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DNA damage and repair in human oocytes and embryos: a review

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Summary

The genome of all cells is protected at all times by mechanisms collectively known as DNA repair activity (DRA). Such activity is particularly important at the beginning of human life, i.e. at fertilization, immediately after and at the very onset of embryonic development. DRA in early development is, by definition, of maternal origin: the transcripts stored during maturation, need to control the integrity of chromatin, at least until the maternal/zygotic transition at the 4- to 8-cell stage in the human embryo. Tolerance towards DNA damage must be low during this critical stage of development. The majority of DNA damage is due to either apoptosis or reactive oxygen species (ROS). Apoptosis, abortive or not, is a common feature in human sperm, especially in oligoasthenospermic patients and FAS ligand has been reported on the surface of human spermatozoa. The susceptibility of human sperm to DNA damage is well documented, particularly the negative effect of ROS (Kodama *et al*., 1997; Lopes *et al*., 1998a,b) and DNA modifying agents (Zenzes *et al*., 1999; Badouard *et al*., 2007). DNA damage in sperm is one of the major causes of male infertility and is of much concern in relation to the paternal transmission of mutations and cancer (Zenzes, 2000; Aitken *et al*., 2003; Fernández-Gonzalez, 2008). It is now clear that DNA damaged spermatozoa are able to reach the fertilization site *in vivo* (Zenzes *et al*., 1999), fertilize oocytes and generate early embryos both *in vivo* and *in vitro*. The effect of ROS on human oocytes is not as easy to study or quantify. It is a common consensus that the maternal genome is relatively well protected while in the maturing follicle; however damage may occur during the long quiescent period before meiotic re-activation (Zenzes *et al*., 1998). In fact, during the final stages of follicular growth, the oocyte may be susceptible to damage by ROS. With regards to the embryo there is active protection against ROS in the surrounding environment i.e. in follicular and tubal fluid (El Mouatassim *et al*., 2000; Guerin *et al*., 2001). DNA repair activity in the zygote is mandatory in order to avoid mutation in the germ line (Derijck *et al*., 2008). In this review we focus on the expression of mRNAs that regulate DNA repair capacity in the human oocyte and the mechanisms that protect the embryo against *de novo* damage.

Keywords: DNA repair, Human, Oocyte, Preimplantation embryo

What is DNA repair?

DNA repair activities are a number of processes that allow the cell to identify and then correct damage to DNA molecules. It is the current consensus that in a somatic cell several hundred thousand DNA repair processes are performed per day. In the embryo, during the first 24 h, immediately before and after the S-phase, the number of lesions to be repaired can reach easily this number, especially if we consider both the maternal and the paternal genome. There are three options for a cell, somatic or embryonic, that is facing DNA damage (Fig. 1): The first is to activate the apoptotic pathways; this activation leads to cell death (Jurisicova & Acton, 2004) that will destroy one or more cells and impair viability. Cell survival will be the result of the balance between pro- and anti-apoptotic factors present in the oocyte. This parameter is often expressed in human embryos when fragmentation occurs. The second option is to tolerate the lesion; this option may lead to mutations and

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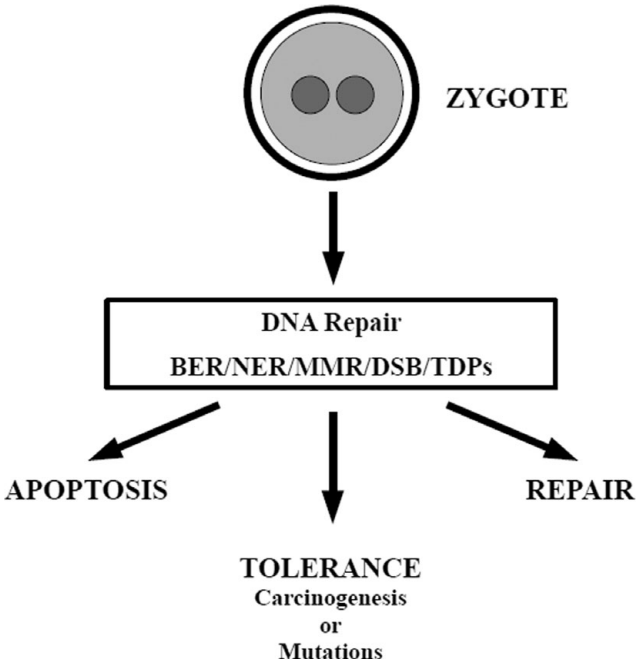

Figure 1 Options for a zygote facing DNA damage.

eventual carcinogenesis in the next generation. The third, and best, option is to repair the lesion.

