membrane potential (Zhang *et al.* 2011) and a decline in levels of ATP production (Chi *et al.* 1988).

As damage to the mitochondria is known to cause an increased production of reactive oxygen species (ROS)

and the release of pro-apoptotic factors such as cytochrome *c* (Liu *et al.* 2009a), which recruit members of apoptotic machinery such as caspases (Takai *et al.* 2007) and endonucleases (Fujino *et al.* 1996), it is likely that mitochondrial dysfunction is the link between post-ovulatory oocyte ageing and apoptosis. Additionally, loss of mitochondrial integrity can result in aberrant protein synthesis and inactivation or loss of mitochondrial DNA (mtDNA; reviewed by Shigenaga *et al.* (1994)).

The pronounced control that mitochondrial function has over post-ovulatory ageing has been demonstrated in studies utilizing microinjection of isolated mitochondria into *in vitro* aged oocytes. During a 24-h culture period, MII oocytes whose mitochondrial pool was increased by 5% via microinjection showed a significant decline in levels of programmed cell death when compared with control oocytes (Perez *et al.* 2000).

Oxidative stress: the initiator of post-ovulatory ageing?

It has been demonstrated by several research groups that ROS – particularly hydrogen peroxide (H_2O_2), superoxide anion ($\text{O}_2^{\bullet-}$) and peroxynitrite (ONOO^-) – accumulate in oocytes with increasing amounts of time following ovulation both *in vitro* and *in vivo* (Takahashi *et al.* 2003, Tatone *et al.* 2011, Lord *et al.* 2013). ROS production within the MII oocyte likely occurs as a by-product of oxidative phosphorylation; however, environmental factors that oocytes are subjected to *in vitro* (exposure to light, lack of antioxidant rich follicular and tubal fluids and increased oxygen tension) are also thought to facilitate ROS production (Mastroianni & Jones 1965, Mass *et al.* 1976, Goto *et al.* 1993, Guerin *et al.* 2001). While the oocyte does offer some intracellular defence against oxidative attack in the form of the antioxidant glutathione, these resources are depleted with post-ovulatory age (Boerjan & de Boer 1990, Yoshida *et al.* 1993). As a consequence of the progressive increase in ROS production and the concomitant depletion of antioxidant protection, the post-ovulatory aged oocyte experiences a state of oxidative stress. We hypothesize that this oxidative stress may act as the ‘trigger’ for a cascade of other factors that orchestrate post-ovulatory ageing, as well as directly affect multiple aspects of oocyte biochemistry and functionality. The idea that oxidative stress may be the initiator of ageing in the MII oocyte is supported by recent findings indicating that the onset of oxidative stress is a relatively early event in *in vitro* culture (Lord *et al.* 2013). Indeed, links can be established between oxidative stress in the oocyte and the aforementioned decline in critical cell cycle factors, mitochondrial dysfunction, apoptosis, impaired Ca^{2+} homeostasis, decreased fertilization rate, poor embryo quality and abnormalities in offspring. These associations will be discussed below.

We propose that the decline in the critical cell cycle factor, MPF, which reportedly orchestrates some facets of

post-ovulatory ageing, may be a symptom of oxidative stress in the MII oocyte. As mentioned previously, the accumulation of ‘pre-MPF’ occurs with increasing periods following ovulation, with phosphorylation at multiple sites on the catalytic subunit of MPF effectively inactivating this compound (Kikuchi *et al.* 2000, 2002). The enzymes that purportedly control these phosphorylation events are cdc25 (a tyrosine phosphatase) and Wee1/Myt1 (tyrosine kinases) (Kikuchi *et al.* 2002). Interestingly, ROS have previously been shown to have a capacity for both inhibition of tyrosine phosphatases (Monteiro *et al.* 1991, Sullivan *et al.* 1994), including cdc25 specifically (Brisson *et al.* 2007), and stimulation of tyrosine kinases (Chan *et al.* 1986). As a result, the induction of oxidative stress in the post-ovulatory oocyte may directly affect levels of MPF within the cell, resulting in the onset of associated downstream symptoms of oocyte ageing such as parthenogenesis and fragmentation (Kikuchi *et al.* 2002).

Oxidative stress that occurs with post-ovulatory age also has the potential to directly affect mitochondrial function. The DNA, proteins and lipids within the mitochondria are particularly susceptible to oxidative attack, not only because of their close proximity to the source of ROS production (the electron transport chain (ETC)) but also in the case of mtDNA, because of the absence of protective histones and mechanisms for DNA repair (reviewed by Shigenaga *et al.* (1994)). Oxidative stress has been linked with mtDNA damage and deletions (Sohal & Dubey 1994), loss of mitochondrial membrane potential (Liu *et al.* 2000), increased ROS generation by the ETC (Liu *et al.* 2009a) and a decline in ATP production (Melov *et al.* 1999). Importantly, factors such as increased ROS generation and a decline of ATP production are also known to be associated with post-ovulatory ageing (Chi *et al.* 1988, Lord *et al.* 2013), suggesting that a link between oxidative stress and age-related mitochondrial dysfunction is certainly plausible. Notably, oxidative damage to mtDNA in the oocyte could potentially be the basis for the aforementioned abnormalities associated with impaired mitochondrial function in offspring originating from aged oocytes (Tarin *et al.* 2002).

Oxidative stress also has the capacity to directly influence the onset of apoptosis in post-ovulatory aged oocytes. H_2O_2 treatment of MII oocytes *in vitro* has been shown to cause a decline in levels of the anti-apoptotic molecule Bcl-2 (Takahashi *et al.* 2009), induce expression of pro-apoptotic Bax and caspase-3, and precipitate both DNA fragmentation (Chaube *et al.* 2007) and cytochrome *c* release (Liu *et al.* 2000). Importantly, these pathologies are common to both oxidative stress and oocyte ageing, and relief from oxidative stress by way of antioxidant treatment has been shown to delay the onset of fragmentation and caspase activation associated with post-ovulatory ageing (Lord *et al.* 2013).

