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Embryo culture at a reduced oxygen concentration of 5%: a mini review

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Review

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Summary

The optimum oxygen tension for culturing mammalian embryos has been widely debated by the scientific community. While several laboratories have moved to using 5% as the value for oxygen tension, the majority of modern *in vitro* fertilization (IVF) laboratory programmes still use 20%. Several *in vivo* studies have shown the oxygen tension measured in the oviduct of mammals fluctuates between 2% and 8% and in cows and primates this values drops to <2% in the uterine milieu. In human IVF, a non-physiological level of 20% oxygen has been used in the past. However, several studies have shown that atmospheric oxygen introduces adverse effects to embryo development, not limited to numerous molecular and cellular physiology events. In addition, low oxygen tension plays a critical role in reducing the high level of detrimental reactive oxygen species within cells, influences embryonic gene expression, helps with embryo metabolism of glucose, and enhances embryo development to the blastocyst stage. Collectively, this improves embryo implantation potential. However, clinical studies have yielded contradictory results. In almost all reports, some level of improvement has been identified in embryo development or implantation, without any observed drawbacks. This review article will examine the recent literature and discusses ongoing efforts to understand the benefits that low oxygen tension can bring to mammal embryo development *in vitro*.

Introduction

The role of oxygen in embryonic metabolism and development is critical; its effects are a balance between useful and harmful factors. Oxygen is consumed in oxidative phosphorylation and free radicals are generated from the 'leakage' of high-energy electrons as they pass down the electron transport chain. The free radicals resulting from this process, the reactive oxygen species (ROS), are a threat to cellular biochemistry, physiology and genomic integrity. In the early days of *in vitro* fertilization (IVF), attention was directed to provide culture conditions comparable with those in the oviduct and uterus to the highest degree possible. This led to laboratories being equipped with CO_2 incubators with a reduced oxygen atmosphere. It is not surprising that the first paper in IVF history, published in *Nature* by Patrick Steptoe and Robert Edwards in 1971 described the use of low oxygen tension for the successful culture of a human embryo to the blastocyst stage. In that report, the authors illustrated the culture conditions that motivated this historical change. Specifically, the investigators reported the gas phase as consisting of 5% oxygen, 5% carbon dioxide, and 90% nitrogen (Steptoe *et al.*, 1971). However, it was also reported that successful pregnancies were obtained after the transfer of embryos that were cultured in atmospheric concentrations of 20% oxygen; causing many to abandon low oxygen equipment (Edwards *et al.*, 1981). The birth of millions of children conceived through high oxygen culture concentrations demonstrated that human embryos can adapt to the atmospheric oxygen tension. In the last 40 years, impressive advances in embryo culture have been made by the scientific community, but still a dominant question facing different units around the world is how to maintain a satisfactory pregnancy rate. Embryo culture is a very complex process with several meticulous aspects to consider. Each of these are assembled together to support metabolic embryo requirements and the biosynthetic pathway of the preimplantation embryo by imitating the *in vivo* environment to the highest degree possible. Exhaustive studies of the normal conditions of the female reproductive tract have produced our contemporary culture systems, with specific amino acid and carbohydrate substrate levels, precise pH control, and tight temperature regulation. However, oxygen tension is crucial and can influence *in vitro* embryo development (Gardner *et al.*, 2001). Several studies have reported the detrimental effect of atmospheric oxygen tension on embryo development *in vitro* (Catt and Henman, 2000; Meintjes *et al.*, 2009). In addition, the reduction of oxygen tension to 5% has been associated with an improvement in embryo quality and pregnancy rates, especially when embryos were transferred at the blastocyst stage (Catt and Henman, 2000; Feil *et al.*, 2006; Kea *et al.*, 2007; Kovačič and Vlaisavljević, 2008; Bontekoe *et al.*, 2012).

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This review analyzes how oxygen usage affects the physiology of the preimplantation embryo, defines stress mechanisms activated at 20% oxygen and discusses the adverse effects of ROS in physiological, pathological, and clinical processes related to implantation, fertility and reproductive capacity. Focal points include physiological animal studies that have investigated oxygen tensions in the oviduct and the uterus.

In vivo oxygen concentration

Since the 1950s, intense research has been performed to identify the oxygen tension in the female reproductive tract. Historically, embryo culture has been performed at atmospheric oxygen. Several studies have investigated the luminal fluids of mammals oviduct, and have reported an oxygen concentration between 2% and 8% (Mastroianni and Jones, 1965; Fischer and Bavister, 1993; Kovačič, 2012). There is good evidence to suggest that, in the female reproductive tract, the developing embryo is exposed to concentrations below 5% (Fischer and Bavister, 1993; Ng *et al.*, 2018). There is increasing evidence in the published literature on mammalian species, including humans, to suggest the adverse effects of atmospheric oxygen on embryo development by changes to the transcriptome (Gardner and Lane, 2005) and alterations to the proteome (Katz-Jaffe *et al.*, 2005) and gene expression (Rinaudo *et al.*, 2006) that compromise both carbohydrate and amino acid metabolism (Wale and Gardner, 2012), impacting the epigenome (Morgan *et al.*, 2005; Ventura-Juncá *et al.*, 2009; Li *et al.*, 2014a; Marcho *et al.*, 2015) and inducing premature X-chromosome inactivation (Lengner *et al.*, 2010). It has been suggested that culture in reduced oxygen tension is crucial to keep physiological embryo development and increase reproductive competence. In the cytoplasm, the oxidative stress resulting from accumulation of ROS is likely to be a mechanism by which high oxygen concentration weakens the embryo, reducing its implantation potential and its capacity to generate a pregnancy (Kwon *et al.*, 1999). Meuter and colleagues (2014) showed that cultured mouse blastocysts might express markers of senescence, generated by oxidative stress when cultured in suboptimal culture conditions.