Apoptosis

Caspases (cysteinyl-aspartate-cleaving proteases) are a group of cysteine proteases that are essential in apoptosis. In the human oocyte, caspases 1 and 10 are not expressed, however caspases 2, 3, 6 and 9 are expressed at 30 to 60 times the background signal. It has been shown that fragmented embryos exhibit a high degree of apoptosis compared with normal embryos (Jurisicova *et al*., 1996, Warner *et al*., 1998). Expression of pro-apoptotic (*BAD*, *BAK*, *BAX*) and anti-apoptotic (*BCL2*, *BCLW*) genes has been observed in the human oocyte (Ménézo *et al*., 2007a; Jaroudi *et al*., 2009) and preimplantation embryo (Jurisicova *et al*. 2003). Defender against apoptosis-1 (Dad-1) is also present in human oocytes at 40 times the background signal, while surviving factor IAP, (inhibitor of apoptosis) was observed at 30 times background. The p53 tumor suppressor, which limits cellular proliferation by inducing cell cycle arrest and apoptosis, is also moderately expressed in the human oocyte (see also Wells *et al*., 2005; Jaroudi *et al*., 2009). Heat shock proteins are members of a complex family that preserves cell survival and may inhibit apoptosis (Mosser *et al*., 1997). We did not find any expression of HSP70 but high expression of its cofactor HSP40 in human oocytes (Ménézo *et al*., 2007a). Apoptosis may occur immediately at the time of fertilization, due to the poor quality of sperm and also during preimplantation development if the DNA repair capacity is inadequate. As embryo fragmentation is an expression of apoptosis it is not clear how the technique of embryo fragment removal (Alikani *et al*., 1999) improves embryo quality. Finally, although inhibition of apoptosis in the bovine embryo leads to increased blastocyst formation, the chromosomal content is altered (Byrne *et al*., 2002). A balance between survival and cell death has to be respected in some way.

DNA repair

First, we should distinguish damage from mutation. Damage is a physical problem: lack of a base or a break in a strand. A mutation is a modification of several bases in a strand. DNA repair mechanisms are not able to recognize mutations and so repair is not possible.

Origin of damage

Most damage originates in the male gamete. Unfortunately little is known about the quality of DNA in human oocytes presumably due to the lack of material available for study. A summary of the major causes of DNA damage is shown in Figure 2.

Oxidation of bases is probably the most important source. This damage may be of environmental origin such as UV-A and B, ionizing radiations, or of endogenous origin. The oxidases xanthine, glycolate, amine (EC1.4.3.6), membrane NADPH oxidases and mitochondrial metabolic activity (OXPHOS) generate ROS. The most common damage observed in DNA oxidation is the formation of 8-oxo-deoxyguanosine (8-OHdG), however other oxidation products may also be detected (Table 1). ROS induces the formation of AP (apurinic/apyrimidic) sites, the most common decay in damaged DNA. ROS can also induce the formation of adducts (Table 1). Peroxidation of membrane polyunsaturated fatty acid leads to malondialdehyde (MDA), 4-hydroxynonenal (4-HNE) and 2,3-epoxy-4-HNE degradation products which form MDA guanine, 4-HNE guanine and 1,N^2-εguanine and 1,N^6-εadenine (Table1). The formation of these adducts and sperm DNA fragmentation appear independent, even if both originate from the action of ROS (Montjean *et al*., 2010). Oxidation processes can also lead to de-amination of the bases (Table 1), with replication of damaged bases leading to wrong opposing bases. Exogenous agents others than those originating from lipid peroxidation can form DNA adducts: e.g. vinyl chloride or benzopyrene in heavy smokers. Adducts

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Table 1 Degradation of bases by oxidation.