In addition to potentially being a precursor to MPF/MAPK depletion, mitochondrial dysfunction and subsequent apoptosis, the damaging nature of oxidative stress itself is likely to be directly involved in generation of the 'aged oocyte' phenotype. First, oxidative stress may be accountable for a perturbation of Ca^{2+} homeostasis in aged oocytes. Previous research has demonstrated that these two events are implicitly linked, as treatment of fresh oocytes with H_2O_2 results in abnormal Ca^{2+} oscillations following fertilization, which exhibit similar frequency and amplitude to those produced by post-ovulatory aged oocytes (Igarashi *et al.* 1997, Takahashi *et al.* 2003). The basis for this interaction lies with the capacity for ROS to directly affect the Ca^{2+} channels and Ca^{2+} -ATPases of the ER (Rohn *et al.* 1993, Doan *et al.* 1994, Wesson & Elliot 1995), as well as the Ca^{2+} signalling molecule calmodulin (Gao *et al.* 2001). Further facets of oocyte biochemistry have also been shown to be vulnerable to oxidative attack; specifically zona pellucida dissolution time and cortical granule loss; which are significantly altered following treatment with H_2O_2 and $\text{O}_2^{\bullet -}$ (Goud *et al.* 2008a). The mechanisms behind these pathologies remain elusive, although it is plausible that oxidative stress influences processes under the control of cytoskeletal fibres, such as cortical granule exocytosis and polar body extrusion, by generating damage to the mitochondria that results in decreased ATP production (Chi *et al.* 1988). With decreased ATP availability, the dynamics of actin and tubulin assembly–disassembly would likely be impeded (Bershadsky *et al.* 1980).

Finally, oxidative stress is also capable of generating global damage to lipid, protein and DNA constituents of the cell (reviewed by Guerin *et al.* (2001)). As previously mentioned, peroxidation to lipids in the plasma membrane may decrease the oocyte's potential for sperm–oocyte fusion. Additionally, such global acquisition of oxidative damage in aged oocytes is presumably related to poor embryo quality following fertilization, as relief from oxidative stress in the aged MII oocyte has been linked with improved developmental potential (Lord *et al.* 2013).

In conclusion, we propose that the development of oxidative stress is a relatively early event following ovulation that may initiate a decline in MPF levels, generate mitochondrial damage resulting in apoptosis, perturb Ca^{2+} homeostasis and instigate damage to the lipid, protein and DNA components of the cell, as well as to the cytoskeleton and zona pellucida. The acquisition of such oxidative damage post-ovulation may culminate in the observed reduction in fertilization rate, decline in embryo quality and increased likelihood for abnormalities in offspring associated with the aged oocyte (Fig. 1). If oxidative stress is indeed the instigator for post-ovulatory degeneration of the MII oocyte, it would be interesting to establish whether the biochemical changes that occur within the oocyte following timely fertilization

(i.e. prior to the onset of 'ageing') involve increased expression of antioxidant enzymes or enzymes involved in repair of oxidative damage. Certainly, recent research within our laboratory suggests an increased capacity to cope with oxidative insult in oocytes post-fertilization (T Lord and R J Aitken (2013), unpublished observations).

Mechanisms for preventing/delaying oocyte ageing

The final portion of this review looks into current strategies for delaying or preventing post-ovulatory oocyte ageing and assesses their effectiveness and potential for adaptation to clinical use in the future.

Culture conditions and media composition

Alterations to the concentration of metabolites within oocyte culture media has been shown to prominently affect post-ovulatory ageing *in vitro*. Specifically, increasing the concentration of pyruvate within culture media has been shown to delay post-ovulatory ageing by maintaining cortical granule integrity, decreasing susceptibility to activating stimuli, preventing an age-associated decline in levels of MPF and glutathione, improving blastocyst formation rates following fertilization and delaying the onset of apoptosis (Liu *et al.* 2009b, Li *et al.* 2012). As the oocyte and early embryo preferentially utilize pyruvate over glucose to drive metabolism (Leese & Barton 1984), it has been proposed that pyruvate supplementation delays oocyte ageing by fuelling prolonged ATP production within oocytes and also by maintaining intracellular redox potential (Liu *et al.* 2009b, Li *et al.* 2011). Increased pyruvate concentration is particularly effective in preventing accelerated *in vitro* ageing of the cumulus-enclosed MII oocyte, a result likely attributed to the more rapid depletion of pyruvate from culture media in the presence of cumulus cells (Downs *et al.* 2002).

Alteration of culture temperature has also been shown to delay the onset of post-ovulatory ageing *in vitro*. Porcine oocytes supplemented with pyruvate and stored at 15 °C were able to be successfully fertilized and maintain developmental potential after 42 h *in vitro*, whereas oocytes exposed to the same conditions at 37 °C could only be successfully fertilized after 6 h of culture (Li *et al.* 2012). The mechanism behind delayed ageing at low temperatures is likely related to a down-regulation of oocyte metabolism (Chip *et al.* 2011), resulting in reduced levels of ROS production and, subsequently, a decreased likelihood for oxidative damage and ROS-induced apoptosis (Li *et al.* 2012).

Maintenance of MPF levels

Currently, one of the most thoroughly investigated methods for preventing/delaying post-ovulatory oocyte

Figure 1 With increasing amounts of time following ovulation, MII stage oocytes experience an accumulation of ROS and a depletion of GSH resulting in the onset of oxidative stress. Oxidative stress has the potential to initiate the post-ovulatory ageing process via multiple pathways: i) through oxidative damage to the mitochondria resulting in subsequent dysfunction, ii) through inhibition of tyrosine phosphatases and stimulation of tyrosine kinases resulting in a decline in levels of MPF, iii) by causing global damage to proteins and DNA, iv) by reducing the function of Ca²⁺ channels and ATPases in the ER as well as affecting the Ca²⁺ signalling molecule calmodulin resulting in perturbed Ca²⁺ homeostasis and v) through peroxidation of lipids in the plasma membrane, decreasing membrane fluidity and potentially affecting the oocytes' potential for fusion with the spermatozoon. The accumulation of these factors, potentially as a result of oxidative stress, results in the culmination of an 'aged' oocyte.

ageing *in vitro* is by maintaining levels of critical cell cycle factors within the cell. Caffeine is one such compound that has been utilized in this regard, as it acts to inhibit phosphorylation of MPF to pre-MPF by Wee1/Myt1. Supplementation of oocyte culture media with caffeine has indeed been shown to maintain levels of MPF post-ovulation and to subsequently decrease levels of parthenogenesis and fragmentation (Kikuchi *et al.* 2002). Additionally, recent studies have established that the addition of caffeine or MG132 (a proteasome inhibitor) to oocyte culture can increase fertilization rates by ICSI and decrease fragmentation in embryos (Ono *et al.* 2011). Further modes of action for caffeine in relation to post-ovulatory ageing include a delay in the deterioration of Ca²⁺ release mechanisms, although the underlying pharmacology is not yet clear (Zhang *et al.* 2011).