In vitro-produced embryos at the pre- and post-compaction stages differed notably in several crucial features. Cleavage stage embryos have a restricted mechanism to control metabolic homeostasis, and therefore are highly susceptible to conditions that may generate ROS (Umaoka *et al.*, 1992; Johnson and Nasr-Esfahani, 1994; Takahashi *et al.*, 2000; Guérin *et al.*, 2001; Favetta *et al.*, 2007). Post-compaction embryos have more complex systems and their regulation competence could be significantly increased (Lane and Gardner, 2007). Several studies conducted on animal models have demonstrated that reduced oxygen concentration is associated with better pregnancy outcome compared with atmospheric oxygen, especially when embryos are transferred at the blastocyst stage (Umaoka *et al.*, 1992; Booth *et al.*, 2005). The fact that oxygen levels differ throughout the reproductive tract is not a new discovery. This phenomenon has been well described in multiple species. In the human, it has been suggested that during the journey from oviduct to the uterus, the preimplantation embryo proceeds through decreasing oxygen concentrations and reaches the lowest concentration when it starts the compaction process (Thompson *et al.*, 1990; Kovačič, 2012). This notion comes from histological data from salpingectomy specimens, demonstrating that the preimplantation embryo crosses the uterotubal junction late on day 3 (Croxatto, 2002), and evidence that the oxygen tension in the uterus is lower than 5–7% (Fischer and Bavister, 1993). At the morula stage there is a shift in the metabolic pathway from oxidative phosphorylation for energy production in the pre-compaction stage to increased dependence on ATP production during glycolysis in the post-compaction stage (Leese, 1995; Thompson *et al.*, 1996). In rabbits and hamsters, uterine oxygen is similar to that in the oviducts and declines to 3–5% at the point of implantation. Oxygen concentration in the oviduct of rhesus monkey is 5–8.7%, while in the uterine level it is 1.5–2% (Fischer and Bavister, 1993). Indeed, the uterus is a hypoxic environment and remains such for the entire first trimester of pregnancy (Rodesch *et al.*, 1992). Hypoxia is important not only to support vasculogenesis, but also for promoting rapid cell proliferation, similar to tumour growth (Krisher and Prather, 2012). Limited studies are currently available that illustrate the progression of the human embryo through the female reproductive tract and analyze oxygen concentration in the uterine cavity in non-pregnant women (Yedwab *et al.*, 1976; Ottosen *et al.*, 2006). The study by Yedwab and co-workers (1976), reported an average oxygen concentration below 2% in the human uterus. In another study the oxygen tension was measured at the endometrial surface in 21 patients at the time of insemination, with a mean value around 2% (Ottosen *et al.*, 2006). To our knowledge, no studies have been published reporting the exact oxygen tension in the human oviduct.

Oxygen tension and cell physiology

In the *in vivo* environment, the embryo is surrounded by oviductal or uterine fluid, and from these it acquires the substrates and oxygen required for development. The main activity of the early embryo is the metabolic production of energy and gene activation. At an early stage, embryos use aerobic respiration to obtain the oxygen required for the oxidation of substrates such as pyruvate, lactate and amino acids. The production of energy, as ATP molecules, starts once the embryo begins to grow. At the blastocyst stage, it switches from oxidative phosphorylation to aerobic glycolysis to support protein synthesis and ion transport systems (Martin, 2000; Thompson *et al.*, 1998). The embryo obtains its oxygen via passive diffusion with an efficiency linked to the oxygen concentration in the gas phase, the solubility of oxygen in the medium, presumptive and yet full appreciated boundary layers or concentration differences at the embryo/cellular surface, influenced by rates of diffusion, utilization and fluid movement (Smith *et al.*, 2012) around the embryo, and the rate of diffusion through the cytoplasm. Baltz and Biggers (1991) analyzed the oxygen transport in embryo culture in a system in which mouse embryos were contained in microdroplets of culture medium surrounded by a thin layer of mineral oil to prevent evaporation. The source of oxygen for the embryos lay beyond two liquid phases: medium and oil. Transport needs to be sufficiently rapid to replace the oxygen consumed by the embryos, otherwise the medium could become depleted of oxygen. This could generate problems, particularly when large numbers of embryos are present in a single drop, or if the layer of oil is thick, so that oxygen must cross a large barrier before reaching the embryos. They concluded that diffusion through a layer of oil appears not to be rate limiting, even if a large number of embryos are cultured in the same drop, suggesting no risk of anoxia (Baltz and Biggers, 1991). In the mouse embryo, more than 70% of the oxygen is metabolized via oxidative phosphorylation at the blastocyst stage and less than 30% when the embryo is at the 2- to 4-cell stage (Trimarchi *et al.*, 2000). It has been established that trophectoderm cells use significantly more

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oxygen, produce more ATP and contain a greater number of mitochondria compared with the inner cell mass (ICM) (Houghton, 2006).