Base	Oxidation products formed	Adducts
Adenine	8-oxo-adenine; 2-hydroxy A; Fappy A; hypoxanthine (deamination)	1,N^6 ε etheno A
Guanine	8-OHdG,4OH; 8-OHdG; Fappy G, xanthine (deamination)	MDA G; 4-HNE G; 1,N^2ε G
Thymine	5,6 hydroxyT; 5 OH5–6-dihydroT; T glycol	
Cytosine*	5-OH C; 5,6-diOH C; C glycol, uracil (deamination)	

*Methyl cytosine forms thymine after deamination
MDA G, malonaldehyde guanosine; 4-HNE, G adduct derived from hydroxynonenal; Fappy; formamidopyrimidine; 1,N^6ε ethenoA, 1,N^2ε G, derived from epoxy-4-hydroxy-2-nonenal. 8-OHdG

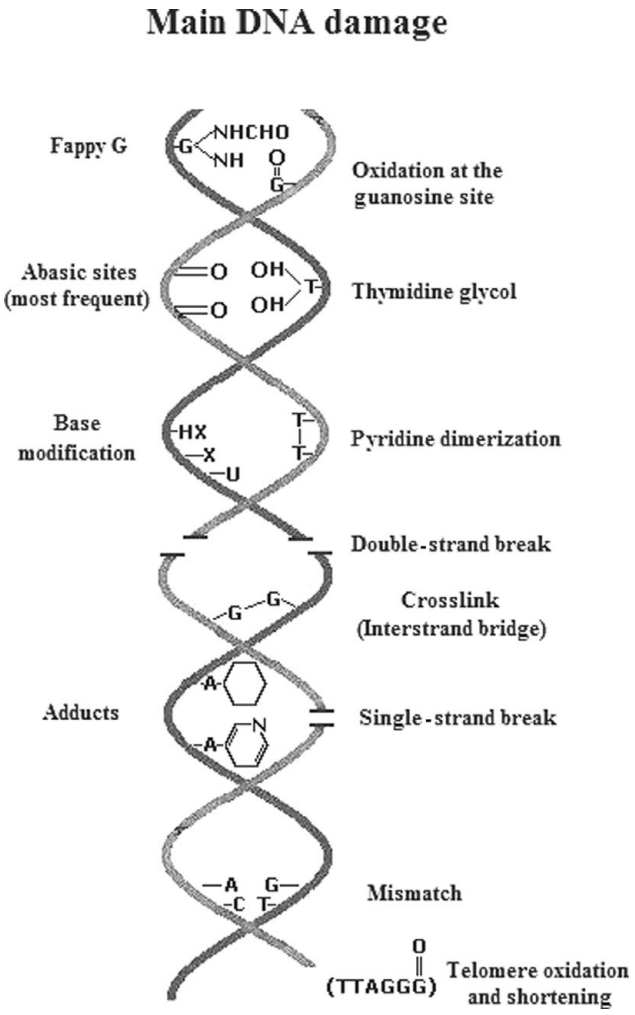

Figure 2 A summary of major causes of DNA damage.

can also lead to the formation of bridges between DNA strands.

Replication errors are also an important cause of AP site formation or mismatched pairing. In fact we may have primary damage, AP site, modification of a base, i.e. oxidation or deamination, that affects the primary structure of DNA. Damage can also affect the secondary structure, single-strand or double-strand breaks and the tertiary structure, i.e., the spatial configuration of the helix: bridges (adducts cancelled). The mechanism of action of DNA repair will depend on the type of decay. There may be direct reversal for lesions in the primary structure, or more complex processes for single-strand damage and double-strand breaks, the most toxic lesions. Decay may be recognized immediately, but more often at the time of replication and/or transcription. As there is none or very little transcription during the first stages of division (Ao *et al*., 1994; that anyway only concerns the sex chromosomes), the transcription block DNA repair activity is probably weakly represented. Shortening of telomeres is a specific DNA decay linked to senescence, which affects both secondary and tertiary structures. Telomeres (TTAGGG) in repeats are moreover very sensitive to oxidation due to the high G content.