While live offspring have been obtained using oocytes that were exposed to caffeine treatment (Ono *et al.* 2011), previously published research has implicated

caffeine as an inhibitor of DNA repair mechanisms (Selby & Sancar 1990). This impairment of DNA repair has been demonstrated to directly affect oocytes, as caffeine-treated golden hamster eggs lost their capacity to repair aberrations in both chromatids and chromosomes following fertilization by spermatozoa possessing DNA damage (Genesca *et al.* 1992). This clearly creates concern as to the safety of utilizing caffeine as an agent for preventing post-ovulatory oocyte ageing *in vitro*, as un-repaired DNA damage within the embryo may result in abnormalities/birth defects in offspring.

In a similar fashion to caffeine, MG132 has also been demonstrated to maintain high MPF levels in oocytes *in vitro*, by preventing degradation of cyclin B. Oocytes treated with MG132 have been shown to produce embryos with improved developmental potential; however, the capacity to produce live offspring is reduced (Yu *et al.* 2005), potentially as a result of inhibition of necessary protein degradation (Gao *et al.* 2005). Again,

MG132 is clearly not a suitable compound for utilization in an ART setting.

In addition to the aforementioned impacts of caffeine or MG132 on cultured oocytes, recent research has suggested that not all facets of post-ovulatory ageing can be controlled by manipulation of cell cycle factors. It has been demonstrated that caffeine-supplemented oocytes do not experience any delay in the onset of age-associated apoptosis, nor any relief from accumulation of oxidative stress (Lord *et al.* 2013). If accumulation of oxidative stress is indeed an upstream event that is intricately linked with age-associated declines in critical cell cycle factors, then relief from oxidative stress by way of antioxidant supplementation should maintain MPF/MAPK levels *in vitro*.

Antioxidants

If oxidative stress acts as a 'trigger' for ageing in post-ovulatory oocytes, then it would be expected that antioxidant treatment would effectively delay this process, either via supplementation of oocyte culture medium to delay ageing *in vitro* or by oral administration to prevent ageing of oocytes *in vivo*.

In vitro studies have indeed demonstrated that antioxidant supplementation can attenuate the process of post-ovulatory oocyte ageing; however, this phenomenon is reliant on the type of antioxidant utilized. Early research by Tarin *et al.* (1998) demonstrated that L-ascorbic acid (vitamin C) and 6-methoxy-2,5,7,8-tetramethylchlormane-2-carboxylic acid (trolox/vitamin E) could not significantly improve fertilization rate nor prevent fragmentation associated with post-ovulatory ageing, while β -mercaptoethanol and L-cysteine in fact decreased the likelihood of aged oocytes reaching the blastocyst stage following fertilization. However, this research also established that addition of the reducing agent dithiothreitol (DTT) to oocyte culture medium resulted in increased fertilization and blastocyst formation rates, potentially by protecting free thiol groups within these 'ageing' oocytes from becoming oxidized. Despite the reported benefits associated with DTT, the application of this method for delaying post-ovulatory ageing in a clinical setting is limited as DTT has been associated with DNA damage within cells (Held *et al.* 1996, Oikawa *et al.* 2002).

Treatment of oocytes with the nitric oxide (NO $\cdot$) donor S-nitroso-N-acetylpenicillamine has also been shown to attenuate certain signs associated with post-ovulatory ageing such as cortical granule exocytosis, zona hardening and poor embryo quality (Goud *et al.* 2008b). NO $\cdot$ has been identified in multiple studies as a free radical crucial to oocyte and embryo function (Gouge *et al.* 1998, Kuo *et al.* 2000). NO $\cdot$ does exhibit antioxidant characteristics – specifically a capacity to scavenge O $_2\cdot^-$ (Robak & Gryglewski 1993);

however, the exact mechanism by which NO $\cdot$ elicits its age-attenuating effects remains elusive.

More recently, a study conducted by our laboratory (Lord *et al.* 2013) has identified that the potent antioxidant melatonin provides protection from post-ovulatory ageing when used to supplement *in vitro* culture medium. Oocytes treated with melatonin experienced a delayed onset of apoptosis, decreased levels of fragmentation, an increased optimal window for fertilization and improved embryo quality when compared with control aged oocyte counterparts. These effects are presumably a direct result of relief from oxidative stress, as melatonin-treated aged oocytes exhibited significantly reduced levels of ROS (Lord *et al.* 2013). Further to this, previous studies have highlighted the ability of melatonin to reverse the deleterious effects of H $_2$ O $_2$ treatment on MII oocytes (Tamura *et al.* 2008) and prevent hypochlorous acid-induced abnormalities in chromosomes and microtubules (Banerjee *et al.* 2012). Importantly, melatonin has a lack of demonstrable toxicity (Jahnke *et al.* 1999), making it a primary candidate for utilization in an assisted reproduction setting.

Although the ability of antioxidants to influence post-ovulatory ageing is well characterized *in vitro*, the capacity for these compounds to delay *in vivo* ageing is poorly defined. Oral administration of antioxidants such as co-enzyme Q10, melatonin and N-acetyl-L-cysteine has been linked with improved oocyte quality, as well as increased fertilization rates and litter sizes in clinical and laboratory studies (Tamura *et al.* 2008, Burstein *et al.* 2009, Liu *et al.* 2012); however, these reports focus primarily on the relationship between antioxidants and ovarian ageing. To the authors' knowledge, no *in vivo* studies have been conducted to establish the effects of orally administered antioxidants on post-ovulatory ageing specifically.