Reactive oxygen species

Preimplantation development is a time of dynamic alteration, involving modifications of the genome, proteome, metabolome and epigenome (Marcho *et al.*, 2015), and therefore embryos have high sensitivity to their external environment. The rationale against the use of atmospheric oxygen tension is that it can be potentially toxic for cells through formation of ROS (Catt and Henman, 2000), and contribute to defective embryo development, with higher rates of fragmentation (Bedaiwy *et al.*, 2004). Gametes and embryos are cultured in a medium that can itself be the source of ROS (Martín-Romero *et al.*, 2008) and this may negatively influence pregnancy outcomes (Thompson *et al.*, 1990; Johnson and Nasr-Esfahani, 1994; Blondin *et al.*, 1997; Kwon *et al.*, 1999; Takahashi *et al.*, 2000; Meuter *et al.*, 2014). Oxidative stress is considered to be a principal cause of the 2-cell developmental block (Johnson and Nasr-Esfahani, 1994; Favetta *et al.*, 2007). Although embryos are equipped with several endogenous means of countering oxidative stress, many antioxidant genes are not expressed until the later stages of preimplantation development (Harvey *et al.*, 1995). Production and accumulation of ROS from exposure to atmospheric oxygen concentrations during culture may therefore overwhelm embryo defence mechanisms. Physiologically, ROS may originate from embryo metabolism and/or the surroundings and are produced during oxygen reduction in the mitochondria (Guérin *et al.*, 2001). Therefore, when cells are exposed to ROS, DNA damage frequently occurs, producing distinctive patterns of chemical modification. Oxidative stress opens the mitochondrial permeability transition pores. As a result, a large loss of ions and metabolites from the mitochondrial matrix occurs, causing mitochondrial alterations, ATP depletion, and finally triggers the apoptotic mechanism (Takahashi *et al.*, 2000; Guérin *et al.*, 2001). Electron transport chain respiration has been suggested to be a significant pathway of mitochondrial superoxide production (Barja, 1999). Importantly, superoxide and hydroxyl anions, being polar molecules, do not pass the inner mitochondrial membrane, and their efflux is regulated by ion channels (Lustgarten *et al.*, 2012). Furthermore, superoxide is rapidly converted to hydrogen peroxide by superoxide dismutases antioxidant enzymes (SOD) (Takahashi and Asada, 1983; Missirlis *et al.*, 2003). Together these barriers make it unlikely that matrix-derived superoxide could escape and exit from the mitochondria. It has been reported that elevated mitochondrial superoxide production might negatively affect the genome, causing DNA damage and tumourigenesis (Ishii, 2007). Nakada and colleagues (1995) described an inverse association between decreased SOD activity and the presence of malignant neuroendocrine tumours located in the medulla. This inverse association suggests that deficits in SOD, and potentially, elevated superoxide, may be related to the pathobiology of the tumour (Nakada *et al.*, 1995).

In addition, ROS can also modify most types of bio-molecules such as lipids and proteins; this alteration negatively affects membrane stability and permeability and induces damage to several sub-cellular organelles (Guérin *et al.*, 2001; Batty *et al.*, 2009). A study published by Ma and co-workers (2017) on a mouse model investigated the mechanisms behind the beneficial effects of reduced oxygen tension in embryogenesis. The study showed that low oxygen tension might improve embryo viability by increasing expression of antioxidant enzymes and glucose transporter activities. Analysis of expression of antioxidant genes showed that they were 8- to 10-fold higher in the 3% oxygen group compared with the 20% group. This upregulation could enhance implantation potential and reduce apoptosis in mouse blastocysts (Ma *et al.*, 2017).