Direct reversal of damage (DDR)

In the oocyte and early embryo, this type of repair mechanism is involved in anomalies in the methylation of bases. As oocytes have high methylation activity and imprinting is mainly the result of methylation, this process is of extreme importance (Ménézo *et al*. 1989; Benkhalifa *et al*., 2009). A rather high expression of the enzymes ABH2 (60 times background) and ABH3 (20 times background) involved in DDR has been observed in human oocytes. These di-oxygenases remove 1-methyladenine and 3-methylcytosine from methylated polynucleotides and then un-modified bases are replaced. Methylguanine methyltransferase (MGMT) that reverses 6-*O*-methylguanine is also expressed in human oocytes at 30 times background signal. It can remove alkyl substituent up to benzyl (C^6 aromatic). The correct duplication of the genome is particularly important at this time in development and it is not surprising that several mechanisms are employed for repair.

Single-strand damage repair (SSR)

This pathway is used when one strand only has been damaged. Although less important than double-strand breaks (DSB), SSR needs to be carried out rapidly

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in order to avoid the creation of DSB at replication. Damage can occur at the level of the sugar moiety as well as at the base (see earlier). Topoisomerase may be a source of single-strand breaks (SSB). First the damage needs to be recognised, followed by an incision and then an excision. The complementary strand is used as a pattern to fix the repair. When the repair involves large pieces of strand, following the removal of the damaged zone, polymerase(s) fills the hole and ligase(s) complete the repair.

Base excision repair (BER)

Removes the damage at a single base level (primary structure oxidation, adduct…). After recognition of specific lesions in the DNA, excision is performed near the damage site. As seen in Table 1, uracil can be formed after deamination of cytosine. Uracil-DNA glycosylase UNGs recognize the U damages. The mitochondrial protein, UNG1 is found at 200 times background in human oocytes, while the nuclear protein UNG2 is expressed 100 times background in human oocytes (Ménézo *et al*., 2007a). *N*-methylpurine-DNA glycosylase (MPG), that recognizes ethenoadenosine, hypoxanthine and 3-Me-A, is highly expressed at 100 times background, while OGG1 (8-oxoguanine DNA glycosylase), involved in 8-OHdG and Fappy G damage recognition is moderately expressed and found at 15 times background. For other recognition proteins see (Ménézo *et al*., 2007a). After damage identification, a scaffold is built. AP1 (or APEX/ref1) the major endonuclease initiates the repair, in association with XRCC1, the ligase accessory factor having a main ligation function for ligases 1 and 3, that manage sealing after polymerase beta action. All these components are expressed at a medium intensity at 15 times background in the oocyte except for polymerase beta, which is expressed at 350 times background.

Nucleotide excision repair (NER)

The NER pathway repairs bulky zones of damage that distort the shape of the helix. Large excisions of up to 30 nucleotides including the damaged zone may be repaired. In human oocytes two pathways are active: global genomic NER (GG-NER) and transcription coupled NER (TC-NER), which differ only in their recognition of helix distortion. These pathways are important as severe human diseases result from in-born genetic mutations of NER proteins. Pyrimidine dimers are the main targets removed by NER. In GG-NER, a complex between RAD23, an excision repair protein and xeroderma pigmentosum, complementation group C (XPC), a protein binding to damaged DNA is formed for distortion recognition. Both are highly expressed in the oocyte. Damage-specific DNA binding proteins 1 and 2 (DDB1 and DDB2) can also recognize certain types of damage. DDB1 is expressed at 50 times background; DDB2 is absent. This pathway is preferentially used for non-transcribed strands and transcriptionally inactive DNA. DNA-damage binding (DDB) and XPC–Rad23B complexes permanently check helix distortions in the genome.

The TC-NER pathway repairs the transcribed strands of transcriptionally active genes; it is more rapid than the GG-NER pathway. During pronucleus formation only sexual chromosomes are transcriptionally active. TC-NER and GG-NER pathways differ only in their initial steps of DNA damage recognition. TC-NER starts when RNA polymerase is blocked at the DNA lesion, without any further recognition signal. Next, a complex of human repair protein XPA (xeroderma pigmentosum, complementation group A) and replication protein A (RPA) is formed: surprisingly its expression is very high (45 times and 500 times background), much higher than the XPC–RAD23 complex. The repair patch size for mammalian cells *in vivo* is around 30 nucleotides.