Although the beneficial effects of antioxidants on post-ovulatory oocyte ageing are dependent on the antioxidant utilized, these compounds may be at the forefront in terms of delaying *in vitro* ageing prior to IVF or ICSI, particularly if oxidative stress does act as the 'trigger' for downstream pathological changes. The variation in therapeutic potential between antioxidants trialled to date may be attributed to the different reactive species scavenged by each compound, their stability in *in vitro* culture media, their capacity to infiltrate multiple organelles within the cell and also their potency at concentrations that are considered safe for clinical use. Following the success of antioxidant supplementation *in vitro*, it would be beneficial to establish the capability of these compounds to delay the onset of senescence in MII oocytes *in vivo*.

Concluding remarks

Ageing of post-ovulatory oocytes clearly has a major impact on fertilization potential, the quality of resulting

embryos, the likelihood of carrying a pregnancy to term and the health and well-being of offspring. Although factors such as MPF/MAPK decline and mitochondrial dysfunction are known to be involved in orchestrating post-ovulatory ageing, the exact molecular mechanisms that control these processes remain elusive. This review has proposed that oxidative stress may act as the 'initiator' of a chain of events that create the aged oocyte phenotype. Oxidative stress can be directly linked not only with MPF decline and mitochondrial dysfunction but also with the acquisition of many abnormalities in oocyte biology such as aberrant Ca^{2+} homeostasis and damage to proteins, lipids and DNA. We have proposed that relieving oxidative stress using antioxidant supplementation may provide a safe and effective avenue for delaying post-ovulatory ageing, specifically in an assisted reproduction setting where extended periods of time between ovulation and fertilization may be unavoidable.

Declaration of interest

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

Funding

The research underlining this review was funded by grants from the National Health and Medical Research Council of Australia and the Australian Research Council to R J Aitken. T Lord is the recipient of an Australian Postgraduate Award PhD scholarship.

References

- Badenas J, Santalo J, Calafell JM, Estop AM & Egozcue J 1989 Effect of the degree of maturation of mouse oocytes at fertilization: a source of chromosome imbalance. *Gamete Research* **24** 205–218. (doi:10.1002/mrd.1120240208)
- Banerjee J, Maitra D, Diamond MP & Abu-Soud HM 2012 Melatonin prevents hypochlorous acid-induced alterations in microtubule and chromosomal structure in metaphase-II mouse oocytes. *Journal of Pineal Research* **53** 122–128. (doi:10.1111/j.1600-079X.2012.00977.x)
- Ben-Rafael Z, Kopf GS, Blasco L, Tureck RW & Mastroianni LJ 1986 Fertilization and cleavage after reinsemination of human oocytes *in vitro*. *Fertility and Sterility* **45** 58–62.
- Bentov Y & Casper RF 2013 The aging oocyte – can mitochondrial function be improved? *Fertility and Sterility* **99** 18–22. (doi:10.1016/j.fertnstert.2012.11.031)
- Bershadsky AD, Gelfand VI, Svitkina TM & Tint IS 1980 Destruction of microfilament bundles in mouse embryo fibroblasts treated with inhibitors of energy metabolism. *Experimental Cell Research* **127** 421–429. (doi:10.1016/0014-4827(80)90446-2)
- Boerjan ML & de Boer P 1990 First cell cycle of zygotes of the mouse derived from oocytes aged postovulation *in vivo* and fertilized *in vivo*. *Molecular Reproduction and Development* **25** 155–163. (doi:10.1002/mrd.1080250208)
- Brisson M, Foster C, Wipf P, Joo B, Tomko RJJ, Nguyen T & Lazo JS 2007 Independent mechanistic inhibition of cdc25 phosphatases by natural product caulibugulone. *Molecular Pharmacology* **71** 184–192. (doi:10.1124/mol.106.028589)
- Budd SL & Nicholls DG 1998 Mitochondria in the life and death of neurons. *Essays in Biochemistry* **33** 43–52.
- Burstein E, Perumalsamy A, Bentov Y, Esfandiari N, Jurisicova A & Casper RF 2009 Co-enzyme Q10 supplementation improves ovarian response and mitochondrial function in aged mice. *Fertility and Sterility* **92** S31. (doi:10.1016/j.fertnstert.2009.07.121)
- Chan TM, Chen E, Tatoyan A, Shargill NS, Pleta M & Hochstein P 1986 Stimulation of tyrosine-specific protein phosphorylation in the rat liver plasma membrane by oxygen radicals. *Biochemical and Biophysical Research Communications* **139** 439–445. (doi:10.1016/S0006-291X(86)80010-9)
- Chaube SK, Prasad PV, Thakur SC & Shirastav TG 2007 Hydrogen peroxide modulates meiotic cell cycle and induces morphological features characteristic of apoptosis in rat oocytes cultured *in vitro*. *Apoptosis* **10** 863–874. (doi:10.1007/s10495-005-0367-8)
- Chen HL, Copperman AB, Grunfeld L, Sandler B, Bustillo M & Gordon JW 1995 Failed fertilization *in vitro*: second day micromanipulation of oocytes versus reinsemination. *Fertility and Sterility* **63** 1337–1340.
- Chi M, Manchester JK, Yang VC, Curato AD, Strickler RC & Lowry OH 1988 Contrast in levels of metabolic enzymes in human and mouse ova. *Biology of Reproduction* **39** 295–307. (doi:10.1095/biolreprod39.2.295)
- Chian RC, Nakahara H, Niwa K & Funahashi H 1992 Fertilization and early cleavage *in vitro* of ageing bovine oocytes after maturation in culture. *Theriogenology* **37** 665–672. (doi:10.1016/0093-691X(92)90146-I)
- Chip S, Zelmer A, Ogunshola OO, Felderhoff-Mueser U, Nitsch C, Buhrer C & Wellmann S 2011 The RNA-binding protein RBM3 is involved in hypothermia induced neuroprotection. *Neurobiological Disorders* **43** 388–396. (doi:10.1016/j.nbd.2011.04.010)
- Doan TN, Gentry DL, Taylor AA & Elliot SJ 1994 Hydrogen peroxide activates agonist-sensitive Ca^{2+} -flux pathways in canine venous endothelial cells. *Biochemical Journal* **297** 209–215.
- Dodson MG, Minhas BS, Curtis SK, Palmer TV & Robertson JL 1989 Spontaneous zona reaction in the mouse as a limiting factor for the time in which an oocyte may be fertilized. *Journal of In Vitro Fertilization and Embryo Transfer* **6** 101–106. (doi:10.1007/BF01130735)
- Downs SM, Humpherson PG & Leese HJ 2002 Pyruvate utilization by mouse oocytes is influenced by meiotic status and the cumulus oophorus. *Molecular Reproduction and Development* **62** 113–123. (doi:10.1002/mrd.10067)
- Ducibella T, Duffy P, Reindollar R & Su B 1990 Changes in the distribution of mouse oocyte cortical granules and ability to undergo the cortical reaction during gonadotropin-stimulated meiotic maturation and aging *in vivo*. *Biology of Reproduction* **43** 870–876. (doi:10.1095/biolreprod43.5.870)
- Dumollard R, Ward Z, Carroll J & Duchon MR 2007 Regulation of redox metabolism in the mouse oocyte and early embryo. *Development* **134** 455–465. (doi:10.1242/dev.02744)
- Fujino Y, Ozaki K, Yamamasu S, Ito F, Matsuoka I, Hayashi E, Nakamura H, Ogita S, Sato E & Inoue M 1996 DNA fragmentation of oocytes in aged mice. *Human Reproduction* **11** 1480–1483. (doi:10.1093/oxfordjournals.humrep.a019421)
- Gao J, Yao Y & Squier TC 2001 Oxidatively modified calmodulin binds to the plasma membrane Ca-ATPase in a nonproductive and conformationally disordered complex. *Biophysical Journal* **80** 1791–1801. (doi:10.1016/S0006-3495(01)76149-8)
- Gao S, Zhiming H, Kihara M, Adashi E & Latham KE 2005 Protease inhibitor MG132 in cloning: no end to the nightmare. *Trends in Biotechnology* **23** 66–68. (doi:10.1016/j.tibtech.2004.12.007)
- Genesca A, Caballin MR, Miro R, Benet J, Germa JR & Egozcue J 1992 Repair of human sperm chromosome aberrations in the hamster egg. *Human Genetics* **89** 181–186. (doi:10.1007/BF00217120)
- Gordo AC, Wu H, He CL & Fissore RA 2000 Injection of sperm cytosolic factor into mouse metaphase II oocytes induces different developmental fates according to the frequency of $[\text{Ca}^{2+}]$ oscillations and oocyte age. *Biology of Reproduction* **62** 1370–1379. (doi:10.1095/biolreprod62.5.1370)
- Gordo AC, Rodrigues P, Kurokawa M, Jellerette T, Exley GE, Warner C & Fissore RA 2002 Intracellular calcium oscillations signal apoptosis rather than activation in *in vitro* aged mouse eggs. *Biology of Reproduction* **66** 1828–1837. (doi:10.1095/biolreprod66.6.1828)

