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REVIEW

Potential effects of age-associated oxidative stress on mammalian oocytes/embryos

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This bioessay aims to explain the different effects of maternal ageing and postovulatory oocyte ageing on mammalian oocytes/embryos under the scope of 'the oxygen radical–mitochondrial injury hypothesis of ageing'. This hypothesis assumes a key role in the senescent process of oxygen radical damage to mitochondrial DNA, proteins and lipids. It is proposed that a decrease in intracellular ATP concentrations and glutathione (GSH)/glutathione disulphide (GSSG) ratio together with a concomitant increase in cytosolic Ca^{2+} are major factors causing the observed detrimental effects of ageing on cytoskeletal fibres, fertilization and embryo development.

Key words: ageing/embryo development/fertilization/mitochondria/oxidative stress

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Introduction

During this century a huge number (more than 300) of theories of ageing have been put forward. This is not surprising if we take into account the fact that in nature ageing exists in many diverse forms and variations making it impossible to postulate a common cause of ageing. For instance, mechanisms of ageing might be different for plants and animals, invertebrates and vertebrates. In addition, organs, tissues and differentiated cells usually have very different patterns of age change and different rates of ageing (for review, see Medvedev, 1990). Although it is doubtful that a single theory can explain all the mechanisms of ageing, there is general consensus that, at least in mammals, an increased accumulation of intracellular oxidative damage with time may play an important role in the process of ageing.

The free radical theory of ageing (Harman, 1956) highlights peroxidative damage to nuclear DNA or cell membranes as the main mechanism for the decay of cellular function with age. This hypothesis, however, has been subsequently modified to assign the key role in the senescent process to oxygen radical damage to mitochondria (Miquel *et al.*, 1980; Miquel and Fleming, 1986; Shigenaga *et al.*, 1994). According to this view, reactive oxygen species (ROS) such as superoxide anion (O^{2-}), hydrogen peroxide (H_2O_2) and the highly toxic hydroxyl free radical (OH˙) are produced continuously in mitochondria because of the 'leakage' of high energy electrons along the electron transport chain. Although mitochondrial proteins and lipids can be damaged, mitochondrial DNA (mtDNA) is a major target for oxidative attack because of its location near the inner mitochondrial membrane sites where oxidants are formed, as well as its lack of both protective histones and of DNA repair activity (for review, see Shigenaga *et al.*, 1994). Accumulated oxidative damage to mtDNA, proteins and lipids may result in mutation, inactivation or loss of mtDNA, synthesis of abnormal proteins, accumulation of oxidized dysfunctional proteins and changes in membrane lipid composition. All these effects may inhibit mitochondrial replication, reduce the efficiency of the electron transport chain, increase the likelihood of ROS formation, inactivate key metabolic enzymes and increase membrane permeability, resulting in a reduction in the number of functionally-intact mitochondria and hence in ATP concentrations (for reviews see Miquel and Fleming, 1986; Shigenaga *et al.*, 1994). The increased production of ROS by defective mitochondria may decrease intracellular glutathione (GSH)/glutathione disulphide (GSSG) ratio. This, in turn, may cause impairment of Ca^{2+} transport and subsequent perturbation of intracellular Ca^{2+} homeostasis, resulting in a sustained increase in cytosolic Ca^{2+} concentration (Beatrice *et al.*, 1984) (for review, see Orrenius *et al.*, 1992).

Accumulation of oxygen radical damage to mitochondria may be especially patent in post-mitotic differentiated cells such as neurons. Although rates of repair may be low in post-mitotic cells, fast-replicating cells such as spermatogonia age more slowly because renewal of macromolecules in each cellular cycle may aid organelle protection (Miquel *et al.*, 1978). Endometrial stem cells also appear to age more slowly than post-mitotic cells. They retain the potential to reorganize and regenerate an entire functional endometrium until at least the sixth decade if they are stimulated to divide with appropriate hormonal replacement therapy (Antinori *et al.*, 1995). However, primary oocytes surrounded by a single squamous layer of pregranulosa cells (primordial follicles) are arrested at the