For both GG-NER and TC-NER, DNA polymerase delta or epsilon fills the gap using the other strand as a template by copying the undamaged strand. Proliferating cell nuclear antigen (PCNA) assists DNA polymerase in the reaction and RPA protects the other DNA strand from degradation during NER. Finally, DNA ligase seals the nicks to finish NER. Whereas expression of PCNA is high (800 times background), the expression of both polymerase delta or epsilon is of medium intensity (10 to 30 times background).

Mismatch repair (MMR)

Mismatch repair fixes errors in DNA replication (mis-pairing including G/T or A/C pairing). Mismatches are commonly due to tautomerization of bases during synthesis. During this process, large regions (of up to 1000 base pairs) of DNA containing the damage are involved and can be removed: much in excess of the mismatched nucleotides only. This system repairs abnormal insertion, mis-pairing, but also O^6-methylG, O^4-methylG, 8-oxoG, interstrand crosslinks, thymidine dimerization and deletions. The damage is recognised in relation to the deformation and anomalies of the helical tertiary (spatial) conformation. An important difference with NER is that the removal process may involve from a few nucleotides up to 100s and even a 1000 base nucleotides. The damage has to be first recognised. MSH2–MSH6 (MutSα), for base substitution and MSH2–MSH3 (MutSβ), for loop repair, are protein complexes that recognise the error on the daughter strand and bind the failed DNA. MSH 2, 3 and 6 are highly expressed at 50 times background for each. The DNA clamp that increases the efficiency

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of DNA synthesis, PCNA (proliferating cell nuclear antigen) is very intensively expressed (>800-fold the background signal). PCNA is the sliding clamp for DNA polymerase delta or epsilon in this process. This process minimizes the risk of induction of the apoptosis pathway. The exonuclease 1 (40 times background), the flap structure-specific endonuclease FEN1 (200 times background), the replication protein, RPA1, (500 times background), replication factors (RFCs) and DNA ligase 1, ATP dependant (LIG1, moderately expressed at 15 times background), are all active in this process. Removal of the main oxidation product 8-OHdG is performed by this process rather than the OGG1 base excision repair process.

Double-strand breaks (DSBs)

The precise replication of the genome during S-phase is of fundamental importance especially in the 1-cell embryo. Double-strand breakage is probably the most important type of DNA damage as it induces chromosomal instability and failed rearrangements. DSB repair is performed by two sub-pathways. Non-homologous end joining (NHEJ) and homologous recombination (HR). These pathways have different relative importance. HR is active during the late part (S/G_2) of the cycle, when sister chromatids are available as a template for repair (Rothkamm *et al*., 2003), whereas NHEJ, predominantly active in G_1, is also active throughout the cell cycle. Thus, the phase of the cell cycle determines by and large what type of repair mechanism is operative. HR seems to be the most active system in mammalian cells, when replication fails or blocks (replication fork or replication stalling, see Arnaudeau *et al*., 2001). Finally, NHEJ appears a less reliable mechanism with deletions appearing at the site of the break leading to translocations.

Homologous recombination (HR)

The cell cycle arrest checkpoint at G_1/S or G_2/M allows for correction of error or damage before cell division, especially when stored mRNAs can be a limiting factor. Ataxia telangiectasia mutated (ATM) is a sensor activated by DNA double-strand breaks. It initiates activation of the DNA damage checkpoint Chek2, which is highly expressed. RAD51C, also highly expressed, interacts with BRCA2 or 1, to form complexes for homologous pairing (Yoshida *et al*., 2004). The complex formed uses the undamaged strand as a template for repair. HR passes also through the formation of a complex including MRE11A (3′ exonuclease, highly expressed), Rad50 (ATPase, moderately expressed) and NSB1. Failed damage repair may lead to cell cycle arrest or apoptosis (p53 linked). Another recruitment for HR passes through interaction of BRCA1 and FANC1/FANCD2.

Non-homologous end joining (NHEJ)

The sensor is the heterodimer KU70/80 (helicase) having a low (ku80, less than 10 times background) or nil (Ku70) expression in the oocyte. The DNA cross-link repair 'Artemis' (DCLRE1C, 15 times): DNA-PKcs endonuclease, which cleaves DNA loops, flaps and gaps, has an intermediate expression level. The flap structure-specific endonuclease FEN1 is highly expressed as well (200 times background). The recruited ligase LIG4 (20 times background) and its accessory factor XRRCC4 (80 times background) are well expressed, but their interacting factor (NHEJ1) is not expressed. This pathway is probably not very active as some 'pieces of the puzzle' appear to be missing. This situation is in contrast to the mouse model in which sperm-derived DSB are mainly repaired by NHEJ (Derijck *et al*., 2009). KU70 expression increases significantly at the blastocyst stage in the human (Jaroudi *et al*., 2009) probably indicating a switch in DNA repair pathways at this time in development.