- Goto K, Noda Y, Mori T & Nakano M 1993 Increased generation of reactive oxygen species in embryos cultured *in vitro*. *Free Radical Biology & Medicine* **15** 69–75. (doi:10.1016/0891-5849(93)90126-F)
- Goud AP, Goud PT, Diamond MP, Gonik B & Abu-Soud HM 2008a Reactive oxygen species and oocyte aging: role of superoxide, hydrogen peroxide and hypochlorous acid. *Free Radical Biology & Medicine* **44** 1295–1304. (doi:10.1016/j.freeradbiomed.2007.11.014)
- Goud PT, Goud AP, Diamond MP, Gonik B & Abu-Soud HM 2008b Nitric oxide extends the oocyte temporal window for optimal fertilization. *Free Radical Biology & Medicine* **45** 453–459. (doi:10.1016/j.freeradbiomed.2008.04.035)
- Gouge RC, Marshburn P, Gordon BE, Nunley W & Huet-Hudson YM 1998 Nitric oxide as a regulator for embryonic development. *Biology of Reproduction* **58** 875–879. (doi:10.1095/biolreprod58.4.875)
- Guerin P, Mouatassim SE & Menezo Y 2001 Oxidative stress and protection against reactive oxygen species in the pre-implantation embryo and its surroundings. *Human Reproduction Update* **7** 175–189. (doi:10.1093/humupd/7.2.175)
- Hamatani T, Falco G, Carter MG, Akutsu H, Stagg CA, Sharov AA, Dudekula DB, VanBuren V & Ko MSH 2004 Age-associated alteration of gene expression patterns in mouse oocytes. *Human Molecular Genetics* **13** 2263–2278. (doi:10.1093/hmg/ddh241)
- Held KD, Sylvester C, Hopcia KL & Biaglow JE 1996 Role of fenton chemistry in thiol-induced toxicity and apoptosis. *Radiation Research* **145** 542–553. (doi:10.2307/3579272)
- Hutchins T & Cortopassi G 1995 A mitochondrial DNA clone is associated with increased risk for Alzheimer disease. *PNAS* **92** 6892–6895. (doi:10.1073/pnas.92.15.6892)
- Igarashi H, Takahashi E, Hiroi M & Doi K 1997 Aging related changes in calcium oscillations in fertilized mouse oocytes. *Molecular Reproduction and Development* **48** 383–390. (doi:10.1002/(SICI)1098-2795(199711)48:3<383::AID-MRD12>3.0.CO;2-X)
- Jahnke G, Marr M, Myers C, Wilson R, Travlos C & Price C 1999 Maternal and developmental toxicity evaluation of melatonin administration orally to pregnant Sprague–Dawley rats. *Toxicological Sciences* **50** 271–279. (doi:10.1093/toxsci/50.2.271)
- Kikuchi K, Naito K, Noguchi J, Shimada A, Kaneko H, Yamashita M, Aoki F, Tojo H & Toyoda Y 2000 Maturation/M-phase promoting factor: a regulator of aging in porcine oocytes. *Biology of Reproduction* **63** 715–722. (doi:10.1095/biolreprod63.3.715)
- Kikuchi K, Naito K, Noguchi J, Kaneko H & Tojo H 2002 Maturation/M-phase promoting factor regulates aging of porcine oocytes matured *in vitro*. *Cloning and Stem Cells* **4** 211–222. (doi:10.1089/15362300260339494)
- Kuijjo LL & Perez GI 2012 Ceramide and mitochondrial function in aging oocytes: juggling a new hypothesis and old players. *Reproduction* **143** 1–10. (doi:10.1530/REP-11-0350)
- Kuo RC, Baxter GT, Thompson SH, Stricker SA, Patton C, Bonaventura J & Epel D 2000 NO is necessary and sufficient for egg activation at fertilization. *Nature* **406** 633–636. (doi:10.1038/35020577)
- Kuroda K 2010 Sperm factor, PLC zeta, and egg activation. *Journal of Mammalian Ova Research* **27** 198–203. (doi:10.1274/jmor.27.198)
- Lash J & Whittaker JR 1974. Concepts of development. In *Fertilization*. Series editor: C R Austin Publisher: Sinauer Associates Inc.
- Leese HJ & Barton AM 1984 Pyruvate and glucose uptake by mouse ova and preimplantation embryos. *Journal of Reproduction and Fertility* **72** 9–13. (doi:10.1530/jrf.0.0720009)
- Li Q, Miao D, Zhou P, Wu Y, Gao D, Wei D, Cui W & Tan J 2011 Glucose metabolism in mouse cumulus cells prevents oocyte aging by maintaining both energy supply and the intracellular redox potential. *Biology of Reproduction* **84** 1111–1118. (doi:10.1095/biolreprod.110.089557)
- Li Q, Wang C, Zhang J, Zhou P, Wang T, Cui W, Luo M & Tan J 2012 Combined inhibitory effects of pyruvate and low temperature on postovulatory aging of mouse oocytes. *Biology of Reproduction* **87** 105. (doi:10.1095/biolreprod.112.103127)
- Liang XW, Zhu JQ, Miao Y, Liu J, Wei L, Lu S, Hou Y, Schatten H, Lu K & Sun Q 2008 Loss of methylation imprint of Snrpn in postovulatory aging mouse oocyte. *Biochemical and Biophysical Research Communications* **371** 16–21. (doi:10.1016/j.bbrc.2008.03.105)