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diplotene stage of the first meiotic prophase from fetal stages, or soon after birth, until stimulated to grow and resume meiosis at the periovulation time. During this 'resting' period, which in women may last ⩾45 years, oxidative stress may be generated in oocytes and/or surrounding ovarian cells and cause a decrease in oocyte/embryo viability with the resulting reduction in pregnancy rates as well as increase in spontaneous abortion rates (for reviews on effects of maternal ageing on reproductive function see Meldrum, 1993; Vom Saal *et al.*, 1994; Yaron *et al.*, 1994). It is important to state at this point that the term 'resting' refers only to meiotic inactivity since 'quiescent' oocytes appear to be metabolically active. They apparently synthesize RNAs such as epidermal growth factor (EGF) (Singh *et al.*, 1995) and cholinesterase (Malinger *et al.*, 1989) mRNAs as well as proteins such as EGF (Singh *et al.*, 1995) and the rabbit 55 kDa zona pellucida protein (Lee and Dubar, 1993).

Recently, it has been shown that oocytes from older women (⩾38 years of age) are 3.3 times more likely to contain the 5 kb 'common' mtDNA deletion than oocytes from younger women (Keefe *et al.*, 1995). In addition, preliminary data show that oocytes from aged (40–48 week old) mice do indeed suffer oxidative stress. They exhibit lower GSH concentration and decreased GSH/GSSG ratio when compared to oocytes from young (8–12 week old) and prepubertal (21–25 day old) mice (J.J.Tarín, J.Sastre, F.J.Vendrell *et al.*, unpublished). Boerjan and de Boer (1990) also reported that ageing of mouse oocytes *in vivo* is associated with decreased cytoplasmic concentration of total glutathione (GSH plus GSSG), suggesting a concomitant decline in GSH/GSSG ratio.

In the first part of this bioessay, I review the effects of both maternal ageing and postovulatory oocyte ageing on cytoskeleton, organelle distribution, fertilization, and development *in vitro* and *in vivo* of mammalian oocytes/embryos. Then, I propose mechanisms by which these effects are produced. My point of view is framed within the 'oxygen radical–mitochondrial injury hypothesis of ageing'.

Effects of maternal and oocyte ageing on mammalian oocytes/embryos

The main effects of maternal and oocyte ageing on mammalian oocytes embryos are shown in Table I. In humans, maternal ageing does not appear to affect cleavage rate and embryo morphology (Feldberg *et al.*, 1990; Yaron *et al.*, 1993). Reduced cleavage rates of embryos from middle-aged women may be overlooked because of the low frequency of observations made (at best, once a day) as well as the short-term culture period (usually until the 4–8-cell stage) employed before transfer or cryopreservation. This short period of embryo culture precludes cell counting at the morula and blastocyst stages, points of embryo development *in vitro* where differences in cleavage rates between groups would be maximum. On the other hand, a lack of effect of maternal age on embryo morphology may be explained by the short-term culture period, the rather subjective methods for classification of embryo morphology utilized in in-vitro fertilization (IVF) centres, and the high degree of cellular fragmentation that characterizes human embryo development *in vitro*. All these factors may conceal subtle or even gross morphological differences between embryos from young and middle-aged women.

Unfortunately, there is a limited number of studies analysing the ultrastructure of oocytes from middle-aged females. In the rat, maternal age is associated with an increased proportion of germinal vesicle oocytes exhibiting undulations in the nuclear membrane. The nucleoplasm appears more dense and the nuclear membrane thicker than in control oocytes. The stacking arrangement of 'sheets' of intermediate filaments typical of mammalian oocytes (Gallicano *et al.*, 1994) deteriorates, with only small single filaments remaining. The number of cortical granules is decreased. The microvilli are shorter and their frequency along the plasma membrane is reduced. Furthermore, the fibre network of the zona pellucida is arranged into continuous bands and the cytoplasmic projections of the cumulus cells which penetrate the zona pellucida are retracted (Peluso *et al.*, 1980).