Embryo culture

To obtain an optimal embryo development and improve pregnancy outcomes, one approach is to keep conditions as similar as possible to the *in vivo* environment. It is evident from the literature that the reduction of oxygen in culture is directly proportional to the reduction of ROS. The negative effect of atmospheric oxygen and oxidative stress on embryo development *in vitro* has been reported (Bontekoe *et al.*, 2012, Catt and Henman, 2000; Bedaiwy *et al.*, 2004; Marcho *et al.*, 2015). However, oxygen concentration studies on human embryos reported controversial results. No significant differences were found in fertilization, cleavage, pregnancy and implantation rates during the culture of embryos up to day 2 or day 3 when using oxygen concentrations of 5% or 20% (Dumoulin *et al.*, 1995; Dumoulin *et al.*, 1999). Conversely, significantly more surplus embryos reached the blastocyst stage when cultured at 5% oxygen compared with 20%. A study published by Waldenström and associates (2009), described a better blastocyst outcome and a significant improvement in pregnancy and birth rates when embryos were cultured under low oxygen concentrations. Kea *et al.* (2007) showed that different oxygen concentrations did not significantly influence fertilization rates, blastocyst formation and quality, or pregnancy rates, but that there was a significant difference in the mean embryo score on day 3 in favour of the reduced oxygen concentration. Kovačič and Vlaisavljević (2008) showed that a lower oxygen concentration increased the proportion of embryos reaching the blastocyst stage. Additionally, the same authors in a subsequent study reported that although the ongoing pregnancies and implantations were similar in the two oxygen concentration groups, the cumulative pregnancy rate (fresh and frozen–thawed embryos) was significantly in favour of the 5% oxygen group (Kovačič *et al.*, 2010). A study published by Ciray and colleagues (2009) on sibling oocytes demonstrated that 5% oxygen tension significantly improved the total blastocyst yield as well as the quality of day 3 and day 5 embryos. Meintjes *et al.* (2009) showed an overall increase in live births when embryos were cultured in reduced oxygen concentrations. Finally, a meta-analysis on four studies confirmed a beneficial effect of culturing embryo in low oxygen concentrations for live birth rates (Bontekoe *et al.*, 2012). These studies analyzed certain characteristics of embryo development, and only compared the clinical outcome, implantation, pregnancy and live birth rates. However, none of these showed a negative correlation with low oxygen culture (Kovačič and Vlaisavljević, 2008; Ciray *et al.*, 2009; Kovačič *et al.*, 2010; de los Santos *et al.*, 2013). Another important aspect to consider is that exposure of embryos to atmospheric oxygen may make them more vulnerable to other *in vitro* stressors, such as ammonium accumulation in the surrounding medium (Wale and Gardner, 2013). Therefore, the negative repercussion could be that when minor problems arise in the embryology laboratory, extended exposure to atmospheric oxygen will compromise embryo development. This molecular mechanism has been investigated by Wale and Gardner (2013). The concept is that when stressors adhere together they bring synergistically adverse effects to embryo development, this is a crucial issue to be taken into consideration to optimize and enhance IVF outcome (Swain *et al.*, 2012, 2013).

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Ultra-low oxygen concentration

Recently a new trend has been reported, investigating the effect of a very low oxygen concentration of 2% on *in vitro* human embryo development. The debate is based on evidence that culture conditions should mimic as accurately as possible *in vivo* conditions. At the moment every incubator used for human embryo culture provides a static environment, without any signs of movement. All the embryology procedures in the IVF laboratory are still carried out in culture dishes. New technological possibilities exist with advancement of microfluidic technology, which is based on the behaviour of liquids in a microenvironment and might be applied to embryo culture in the future and provide dynamics and movement to embryo development (Swain *et al.*, 2013; Smith and Takayama, 2017). Regarding oxygen concentration, it is evident that, *in vivo*, the embryo in the reproductive tract is exposed to oxygen concentrations lower than 5% (Mastroianni and Jones, 1965; Fischer and Bavister, 1993; Kovačič, 2012). Therefore, it would be interesting to culture embryos in very low oxygen concentrations of 2% to evaluate embryo development and blastocyst formation. The published literature is not clear on this debate, perhaps because very few studies are available and conclusions are still inconsistent. A study published by Thompson and co-workers (1990), using sheep and cattle models is summarized here. Two-cell sheep embryos and 8-cell cow embryos were cultured for 5 days in medium previously equilibrated at different oxygen concentrations: sheep 0, 2, 4, 6, 8, 10, 12, 17 or 20%; and cow at 0, 4, 8, 12, 17 or 20%. At the end of 5 days of embryo culture, morphology was analyzed and the embryos were stained to evaluate the number of nuclei. Sheep embryos showed the highest number of morula (nearly 60%) at oxygen concentrations between 6% and 10%. Low quality and poor embryo development was detected at the 0%, as well as at the 20%, oxygen concentration indicating that oxygen is vital and necessary for embryo development. The same trend was observed for cattle embryos. Different results were reported by an Australian group (Feil *et al.*, 2006) in the mouse model. The study observed the effect of embryo culture under different oxygen concentrations and subsequent fetal and placental development at day 18 of pregnancy. Embryos were cultured from zygote to morula at 7% oxygen concentration followed by 20, 7 or 2% to the blastocyst stage. Implantation rates was not influenced by oxygen concentration, but resorption rates were increased in embryos cultured under 2% oxygen, compared with 7% oxygen. The retained placenta is a significant cause of maternal mortality. Day 18 fetal weights were reduced following culture under 2%, compared with 7% or 20% oxygen, or *in vivo* development. Placental weight in the mouse model was not influenced by oxygen tension. Another study performed in the mouse model reported better blastocyst development and hatching rates for embryos cultured in 3% oxygen compared with 20% oxygen. Those data were obtained from different experiments, in which 2-cell embryos (a total of 185) were cultured in 3% oxygen and 189 embryos were cultured in 20% oxygen. Results reported that embryos cultured in 3% oxygen tension had significantly higher rates of blastocyst development (92.3% vs. 79.4%) and hatching (80% vs. 70.4%) compared with those cultured in 20% (Ma *et al.*, 2017). Limited data are available on humans: a study published by Yang and colleagues (2016) analyzed 155 embryos donated to research. All embryos in the study were frozen on day 3 and all were cultured at 20% oxygen concentration from fertilization until day 3. The surviving 120 embryos, after thawing, were allocated to 2, 5 or 20% oxygen concentration for further culture to the blastocyst stage. The rates of blastocyst formation and good quality blastocyst were not statistically different among the three oxygen concentration groups. However, the study presents a low number of embryos, (only 120) and the randomization showed uneven numbers among the groups (Yang *et al.*, 2016). Another study on human embryos was presented by Kaser and collaborators at the American Society for Reproductive Medicine (ASRM) meeting in 2016 (Kaser *et al.*, 2016). Donated embryos at the pronuclear (2PN) stage were randomized to either 5% oxygen from days 1 to 5 (102 embryos) or 5% oxygen from days 1 to 3 and moved to 2% oxygen from day 3 to day 5 (101 embryos). The percentage of good quality embryo on day 3 was similar between the two groups. Quite interestingly, the authors found in the 5–2% group a two-fold improvement in blastulation rate (40.2% versus 22.5%, $P = 0.02$). The same group published another study evaluating the effect of sequential oxygen tension on human blastocysts (5% oxygen from day 1 to day 3 and subsequently 2% from day 3 to day 5). This was a randomized controlled trial in which embryos were donated to research and a good number were tripronucleate (3PN) zygotes. Sibling zygotes were randomized to culture in 5% oxygen from day 1 until day 5 ($n = 102$) or 5% oxygen from day 1 to day 3, then 2% from day 3 to day 5 ($n = 101$). Although this was a small study, the results supported the hypothesis that the total proportion of blastocysts is superior when oxygen tension is reduced from 5% to 2% on day 3 for extended culture to day 5. In addition, they observed a significant increase in the proportion of 2PN and 3PN embryos that blastulated in 2% oxygen, demonstrating improved utilization of key anabolic amino acids and signs of reduced redox stress (Kaser *et al.*, 2018). A study presented at the European Society of Human Reproduction and Embryology (ESHRE) meeting in 2017 by Ferrieres-Hoa and colleagues (Ferrieres-Hoa *et al.*, 2017) analyzed the effect of ultra-low oxygen tensions of 2% on the development of human blastocysts. Blastocyst formation rate was 60.1% (143/238) in the 2% oxygen group compared with 52.1% (88/169) in the control group (5%). Blastocyst utilization rate was significantly higher in the 2% oxygen group (72% vs. 45.5%, $P < 0.0001$). According to these preliminary studies, it seem that the culture of human embryos in a very low oxygen tension of 2% may improve IVF outcomes. Culture in sequential oxygen concentration, cleavage stage until day 3 at 5% oxygen and extended culture to blastocyst stage at 2% is an interesting concept from both a biological and clinical outcome perspective. However, more work is needed to confirm this trend, before translating this regime to clinical use.