- Liang XW, Ge ZL, Wei L, Guo L, Han ZM, Schatten H & Sun QY 2011 The effects of postovulatory aging of mouse oocytes on methylation and expression of imprinted genes at mid-term gestation. *Molecular Human Reproduction* **17** 562–567. (doi:10.1093/molehr/gar018)
- Liu L, Trimarchi JR & Keefe DL 2000 Involvement of mitochondria in oxidative stress-induced cell death in mouse zygotes. *Biology of Reproduction* **62** 1745–1753. (doi:10.1095/biolreprod62.6.1745)
- Liu H, Wang T & Huang K 2009a Cholestane-3B, 5a, 6B-triol-induced reactive oxygen species production promotes mitochondrial dysfunction in isolated mice liver mitochondria. *Chemico-Biological Interactions* **179** 81–87. (doi:10.1016/j.cbi.2008.12.003)
- Liu N, Wu Y, Lan G, Sui H, Ge L, Wang J, Liu Y, Qiao T & Tan J 2009b Pyruvate prevents aging of mouse oocytes. *Reproduction* **138** 223–234. (doi:10.1530/REP-09-0122)
- Liu J, Liu M, Ye X, Liu K, Huang J, Wang L, Ji G, Liu N, Tang X, Baltz JM *et al.* 2012 Delay in oocyte ageing in mice by the antioxidant N-acetyl-L-cysteine (NAC). *Human Reproduction* **27** 1411–1420. (doi:10.1093/humrep/des019)
- Longo FJ 1981 Changes in the zones pellucidae and plasmalemma of aging mouse eggs. *Biology of Reproduction* **25** 399–411. (doi:10.1095/biolreprod25.2.399)
- Lord T, Nixon B, Jones KT & Aitken RJ 2013 Melatonin prevents post-ovulatory oocyte aging in the mouse and extends the window for optimal fertilization *in vitro*. *Biology of Reproduction* **88** 1–9. (doi:10.1095/biolreprod.112.106450)
- Ma W, Zhang D, Hou Y, Li Y, Sun Q, Sun X & Wang W 2005 Reduced expression of MAD2, BCL2, and MAP kinase activity in pig oocytes after *in vitro* aging are associated with defects in sister chromatid segregation during meiosis II and embryo fragmentation after activation. *Biology of Reproduction* **72** 373–383. (doi:10.1095/biolreprod.104.030999)
- Mailhes JB, Young D & London SN 1998 Postovulatory ageing of mouse oocytes *in vivo* and premature centromere separation and aneuploidy. *Biology of Reproduction* **58** 1206–1210. (doi:10.1095/biolreprod58.5.1206)
- Marston JH & Chang MC 1964 The fertilizable life of ova and their morphology following delayed insemination in mature and immature mice. *Journal of Experimental Zoology* **155** 237–251. (doi:10.1002/jez.1401550211)
- Mass DHA, Storey BT & Mastroianni L 1976 Oxygen tension in the oviduct of the rhesus monkey (*Macaca mulatta*). *Fertility and Sterility* **27** 1312–1317.
- Mastroianni L & Jones R 1965 Oxygen tension within the rabbit fallopian tube. *Journal of Reproduction and Fertility* **39** 99–102.
- Mayer W, Nivelelari A, Walter J, Fundele R & Haaf T 2000 Demethylation of the zygotic paternal genome. *Nature* **403** 501–502. (doi:10.1038/35000656)
- Melov S, Coskun P, Patel M, Tuinstra R, Cottrell B, Jun AS, Zastawny TH, Dizdaroğlu M, Goodman SI, Huang TT *et al.* 1999 Mitochondrial disease in superoxide dismutase 2 mutant mice. *PNAS* **96** 846–851. (doi:10.1073/pnas.96.3.846)
- Miao Y, Liu X, Qiao T, Miao D, Luo M & Tan J 2005 Cumulus cells accelerate aging of mouse oocytes. *Biology of Reproduction* **73** 1025–1031. (doi:10.1095/biolreprod.105.043703)
- Minami N, Suzuki T & Tsukamoto S 2007 Zygotic gene activation and maternal factors in mammals. *Journal of Reproduction and Development* **53** 707–715. (doi:10.1262/jrd.19029)
- Monteiro HP, Ivanschenco Y, Fisher R & Stern A 1991 Inhibition of protein tyrosine phosphatase activity by diamide is reversed by epidermal growth factor in fibroblasts. *FEBS Letters* **295** 146–148. (doi:10.1016/0014-5793(91)81405-W)
- Oikawa S, Hiraku Y, Fujiwara T, Saito I & Kawanishi S 2002 Site-specific hydroxylation at polyguanosine in double-stranded DNA by nickel(II) in the presence of SH compounds: comparison with singlet oxygen-induced DNA damage. *Chemical Research in Toxicology* **15** 1017–1022. (doi:10.1021/tx0101752)
- Ono T, Mizutani E, Li C, Yamagata K & Wakayama T 2011 Offspring from intracytoplasmic sperm injection of aged mouse oocytes treated with caffeine or MG132. *Genesis* **49** 460–471. (doi:10.1002/dvg.20756)
- Perez GI, Trbovich AM, Gosden RG & Tilly JL 2000 Mitochondria and the death of oocytes. *Nature* **403** 500–501. (doi:10.1038/35000651)
- Perez GI, Juriscova A, Matikainen T, Moriyama T, Kim MR, Takai Y, Pru JK, Kolesnick RN & Tilly JL 2005 A central role for ceramide in the age-related acceleration of apoptosis in the female germline. *FASEB Journal* **19** 860–862.