Data on the effects of post-ovulatory ageing of human oocytes on embryo development *in vitro* and *in vivo* are contradictory. Whereas some studies report no effect of oocyte ageing on cleavage rate and/or embryo morphology after intracytoplasmic sperm injection (ICSI) (Nagy *et al.*, 1993; Tsirigotis *et al.*, 1995) or subzonal insemination (SUZI) (Wiker *et al.*, 1993; Imoedemhe and Sigue, 1994), others show increased fragmentation following conventional IVF methods (Imoedemhe and Sigue, 1994). Likewise, although most authors point out that aged oocytes have a reduced potential for implantation and development *in vivo* (Chen *et al.*, 1995) (for review, see Winston *et al.*, 1993), others claim that high pregnancy rates can be achieved after reinsemination of aged oocytes following conventional IVF (Ashkenazi *et al.*, 1990; Pool *et al.*, 1990) or assisted fertilization (Wiker *et al.*, 1993; Imoedemhe and Sigue, 1994). Discrepancies among studies may be explained by differences in maturation status of oocytes at insemination (Winston *et al.*, 1993); sperm source used for reinsemination (fresh versus aged spermatozoa and husband's versus donor's spermatozoa); and criteria used for allocating inseminated–unfertilized oocytes to conventional IVF or assisted fertilization techniques.

Potential effects of age-associated oxidative stress on oocytes/embryos

Cytoskeletal fibres

As mentioned above, oxygen radical damage to mitochondrial DNA, proteins and lipids is associated with decreased intracellular ATP levels, drop in GSH/GSSG ratio and increased cytosolic Ca^{2+}. Each of these parameters acting separately or together may affect the dynamic equilibrium of microtubules (assembly–disassembly of tubulins) and/or microfilaments (assembly–disassembly of actin) and so induce the aforementioned cytoskeletal alterations observed in aged oocytes/embryos (Tarín, 1995). Among these cytoskeletal alterations, the increased frequency of aneuploidy, inhibition of extrusion of the first polar body, and increased incidence of cellular fragmentation and gross abnormal morphology are of particular

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Table I. Effects of maternal and oocyte ageing on mammalian oocytes/embryos

Effect	Maternal ageing	Oocyte ageing
Aggregates of tubuli of smooth endoplasmic reticulum	–	Yes[1]
Aggregates of small mitochondria-vesicle complexes	–	Yes[2]
Loss of actin microfilaments beneath the plama membrane	–	Yes[3,4]
Structural alteration of microvillar extensions of the plasma membrane from a long slender profile to a short and bulbous or club-shaped appearance	–	Yes[1]
Reduction of pole-to-pole distance of the MII spindle	Yes[5]	–
Disruption, structural alterations and centripetal migration of the spindle	–	Yes[3–4, 6–7]
Scattering, decondensation of chromosomes and formation of a single chromatid mass (restitution nucleus) in which individual chromosomes are not discernible	–	Yes[6–8]
Chromosome fragmentation	Yes[9]	Yes[10]
Shorten the duration of prophase I	Yes[11]	–
C-meiosis	Yes[5]	–
Aneuploidy	Yes[12–14]	No[15–17]
Non-extrusion of the first polar body	Yes[18–19]	–
Non-extrusion of the second polar body	–	Yes[3, 15–16]
Polyspermy	Yes[20–21]	Yes[17, 22–23]
Cytoplasmic fragmentation	Yes[24–27]	Yes[28]
Spontaneous parthenogenetic activation	–	Yes[29–30]
Centripetal migration and/or spontaneous release of cortical granules into the perivitelline space	–	Yes[31–33]
Structural alteration and hardening of the zona pellucida	Yes[34–36]	Yes[31, 34, 37–39]
Alteration in the carbohydrate composition of the plasma membrane	–	Yes[37]
Increased uptake of leucine	–	Yes[40]
Rise in acid phosphatase positive organelles (lysosomes)	–	Yes[28]
Changes in the electrophoretic pattern of synthesized proteins	–	Yes[41–42]
Decreased fertilization	Yes[20, 27, 43–45]	Yes[49–51]
	No[21, 26, 46–48]	–
Shortening of the duration of the first cell cycle	–	Yes[52–53]
Inhibition of full pronuclear development	–	Yes[50, 54–55]
Formation of multiple small-sized pronuclei	–	Yes[56–57]
Abnormal development of embryos	Yes[24–27]	Yes[49–51]
Increased embryonic mortality	Yes[58–60]	Yes[49–51]