Cell cycle checkpoints are unlikely to function in the early preimplantation embryo (Delanthy *et al*., 1995; Harrisson *et al*., 2000) as the mRNA turnover is ineluctable and there is a race against time in order to avoid loss of mRNAs. Furthermore, the mechanism may induce too much delay at the G_1/S and G_2/M checkpoints and cell division is already slow. The cell cycle checkpoints required for cell cycle arrest are rather well expressed, Chek1 at 100 times background signal and Chek2 at 10 times background signal.

Repair of DNA- protein crosslink

TDP1 (tyrosyl-DNA phosphodiesterase) hydrolyses the phosphodiester bound at 3′. It is found at stalled topoisomerase I (Top1)-DNA covalent complexes. It requires phosphorylation by ATM for optimal function (Das *et al*., 2009). TDP is present at 150 times background.

Reorganization of chromatin structure

After DNA repair (especially DSB) the tertiary structure of the nucleus has to be re-established. H2AFX contributes to histone formation and therefore the structure of DNA. Surprisingly, other key DNA damage response genes *5ATM*, *ChK1* are found on the same region of chromosome 11. It is highly expressed in oocytes at >100 times background. CAF-1 is another complex thought to mediate chromatin assembly in DNA replication and DNA repair with a high expression of 300 times background.

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Telomeres

Telomeres are TTAGGG repeats, capping the chromosome ends and therefore preventing end-to-end fusion. As mentioned earlier, the high number of G moieties renders them highly sensitive to damage from ROS. Defence against ROS decreases with age, which could be a reason for chromosome telomere shortening with age. According to Liu *et al.* (2002) they shorten at each round of DNA replication as mitochondrial dysfunction leads to telomere attrition probably again via insult from ROS. However, oocytes are 'generated' during fetal life. Telomerase, a reverse transcriptase prevents their shortening. Telomeres are short in the oocyte and telomerase activity and mRNA expression not found in the oocyte, start at blastocyst formation. An alternative lengthening of telomeres (ALT) involves telomere–telomere recombination (Keefe & Liu, 2009). Damage repair proteins i.e. RAD50, MRE11 (MRE11 meiotic recombination 11), Bloom and WRN are partners in this process in association with extensive telomere sister-chromatid exchange (T-SCE). They are all expressed in the oocyte (RAD, 100 times background; MRE11, 65 times background; Bloom, 30 times background; WRN, 30 times background). According to Keefe & Liu (2009) and Liu *et al.* (2007), the age-associated meiotic defects observed in oocytes from older patients might be linked to oxidative stress, aberrant DNA damage response and telomere recombination, leading to miscarriages and infertility.

In vivo and *in vitro* protection against ROS in early human preimplantation development

Even under physiological, *in vivo*, conditions, the human genome is continuously subjected to aggression. In IVF/ICSI, *in vitro* manipulations may increase these insults by generating ROS (Pabon *et al.*, 1989; Muratori *et al.*, 2003). For example, spermatozoa are not protected when the seminal plasma is removed. The early embryo has to be protected against newly generated damage, especially as it also generates ROS as a byproduct of its own metabolism. Enzymatic and non-enzymatic defences are present, for example hypotaurine, pyruvate and vitamin E are found in the environment surrounding the male and female gametes. Albumin is also thought to protect the gametes. The oocyte has endogenous protection; mRNAs coding for Cu–Zn (in the cytosol) and Mn SOD (mitochondria) are present in the oocyte, as is glutathione peroxidase. We did not find any transcripts for catalase in human oocytes (El Mouatassim *et al.*, 1999). NADPH is furnished by LDH to regenerate reduced glutathione. Thioredoxins (TRXs) are also highly expressed. TRX increases cell growth and resistance to programmed cell death (Powis *et al.*, 2000). In the tubal fluid, hypotaurine, taurine, catalase, SODs, glutathione synthase and glutathione peroxidase are also actively present, probably released by tubal epithelial cells.