- Qiao T, Liu N, Miao D, Zhang X, Han D, Ge L & Tan J 2007 Cumulus cells accelerate aging of mouse oocytes by secreting a soluble factor(s). *Molecular Reproduction and Development* **75** 521–528. (doi:10.1002/mrd.20779)
- Robak J & Gryglewski RJ 1993 Nitric oxide donors as generators and scavengers of superoxide anion. *Polish Journal of Pharmacology* **45** 51–58.
- Rohn TT, Hinds TR & Vincenzi FF 1993 Ion transport ATPases as targets for free radical damage. Protection by an aminosteroid of the Ca^{2+} pump ATPase and $\text{Na}^{+}/\text{K}^{+}$ pump ATPase of human red blood cell membranes. *Biochemical Pharmacology* **46** 525–534. (doi:10.1016/0006-2952(93)90530-A)
- Schultz GA & Heyner S 1992 Gene expression in pre-implantation mammalian embryos. *Mutation Research* **296** 17–31. (doi:10.1016/0165-1110(92)90029-9)
- Selby CP & Sancar A 1990 Molecular mechanisms of DNA repair inhibition by caffeine. *PNAS* **87** 3522–3525. (doi:10.1073/pnas.87.9.3522)
- Shigenaga MK, Hagen TM & Ames BN 1994 Oxidative damage and mitochondrial decay in aging. *PNAS* **91** 10771–10778. (doi:10.1073/pnas.91.23.10771)
- Sohal RS & Dubey A 1994 Mitochondrial oxidative damage, hydrogen peroxide release, and aging. *Free Radical Biology & Medicine* **16** 621–626. (doi:10.1016/0891-5849(94)90062-0)
- Sont JK & Vandenbroucke JP 1993 Life expectancy and mitochondrial DNA. Do we inherit longevity from our mother's mitochondria? *Journal of Clinical Epidemiology* **46** 199–201. (doi:10.1016/0895-4356(93)90058-9)
- Spielmann H, Kruger C, Stauber M & Vogel R 1985 Abnormal chromosome behavior in human oocytes which remained unfertilized during human *in vitro* fertilization. *Journal of In Vitro Fertilization and Embryo Transfer* **2** 138–142. (doi:10.1007/BF01131500)
- Sullivan SG, Chiu DT, Errasfa M, Wang JM, Qi JS & Stern A 1994 Effects of H_2O_2 on protein tyrosine phosphatase activity in HER14 cells. *Free Radical Biology & Medicine* **16** 399–403. (doi:10.1016/0891-5849(94)90042-6)
- Sun Q, Miao Y & Schatten H 2009 Towards a new understanding on the regulation of mammalian oocyte meiosis resumption. *Cell Cycle* **8** 2741–2747. (doi:10.4161/cc.8.17.9471)
- Szollosi D 1971 Morphological changes in mouse eggs due to aging in the fallopian tube. *American Journal of Anatomy* **130** 209–226. (doi:10.1002/aja.1001300207)
- Takahashi T, Saito H, Hiroi M, Doi K & Takahashi E 2000 Effects of aging on inositol 1,4,5-triphosphate-induced Ca^{2+} release in unfertilized mouse oocytes. *Molecular Reproduction and Development* **55** 299–306. (doi:10.1002/(SICI)1098-2795(200003)55:3<299::AID-MRD8>3.0.CO;2-G)
- Takahashi T, Takahashi E, Igarashi H, Tezuka N & Kurachi H 2003 Impact of oxidative stress in aged mouse oocytes on calcium oscillations at fertilization. *Molecular Reproduction and Development* **66** 143–152. (doi:10.1002/mrd.10341)
- Takahashi E, Igarashi H, Kawagoe J, Amita M, Hara S & Kurachi H 2009 Poor embryo development in mouse oocytes aged *in vitro* is associated with impaired calcium homeostasis. *Biology of Reproduction* **80** 493–502. (doi:10.1095/biolreprod.108.072017)
- Takai Y, Matikainen T, Juriscova A, Kim MR, Trbovich AM, Fujita E, Nakagawa T, Lemmers B, Flavell RA, Hakem R *et al.* 2007 Caspase-12 compensates for lack of caspase-2 and caspase-3 in female germ cells. *Apoptosis* **12** 791–800. (doi:10.1007/s10495-006-0022-z)
- Tamura H, Takasaki A, Miwa I, Taniguchi K, Maekawa R, Asada H, Taketani T, Matsuoka A, Yamagata Y, Shimamura K *et al.* 2008 Oxidative stress impairs oocyte quality and melatonin protects oocytes from free radical damage and improves fertilization rate. *Journal of Pineal Research* **44** 280–287. (doi:10.1111/j.1600-079X.2007.00524.x)
- Tarin JJ, Ten J, Vendrell FJ & Cano A 1998 Dihydrothreitol prevents age-associated decrease in oocyte/conceptus viability *in vitro*. *Human Reproduction* **13** 381–386. (doi:10.1093/humrep/13.2.381)
- Tarin JJ, Perez-Albala S, Aguilar A, Minarro J, Hermenegildo C & Cano A 1999 Long-term effects of postovulatory aging of mouse oocytes on offspring: a two-generational study. *Biology of Reproduction* **61** 1347–1355. (doi:10.1095/biolreprod61.5.1347)