[1]Longo, 1974; [2]Sundstrom *et al.*, 1985; [3]Webb *et al.*, 1986; [4]Pickering *et al.*, 1988; [5]Eichenlaub-Ritter *et al.*, 1988a; [6]Eichenlaub-Ritter *et al.*, 1988b; [7]Van Wissen *et al.*, 1991; [8]Szollosi, 1971; [9]Tarín *et al.*, 1990; [10]Zenzes and Casper, 1992; [11]Eichenlaub-Ritter and Boll, 1989; [12]Plachot *et al.*, 1988; [13]Munné *et al.*, 1995; [14]see Estop (1989) for review; [15]O'Neill and Kaufman, 1988; [16]Zackowski and Martin-Deleon, 1988; [17]Badenas *et al.*, 1989; [18]Peluso *et al.*, 1980; [19]Roberts and O'Neill, 1995; [20]Page *et al.*, 1983; [21]Feldberg *et al.*, 1990; [22]Ben-Rafael *et al.*, 1986; [23]Pool *et al.*, 1990; [24]Parkening and Soderwall, 1973; [25]Matt *et al.*, 1987; [26]Day *et al.*, 1989; 1991; [27]Brinsko *et al*, 1994; [28]Longo, 1980; [29]Edirisinghe *et al.*, 1986; [30]Bergere *et al.*, 1992; [31]Dodson *et al.*, 1989; [32]Ducibella *et al.*, 1990; [33]see Gulyas (1980) for review; [34]Nogués *et al.*, 1988; [35]Cohen *et al.*, 1992; [36]Stein *et al.*, 1995; [37]Longo, 1981; [38]Gianfortoni and Gulyas, 1985; [39]Fukuda *et al.*, 1992; [40]Carroll and Longo, 1981; [41]Golbus and Stein, 1976; [42]Van Blerkom, 1979; [43]Català *et al.*, 1988; [44]Ball *et al.*, 1989; [45]Yie *et al.*, 1996; [46]Parkening and Chang, 1976; [47]Eppig and O'Brien, 1995; [48]Sharma *et al.*, 1988; [49]see Lanman (1968) for review; [50]see Smith and Lodge (1987) for review; [51]see Winston *et al.* (1993) for review; [52]Fraser, 1979; [53]Boerjan and de Boer, 1990; [54]Tesarik, 1989; [55]Boerjan and Saris, 1991; [56]Nagy *et al.*, 1993; [57]Tesarik, 1993; [58]see Meldrum (1993) for review, [59]see Vom Saal *et al.* (1994) for review; [60]see Yaron *et al.* (1994) for review.

concern, especially for those peri-menopausal women planning pregnancies.

It is known that cellular ATP deprivation caused by inhibitors of energy metabolism induces a gradual breakdown of actin-containing microfilament bundles (Bershadsky *et al.*, 1980; Svitkina *et al.*, 1986). Such an effect is accompanied by reduction of monomeric (G) actin and concomitant increase in polymeric or filamentous (F) actin from a detergent-soluble or labile pool (Hinshaw *et al.*, 1993). Interestingly, cytochalasin also shortens actin microfilaments and accelerates the polymerization of actin (for review, see Tait and Frieden, 1982). However, the cytoplasmic microtubule network appears to be unaffected by intracellular ATP deprivation caused by prolonged incubation of cells with metabolic inhibitors (e.g. sodium azide, oligomycin, uncouplers) (for review, see Bershadsky and Gelfand, 1983). This is not the case when intracellular GSH is oxidized to GSSG or values of cytosolic Ca^{2+} increase. Both oxidation of GSH to GSSG and an increase in calcium prevent polymerization of tubulin and cause rapid depolymerization of preformed microtubules *in vitro* (Kirschner and Williams, 1974; Berkowitz and Wolff, 1981; Schliwa *et al.*, 1981) and *in vivo* (Kiehart, 1981; Keith *et al.*, 1983; Hori *et al.*, 1993) (for a review on effects of GSH/GSSG ratio on cytoplasmic microtubules *in vitro* and *in vivo*, see Kosower and Kosower, 1978). Oxidation of GSH to GSSG in diamide-treated cells is associated also with fragmentation and shortening of microfilaments together with an increase in actin polymerization (Hinshaw *et al.*, 1991; Rokutan *et al.*, 1994). Likewise, it has been proposed that increased amounts of Ca^{2+} are involved in alterations of actin microfilaments and actin-binding proteins associated with formation of surface blebs during oxidative cell injury (for review, see Orrenius *et al.*, 1992).