Oxygen tension utilized in different IVF laboratories

Oxygen tension is one of the most important aspects discussed here, but sometimes is also one of the most neglected. In 1971, Edwards and Steptoe performed several experiments comparing embryo development at different oxygen concentrations. They obtained better results under reduced oxygen tension. This finding helped to establish 5% oxygen tension as the standard condition for human embryo culture. Subsequently several studies comparing embryo development under different oxygen concentrations were performed. Haidri and co-workers, (1971) described the negative effect of atmospheric oxygen concentration on the developmental competence of mouse oocytes, in particular oxygen has been shown to affect oocyte nuclear maturation and reduce embryo implantation potential. Eppig and Wigglesworth (1995) showed how increased oxygen tension had a dramatic effect on the development of the mouse embryo. The study reported that 42% of

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oocytes grown in 5% oxygen cleaved to the 2-cell stage compared with only 3% when the oocytes were cultured in a 20% oxygen concentration. The use of low oxygen concentration and embryo culture widely diverged among clinics around the world. Although is not an easy task to carry out a global investigation of all IVF units, a world survey on the oxygen concentration used during embryo culture has been performed and published by Christianson and colleagues (2014). The survey was conducted online and collected data from 265 clinics and 71 different countries. Results showed that only 27% of IVF embryos are cultured exclusively at physiologic oxygen tension of 5%. Some units (34%), reported to use a combination with two gas system, using 5% oxygen only at specific stages during the embryo culture, while the majority of IVF clinics interviewed (39%) did not use 5% oxygen concentration at any time during the culture. Even if this analysis is only representative of a small part of the world's IVF clinics, it is evident from the survey and from the literature that there is no agreement on the oxygen concentration to be used in embryo culture. In addition, the existence of geographical differences about the use of 5% oxygen are clear. Australia, New Zealand, Japan and some Northern European countries seem to use physiologic oxygen concentrations of 5%, while other countries use atmospheric oxygen concentration of 20%. The wide adoption of low oxygen tension in Australian IVF clinics can be attributed to several studies performed in that part of the world showing the advantage of reduced oxygen tension in different mammals and humans (Catt and Henman, 2000). The most influential reason for not using low oxygen might be related to the additional cost. Reduced oxygen in the culture environment requires a nitrogen gas system, specialized incubators, and additional quality assurance associated with oxygen sensors (Higdon *et al.*, 2008). Finally, the oxygen tension in embryo culture may also have safety implications and long-term offspring health implication yet to be fully realized. During embryo culture, major epigenetic reprogramming takes place that is extremely important for the normal fate of the embryo and establishing developmental origin of offspring health and disease. Epigenetic reprogramming is very vulnerable to changes in environmental conditions such as the ones applied in IVF, including oxygen tension. The increased oxidative load derived from excess of oxygen may affect oxidation of methylcytosines leading to cytosine de-methylation and/or deamination to thymine and possibly uracil incorporation. Recently, several studies have reported the influence of oxygen tension and culture medium in epigenetic reprogramming and its effect on early embryo development (Morgan *et al.*, 2005; Lonergan *et al.*, 2006; Ventura-Juncá *et al.*, 2009; Marcho *et al.*, 2015) especially of imprinted genes that play a key role in fetal and placental development (Mann *et al.*, 2004) and glucose metabolism and fetal growth in mice model (Donjacour *et al.*, 2014). The consequences of these perturbations on the outcome of the offspring raises important questions not resolved that still need to be clarified. This calls for the need for more research and data on the effects of oxygen tension on epigenetic processes and on long-term consequences. Should future data suggest an important link between oxygen tension, embryo culture/manipulation, epigenetic modifications, and offspring health, then a shift to a more closely regulated oxygen tension during embryo culture will be needed.