In vitro, the constitution of the culture medium is of paramount importance. Protection against damage may be afforded by addition of antioxidants such as hypotaurine, vitamin C and albumin. As the oxygen tension *in vivo* is less than 10%, thought should be given to this parameter *in vitro* even though it does not appear to be important before genomic activation. The sulphur amino acids are important for regeneration of glutathione, especially as cystathionine beta synthase (the pathway regenerating homocysteine and forming cysteine) is absent in the human oocyte. It has been reported that 'essential amino acids' including cysteine and methionine, are 'toxic' for early embryos (Lane *et al.*, 2001) and many commercial media are produced without methionine. We believe this factor to be an error in human *in vitro* culture medium. For example, lack of methionine prevents formation of endogenous anti-ROS protectants by the embryo and induces caspase-dependent or -independent apoptosis (Liu *et al.*, 2003). Absence of methionine prevents a normal imprinting process, which could lead to genetic problems (Ménézo *et al.*, 2010). Finally, deficiency in folic acid (vitamin B9) in culture medium may induce thymidine starvation (O'Neill, 1998) and a consequent decrease in gene expression. Equally important is the role of folic acid in homocysteine (Hcy) recycling. In addition to defective methylation, Hcy and folate deficiency are involved in cellular dysfunction and ROS linked apoptosis.

Conclusions

The human oocyte is relatively competent to repair DNA damage, thus avoiding tolerance, as a source of mutations. Nonetheless, not all mRNAs for repair are necessarily fully translated, some may be lost before and during translation. The pathways are redundant, but it is not known whether all pathways are used simultaneously at the beginning of development. Part of the mRNAs are translated rapidly and some must be protected and stored, under a non polyadenylated form, until translation at the 4–8-cell stage at the maternal to zygotic transition or even later at the blastocyst stage.

There are little data as to whether it is possible to increase DNA repair capacity in oocytes. Some effectors of oocyte competence may be active (Ménézo & Elder 2010). For example, GH has been described to increase DNA repair capacity in the liver (Thompson *et al.*, 2000), to increase oocyte competence in bovine

oocytes (Izadyar *et al*., 1998) and in poor responders (Kyrou *et al*., 2009). We have detected expression of mRNA for GH receptors in cumulus cells and the oocyte (Ménézo *et al*., 2003). One fact is clear, with increasing maternal age the mRNA stores in oocytes decrease as does the efficiency of DNA repair (Hamatani *et al*., 2004).

Considering *in vitro* culture conditions, the saying of Hippocrates 'primum non nocere' [first do no harm] should be applied. We recommend that all amino acids, especially sulphur amino acids, are present in medium as they are involved in homeostasis and anti-ROS protection: media without amino acids, or lacking some, should be avoided in in vitro fertilizaton (IVF) practices. For example, lack of methionine may lead to mitochondrial dependant apoptosis. IVF is a worldwide accepted practice that has led to the birth of over ten million babies. Any laboratory technique thought to lead to an increase in apoptosis should be carefully screened. Spontaneous ROS formation can also occur in the medium itself, without embryos and since commercially available culture media differ greatly (Martin-Romero *et al*., 2008), added *in situ* protection against ROS is strongly recommended.

Treatment of patients with vitamins in order to avoid DNA damage to sperm has been questionable. In fact ingestion 'larga manu' of anti-oxidants containing vitamins A, C and selenium is perhaps detrimental to the sperm (Ménézo *et al*., 2007b) especially as they induce DNA decondensation, by inducing chromosomal anomalies, which is deleterious for early development (Carell, 2009; Rousseaux *et al*., 2009). We suggest a softer protocol using anti-oxidants associated with folic acid and Zn (Fertibiol®, Procrelia® and Condensyl®; Nurelia), according to the type of decay. Lack of folic acid is linked with poor sperm quality (Boxmeer *et al*. 2009). We have observed (El Mouatassim *et al*., 1999) that some mRNAs (APEX) coding for important steps in DNA repair, can be delivered by the sperm itself.

Finally, we would like to point out that as the repair capacity of the human oocyte may be insufficient to overcome paternally borne damage, *in vivo* improvement of spermatozoa before ART remains of paramount importance.

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