Fertilization

Mammalian oocytes and spermatozoa undergo a gradual although opposite change in their intracellular and plasma membrane redox state as they experience nuclear and/or cytoplasmic maturation. Oocytes exhibit a decrease in their

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intracellular GSSG concentration and an increase in their intracellular GSH concentration and GSH/GSSG ratio as they progress from germinal vesicle to metaphase II stage (J.J.Tarín, J.Sastre, F.J.Vendrell *et al.*, unpublished data). On the other hand, spermatozoa enter an oxidative state during epididymal maturation by generating disulphide (-S-S-) cross-linkings in nuclear protamines and proteins of the perinuclear matrix, outer membrane of mitochondria, plasma membrane and tail organelles including the outer dense fibre and the fibrous sheath of the principal piece (Bedford *et al.*, 1973; Reyes *et al.*, 1976; Shalgi *et al.*, 1989) (for review, see Yanagimachi 1994).

Fertilization, therefore, may be inhibited by any factor capable of preventing or counteracting these opposing redox changes in oocytes and spermatozoa. For instance, treatment of guinea pig spermatozoa with the disulphide-reducing agent dithiothreitol inhibits capacitation, acrosome reaction and binding to the zona pellucida and oocyte plasma membrane (Yanagimachi *et al.*, 1983). Age-associated oxidative stress of oocytes may inhibit fertilization by counteracting the reducing potential of oocytes. In particular, oxidative damage to cytoskeletal fibres may cause deterioration of the machinery involved in cortical granule (CG) exocytosis. It may either induce spontaneous release of CG with the resulting changes in the zona pellucida glycoproteins ZP2 and ZP3 (sperm penetration would be prevented), or inhibit or delay CG release after entry of the fertilizing spermatozoon into the oocyte (polyspermy would be promoted).

Peroxidative damage to lipids of the plasma membrane may change lipid composition (Choe *et al.*, 1995) (for review, see Shigenaga *et al.*, 1994) decreasing the fluidity and elasticity of the membrane (sperm–oocyte plasma membrane fusion would be hampered). Lipid peroxidation of oocyte plasma membrane may have a role in triggering the plasma membrane polyspermy block after entry of an 'oxidizing' spermatozoon into an oocyte. It may also explain the fact that aged hamster and rabbit oocytes expel intact CG enclosed within cytoplasmic blebs (for review, see Gulyas, 1980). According to the present model, lipid peroxidation of both CG and plasma membranes may hinder fusion of CG with the plasma membrane; CG contents would not be released into the perivitelline space and zona reaction would not take place (polyspermy would be promoted).

A reduction in the intracellular GSH/GSSG ratio may deprive the oocyte of sufficient reducing power to be able to decondense the sperm nucleus (Perreault *et al.*, 1984, Calvin *et al.*, 1986). It may also induce spontaneous Ca^{2+} release from internal stores by oxidizing sulphydryl (SH) groups in the permeability transition pore (Beatrice *et al.*, 1984) (for review, see Orrenius *et al.*, 1992). The increased cytosolic Ca^{2+} concentration may not only cause spontaneous parthenogenetic activation of aged oocytes but may also interfere with the mechanism triggering oocyte activation at fertilization. Oxidation of GSH to GSSG may also inhibit synthesis of proteins and other macromolecules including RNA and DNA (for review, see Kosower and Kosower, 1978). This may prevent full pronuclear development. Finally, oxidative damage to cytoskeletal fibres may hinder the extrusion of the second polar body and cause dispersion of chromosomes into smaller groups with the resulting formation of multiple small-sized pronuclei.