Conclusions

Intense research and scientific advancements over the past few decades have helped embryologists to improve markedly the embryo culture system. Oxygen tension in embryo culture has been discussed and investigated since the earliest days of clinical IVF. Culturing embryos in the most physiological oxygen concentration has had marginal uptake across the world, but there is now enough convincing evidence that reduced oxygen tension produces better laboratory and clinical outcomes. The culture of embryos at atmospheric oxygen concentration is thought to be non-physiological and therefore should be avoided (Catt and Henman, 2000). Scientists have tried to make culture as effective as the *in vivo* environment, together with improvements in culture medium; reduced oxygen tension has helped to obtain greater efficiency in culturing embryos to the blastocyst stage and increase the policy of elective single embryo transfer (eSET). A Cochrane database on low oxygen indicated that a reduced oxygen concentration of 5% has a clinical benefit on assisted reproductive technology (ART) outcome compared with embryo culture at atmospheric concentrations (Bontekoe *et al.*, 2012). Low oxygen cultures have a beneficial effect on pregnancy rate, implantation and live birth rates. Clinics following a policy of elective single transfer at the blastocyst stage might benefit from a low oxygen culture system (Catt and Henman, 2000; Feil *et al.*, 2006; Meintjes *et al.*, 2009). However, recently, a new trend has been described consisting of further reduction of oxygen tension in extended culture, and this may become the new standard for IVF culture in the future. Like the debate between 5% and 20% oxygen, which has been going on for the past couple of decades, further oxygen reduction may become indispensable to obtain outstanding blastocyst formations and may be helpful in increasing eSET to reduce multiple pregnancy in ART.

However, it needs to be mentioned that dissolved oxygen concentration during culture of embryos is generally lower than that in the incubator because of the poor solubility of oxygen in the medium (Talevi *et al.*, 2018). In that context, even though culture is performed at a reduced oxygen concentration of 5% or lower, the actual oxygen concentration present immediately close to the embryo might depend on the volume of the drop of culture medium used, the movement of medium at the surface of the embryo, dynamic or static culture (Heo *et al.*, 2010, Swain *et al.*, 2013), and the amount of mineral oil applied over the embryo. Consideration of these inter-related factors will be essential in future experimental designs and data interpretation.

To conclude, although embryos may show a certain degree of plasticity to small changes in oxygen tension, scientists should always focus on providing embryos with the most suitable environment, to avoid suboptimal conditions that may induce embryos to spend energy coping with stress, and which may in turn may have some effect on embryo viability, reduced implantation potential and pregnancy outcome.

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References

Baltz JM and Biggers JD (1991) Oxygen transport to embryos in microdrop cultures. *Mol Reprod Dev* **28**, 351–5.

Barja G (1999) Mitochondrial oxygen radical generation and leak: sites of production in states 4 and 3, organ specificity, and relation to aging and longevity. *J Bioenerg Biomembr* **31**, 347–66.

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Batty E, Jensen K and Freemont P (2009) PML nuclear bodies and their spatial relationships in the mammalian cell nucleus. *Front Biosci* **14**, 1182–96.

Bedaiwy MA, Falcone T, Mohamed MS, Aleem AA, Sharma RK, Worley SE, Thornton J and Agarwal A (2004) Differential growth of human embryos *in vitro*: role of reactive oxygen species. *Fertil Steril* **82**, 593–600.

Blondin P, Coenen K and Sirard MA (1997) The impact of reactive oxygen species on bovine sperm fertilizing ability and oocytes maturation. *J Androl* **18**, 454–60.

Bontekoe S, Mantikou E, van Wely M, Seshadri S, Repping S and Mastenbroek S (2012) Low oxygen concentrations for embryo culture in assisted reproductive technologies. *Cochrane Database Syst Rev* **11**, 7.

Booth PJ, Holm P and Callesen H (2005) The effect of oxygen tension on porcine embryonic development is dependent on embryo type. *Theriogenology* **63**, 2040–52.

Catt JW and Henman M (2000) Toxic effects of oxygen on human embryo development. *Hum Reprod* **15**(Suppl 2), 199–206.