- Tarin JJ, Perez-Albala S & Cano A 2000 Consequences on offspring of abnormal function in ageing gametes. *Human Reproduction Update* **6** 532–549. (doi:10.1093/humupd/6.6.532)
- Tarin JJ, Perez-Albala S, Perez-Hoyos P & Cano A 2002 Postovulatory aging of oocytes decreases reproductive fitness and longevity of offspring. *Biology of Reproduction* **66** 495–499. (doi:10.1095/biolreprod66.2.495)
- Tatone C, Emidio GD, Barbaro R, Vento M, Ciriminna R & Artini PG 2011 Effects of reproductive aging and postovulatory aging on the maintenance of biological competence after oocyte vitrification: insights from the mouse model. *Theriogenology* **76** 864–873. (doi:10.1016/j.theriogenology.2011.04.017)
- Torres-Padilla ME, Bannister AJ, Hurd PJ, Kouzarides T & Zernicka-Goetz M 2006 Dynamic distribution of the replacement histone variant H3.3 in the mouse oocyte and preimplantation embryos. *International Journal of Developmental Biology* **50** 455–461. (doi:10.1387/ijdb.052073mt)
- Wakayama S, Thuan NV, Kishigami S, Ohta H, Mizutani E, Hikichi T, Miyake M & Wakayama T 2004 Production of offspring from one-day-old oocytes stored at room temperature. *Journal of Reproduction and Development* **50** 627–637. (doi:10.1262/jrd.50.627)
- Wang YA, Macaldowie A, Hayward I, Chambers GM & Sullivan EA 2011 Assisted reproductive technology in Australia and New Zealand 2009, *Assisted reproduction technology series no. 15*. AIHW: Canberra, Australia.
- Wesson DE & Elliot SJ 1995 The H_2O_2 -generating enzyme, xanthine oxidase, decreases luminal Ca^{2+} content of the IP_3 -sensitive Ca^{2+} store in vascular endothelial cells. *Microcirculation* **2** 195–203. (doi:10.3109/10739689509146767)
- Whately SA, Curti D & Marchbanks RM 1996 Mitochondrial involvement in schizophrenia and other functional psychoses. *Neurochemical Research* **21** 995–1004. (doi:10.1007/BF02532409)
- Wilcox AJ, Weinberg CR & Baird DD 1998 Post-ovulatory ageing of the human oocyte and embryo failure. *Human Reproduction* **13** 394–397. (doi:10.1093/humrep/13.2.394)
- Wu Y, Wang X, Liu J, Bao Z, Tang D, Wu Y & Zeng SM 2011 BIM EL-mediated apoptosis in cumulus cells contributes to degenerative changes in aged oocytes via a paracrine action. *Theriogenology* **76** 1487–1495. (doi:10.1016/j.theriogenology.2011.06.016)
- Xu Z, Abbott A, Kopf GS, Schultz RM & Ducibella T 1997 Spontaneous activation of ovulated mouse eggs: time-dependant effects on M-phase exit, cortical granule exocytosis, maternal messenger ribonucleic acid recruitment, and inositol 1,4,5-triphosphate sensitivity. *Biology of Reproduction* **57** 743–750. (doi:10.1095/biolreprod57.4.743)
- Yanagida K, Yazawa H, Katayose H, Suzuki K, Hoshi K & Sato A 1998 Influence of oocyte preincubation time on fertilization after intracytoplasmic sperm injection. *Human Reproduction* **13** 2223–2226. (doi:10.1093/humrep/13.8.2223)
- Yoon SY, Jellerette T, Salicioni AM, Lee HC, Yoo MS, Coward K, Parrington J, Grow D, Cibelli JB, Visconti PE *et al.* 2008 Human sperm devoid of PLC, zeta 1 fail to induce Ca^{2+} release and are unable to initiate the first step of embryo development. *Journal of Clinical Investigation* **118** 3671–3681. (doi:10.1172/JCI36942)
- Yoshida M, Ishigati K, Nagai T, Chikyu M & Pursel VG 1993 Glutathione concentration during maturation and after fertilization in pig oocytes: relevance to the ability of oocytes to form male pronucleus. *Biology of Reproduction* **49** 89–94. (doi:10.1095/biolreprod49.1.89)
- Yu Y, Yong J, Li X, Qing T, Qin H, Xiong X, You J, Ding M & Deng H 2005 The proteasomal inhibitor MG132 increases the efficiency of mouse embryo production after cloning by electrofusion. *Reproduction* **130** 553–558. (doi:10.1530/rep.1.00758)
- Zhang N, Wakai T & Fissore RA 2011 Caffeine alleviates the deterioration of Ca^{2+} release mechanisms and fragmentation of *in vitro*-aged mouse eggs. *Molecular Reproduction and Development* **78** 684–701. (doi:10.1002/mrd.21366)

Received 20 March 2013

First decision 14 May 2013

Revised manuscript received 13 August 2013

Accepted 15 August 2013