Cleavage rate and/or arrest in embryo development

A reduction in oocyte/embryo ATP contents with age may inhibit protein synthesis and cellular functions including mitosis, compaction, blastocoel formation and hatching, and so delay and/or arrest embryo development. In fact, in the mouse there is a positive correlation between the amount of ATP in oocytes/embryos and the proportion of those oocytes/embryos reaching the blastocyst stage (Quinn and Wales, 1973) as well as the proportion reaching the expanded and hatched blastocyst stage (Van Blerkom *et al.*, 1995). Furthermore, in humans an association between ATP content of oocytes and later embryo potential for implantation and/or development *in vivo* has been reported (Van Blerkom *et al.*, 1995). In that study, it was found that most of the non-inseminated–unfertilized oocytes from patients who conceived had ATP concentrations in the 2.2–2.6 pmol range. In contrast, the ATP concentrations of the majority of the oocytes from women who did not become pregnant was in the 1.4–1.7 pmol range, with none >2.0 pmol. In the mouse, ATP deprivation appears to increase the incidence of embryos arrested at pre-compaction stages. However, cleavage rate of early human embryos does not appear to be affected by intracellular ATP values (Van Blerkom *et al.*, 1995). As mentioned above, a slower cleavage rate of ATP-deprived embryos may be overlooked because of the limited number of observations made as well as the short-term culture period employed prior to embryo transferral or cryopreservation.

A decreased GSH/GSSG ratio may inhibit not only the synthesis of proteins, RNA and DNA but also the activation of maturation promoting factor (MPF) with the resulting block from ENTRY into the M-phase. This could be achieved by preventing dephosphorylation of the $p34^{cdc2}$ subunit of MPF via inhibition of the tyrosine phosphatase $p80^{cdc25}$, as shown in mouse fibroblast cells treated with the oxidants H_2O_2 (Sullivan *et al.*, 1994) and diamide (Monteiro *et al.*, 1991). This is supported by the fact that addition of superoxide dismutase, a scavenger of O^{2-}, or thioredoxin, a potent protein disulphide reductase, to the culture medium of mouse embryos releases the 2-cell block to development and restores dephosphorylation of the p34cdc2 subunit of MPF at the M-phases of both the first and the second cleavage (Natsuyama *et al.*, 1993). Alternatively, MPF activation may be prevented through activation of proteolysis of the cytostatic factor $p39^{mos}$ by the calcium-dependent cysteine protease calpain (Watanabe *et al.*, 1989; see Orrenius *et al.*, 1992, for review, on involvement of Ca^{2+}-activated proteases in oxidant injury to cells). Paradoxically, prevention of MPF activation may catalyse the EXIT from the M-phase of aged oocytes either spontaneously or subsequent to activation by a spermatozoon at fertilization. Formation of a restitution nucleus in spontaneously activated oocytes (Szollosi, 1971; Eichenlaub-Ritter *et al.*, 1988b; Van Wissen *et al.*, 1991) and shortening of the duration of the first cell cycle in sperm-activated oocytes (Fraser, 1979; Boerjan

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and de Boer, 1990) may be direct consequences of such an event. Likewise, maternal age-associated oxidative stress may alter the balance of phosphorylations and dephosphorylations of cell cycle-related proteins during the first meiotic division resulting in shortening of duration of prophase I. It is worth noting the fact that ROS may affect overall tyrosine phosphorylation not only by inhibiting tyrosine phosphatases (Monteiro *et al.*, 1991; Sullivan *et al.*, 1994) but also by stimulating tyrosine kinases (Chan *et al.*, 1986).