Christianson MS, Zhao Y, Shoham G, Granot I, Safran A, Khafagy A, Leong M and Shoham Z (2014) Embryo catheter loading and embryo culture techniques: results of a worldwide web-based survey. *J Assist Reprod Genet* **31**, 1029–36.

Ciray HN, Aksoy T, Yaramanci K, Karayaka I and Bahceci M (2009) *In vitro* culture under physiologic oxygen concentration improves blastocyst yield and quality: a prospective randomized survey on sibling oocytes. *Fertil Steril* **91**(Suppl 4), 1459–61.

Croxatto HB (2002) Physiology of gamete and embryo transport through the fallopian tube. *Reprod BioMed Online* **4**, 160–9.

de los Santos MJ, Gámiz P, Albert C, Galán A, Viloria T, Pérez S, Romero JL and Remohí J (2013) Reduced oxygen tension improves embryo quality but not clinical pregnancy rates: a randomized clinical study into ovum donation cycles. *Fertil Steril* **100**, 402–7.

Donjacour A, Liu X, Lin W, Simbulan R and Rinaudo PF (2014) *In vitro* fertilization affects growth and glucose metabolism in a sex-specific manner in an outbred mouse model. *Biol Reprod* **90**, 80.

Dumoulin JC, Vanvuchelen RC, Land JA, Pieters MH, Geraedts JP and Evers JL (1995) Effect of oxygen concentration on in vitro fertilization and embryo culture in the human and the mouse. *Fertil Steril* **63**, 115–9.

Dumoulin JC, Meijers CJ, Bras M, Coonen E, Geraedts JP and Evers JL (1999) Effect of oxygen concentration on human in-vitro fertilization and embryo culture. *Hum Reprod* **14**, 465–9.

Edwards RG, Purdy JM, Steptoe PC and Walters DE (1981) *Am J Obstet Gynecol* **141**, 408–16.

Eppig JJ and Wigglesworth K (1995) Factors affecting the developmental competence of mouse oocytes grown *in vitro*: oxygen concentration. *Mol Reprod Dev* **42**, 447–56.

Favetta LA, St John EJ, King WA and Betts DH (2007) High levels of p66shc and intracellular ROS in permanently arrested early embryos. *Free Radic Biol Med* **42**, 1201–10.

Feil D, Lane M, Roberts CT, Kelley RL, Edwards LJ, Thompson JG and Kind KL (2006) Effect of culturing mouse embryos under different oxygen concentrations on subsequent fetal and placental development. *J Physiol* **572**(Pt 1), 87–96.

Ferrieres-Hoa A, Roman K, Mullet T, Gala A and Hamamah S (2017) Ultra-low (2%) oxygen tension significantly improves human blastocyst development and quality. *Hum Reprod* **32**(Supp 1), i26.

Fischer B and Bavister BD (1993) Oxygen tension in the oviduct and uterus of rhesus monkeys, hamsters and rabbits. *J Reprod Fertil* **99**, 673–9.

Gardner DK, Lane M, Stevens J and Schoolcraft WB (2001) Noninvasive assessment of human embryo nutrient consumption as a measure of developmental potential. *Fertil Steril* **76**, 1175–80.

Gardner DK and Lane M, (2005) Ex vivo early embryo development and effects on gene expression and imprinting. *Reprod Fertil Dev* **17**, 361–70.

Guérin P, El Mouatassim S and Ménézo Y (2001) Oxidative stress and protection against reactive oxygen species in the pre-implantation embryo and its surroundings. *Hum Reprod Update* **7**, 175–89.

Haidri AA, Miller IM and Gwatkin RB (1971) Culture of mouse oocytes *in vitro*, using a system without oil or protein. *J Reprod Fertil* **26**, 409–11.

Harvey MB, Arcellana-Panlilio MY, Zhang X, Schultz GA and Watson AJ (1995) Expression of genes encoding antioxidant enzymes in preimplantation mouse and cow embryos and primary bovine oviduct cultures employed for embryo coculture. *Biol Reprod* **53**, 532–40.

Heo YS, Cabrera LM, Bormann CL, Shah CT, Takayama S and Smith GD (2010) Dynamic microfunnel culture enhances mouse embryo development and pregnancy rates. *Hum Reprod* **25**, 613–22.

Higdon HL, Blackhurst DW and Boone WR (2008) Incubator management in an assisted reproductive technology laboratory. *Fertil Steril* **89**, 703–10.

Houghton FD (2006) Energy metabolism of the inner cell mass and trophectoderm of the mouse blastocyst. *Differentiation* **74**, 11–8.

Ishii N (2007) Role of oxidative stress from mitochondria on aging and cancer. *Cornea* **26**, S3–9.

Johnson MH and Nasr-Esfahani MH (1994) Radical solutions and cultural problems: could free oxygen radicals be responsible for the impaired development of preimplantation mammalian embryos *in vitro*? *Bioessays* **16**, 31–8.

Kaser DJ, Bogale B, Sarda V, Farland LV and Racowsky C (2016) Randomized controlled trial of low (5%) vs. ultra-low (2%) oxygen tension for *in vitro* development of human embryos. *Fertil Steril* **106**, e4.

Kaser DJ, Bogale B, Sarda V, Farland LV, Williams PL and Racowsky C (2018) Randomized controlled trial of low (5%) versus ultralow (2%) oxygen for extended culture using bipronucleate and tripronucleate human preimplantation embryos. *Fertil Steril* **109**, 1030–7.