Embryo morphology

The increased incidence of cellular fragmentation and gross abnormal morphology exhibited by embryos derived from aged oocytes may result from oxidative damage to cytoskeletal fibres. The age-associated reduction in GSH/GSSG ratio and the concomitant increase in cytosolic Ca^{2+} concentration may be major factors causing morphological anomalies in embryos. Although based on few experiments, Van Blerkom *et al.* (1995) have shown that in humans ATP content is probably more patient-dependent than related to a specific embryo morphology. They have shown that the ATP concentration of embryos with numerous fragments from patients who conceive is in the 2.6–3.2 pmol range. In contrast, the ATP concentration of embryos with a similar extent of fragmentation from patients who fail to conceive is in the 0.9–1.4 pmol range. The lack of effect by ATP on embryo morphology may be explained by the insensitivity of the cytoplasmic microtubule network to intracellular ATP deprivation.

There is evidence supporting the concept that age-associated oxidative damage to cytoskeletal fibres may result in abnormal embryo morphology. In contrast to the surface of vital blastomeres, which is organized into short, regular microvilli, the surface of cellular fragments exhibits irregularly shaped blebs and protrusions (Alikani and Dale, 1995). Furthermore, in humans the majority of poor quality embryos are either aneuploid or have fragmented chromosomes (Pellestor *et al.*, 1994). As mentioned above, cytoskeletal alterations associated with formation of surface blebs are characteristic signals of oxidative cell injury. Moreover, it has been shown that a number of physical and chemical agents, that are directly or indirectly involved in intracellular free radical production and/or oxidative stress generation, including ionizing (X-rays or γ-rays) and non-ionizing (UV) radiation, quinones, heavy metal ions, anaesthetics, GSH-conjugating and GSH-oxidizing agents, carbamates, organo-mercurial agents, inducers of mixed-function oxidase reaction, cigarette smoke, methylxanthines and oestrogens, are potential inducers of aneuploidy (for reviews see Bond and Chandley, 1983; Onfelt, 1986; Tarín, 1995). Further, a sustained increase in intracellular Ca^{2+} has been shown to promote endonuclease activation and high molecular weight DNA fragmentation in thymocyte apoptosis (Zhivotovsky *et al.*, 1994).

Birth defects, childhood cancer and fertility of offspring

It is well known that paternal ageing leads to heritable mutations responsible for numerous malformations at birth including the dominant conditions achondroplasia, Apert's syndrome, myositis ossificans and Marfan's syndrome, and the recessive condition haemophilia A. Childhood cancer in offspring including bilateral retinoblastoma also appears to be associated with paternal ageing (for reviews see Vogel and Rathenberg, 1975; Auroux, 1983; Ames *et al.*, 1993; Crow, 1993). In contrast, maternal ageing has either no, or much more limited, long-term effects on offspring. This fact is not unexpected if we take into account that, unlike spermatogonia, diplotene oocytes and to less extent metaphase I and II oocytes have an efficient DNA repair system which is essentially independent of maternal age (Guli and Smith, 1989). Thus, any damage to the nuclear DNA of the oocyte caused by either intrinsic (e.g. age-associated oxidative stress) or extrinsic (e.g. maternal exposure to ionizing and non-ionizing radiation or to other mutagenic agents) may be repaired before being transmitted to the offspring. Furthermore, oocytes exhibiting heavy oxidative damage to their intracellular components may be selected against at fertilization and so their traits would not be transmitted to future generations. However, a long-term deletion effect of maternal ageing upon male offspring infertility has been suggested by Cummins *et al.* (1994). The mechanism by which this effect may occur is based on the inheritance of excess loads of maternal defective mitochondria allocated randomly to testis-forming tissues, in particular to precursors of Sertoli and Leydig cells. Prophylactic measures for improving the reproductive function of middle-age women and so reducing the potential short-term (aneuploidy) and long-term (male infertility?) risks to offspring may be based on consumption of dietary antioxidants. However, it is necessary to bear in mind that such a preventive measure may be inefficient if started too late, i.e. at the peri-menopausal period, when extensive oxidative damage to oocytes may have already occurred.

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