Katz-Jaffe MG, Linck DW, Schoolcraft WB and Gardner DK (2005) A proteomic analysis of mammalian preimplantation embryonic development. *Reproduction* **130**, 899–905.

Kea B, Gebhardt J, Watt J, Westphal LM, Lathi RB, Milki AA and Behr B (2007) Effect of reduced oxygen concentrations on the outcome of *in vitro* fertilization. *Fertil Steril* **87**, 213–6.

Kovačič B and Vlaisavljević V (2008) Influence of atmospheric versus reduced oxygen concentration on development of human blastocysts *in vitro*: a prospective study on sibling oocytes. *Reprod BioMed Online* **17**, 229–36.

Kovačič B, Sajko MC and Vlaisavljević V (2010) A prospective, randomized trial on the effect of atmospheric versus reduced oxygen concentration on the outcome of intracytoplasmic sperm injection cycles. *Fertil Steril* **94**, 511–9.

Kovačič B (2012) Culture systems: low-oxygen culture. *Methods Mol Biol* **912**, 249–72.

Krisher RL and Prather RS (2012) A role for the Warburg effect in preimplantation embryo development: metabolic modification to support rapid cell proliferation. *Mol Reprod Dev* **79**, 311–20.

Kwon HC, Yang HW, Hwang KJ, Yoo JH, Kim MS, Lee CH, Ryu HS and Oh KS (1999) Effects of low oxygen condition on the generation of reactive oxygen species and the development in mouse embryos cultured *in vitro*. *J Obstet Gynaecol Res* **25**, 359–66.

Lane M and Gardner DK (2007) Embryo culture medium: which is the best? *Best Pract Res Clin Obstet Gynaecol* **21**, 83–100.

Leese HJ (1995) Metabolic control during preimplantation mammalian development. *Hum Reprod Update* **1**, 63–72.

Lengner CJ, Gimelbrant AA, Erwin JA, Cheng AW, Guenther MG, Welstead GG, Alagappan R, Frampton GM, Xu P, Muffat J, Santagata S, Powers D, Barrett CB, Young RA, Lee JT, Jaenisch R and Mitalipova M (2010) Derivation of pre-X inactivation human embryonic stem cells under physiological oxygen concentrations. *Cell* **141**, 872–83.

Li W, Goossens K, Van Poucke M, Forier K, Braeckmans K, Van Soom A and Peelman LJ (2014a) High oxygen tension increases global methylation in bovine 4-cell embryos and blastocysts but does not affect general retrotransposon expression. *Reprod Fertil Dev* **28**, 948–59.

Lonergan P, Fair T, Corcoran D and Evans AC (2006) Effect of culture environment on gene expression and developmental characteristics in IVF-derived embryos. *Theriogenology* **65**, 137–52.

Lustgarten, M.S., Bhattacharya, A., Muller, F.L., Jang, Y.C., Shimizu, T., Shirasawa, T., Richardson, A., Van Remmen, H. (2012) Complex I generated, mitochondrial matrix-directed superoxide is released from the mitochondria through voltage dependent anion channels. *Biochem Biophys Res Commun* **422**, 515–21.

Ma YY, Chen HW and Tzeng CR (2017) Low oxygen tension increases mitochondrial membrane potential and enhances expression of antioxidant genes and implantation protein of mouse blastocyst cultured *in vitro*. *J Ovarian Res* **10**, 47.

https://doi.org/10.1017/S0967199419000522 Published online by Cambridge University Press

Mann MR, Lee SS, Doherty AS, Verona RI, Nolen LD, Schultz RM and Bartolomei MS (2004) Selective loss of imprinting in the placenta following preimplantation development in culture. *Development* **131**, 3727–35.

Marcho C, Cui W and Mager J (2015) Epigenetic dynamics during preimplantation development. *Reproduction* **150**, R109–120.

Martín-Romero FJ, Miguel-Lasobras EM, Domínguez-Arroyo JA, González-Carrera E and Alvarez IS (2008) Contribution of culture media to oxidative stress and its effect on human oocytes. *Reprod Biomed Online* **17**, 652–61.

Martin KL (2000) Nutritional and metabolic requirements of early cleavage stage embryos and blastocysts. *Hum Fertil (Camb)* **3**, 247–54.

Mastroianni Jr L and Jones R (1965) Oxygen tension within the rabbit fallopian tube. *J Reprod Fertil* **9**, 99–102.

Meintjes M, Chantilis SJ, Douglas JD, Rodriguez AJ, Guerami AR, Bookout DM, Barnett BD and Madden JD (2009) A controlled randomized trial evaluating the effect of lowered incubator oxygen tension on live births in a predominantly blastocyst transfer program. *Hum Reprod* **24**, 300–7.

Meuter A, Rogmann LM, Winterhoff BJ, Tchkonia T, Kirkland JL and Morbeck DE (2014) Markers of cellular senescence are elevated in murine blastocysts cultured *in vitro*: molecular consequences of culture in atmospheric oxygen. *J Assist Reprod Genet* **31**, 1259–67.

Missirlis F, Hu J, Kirby K, Hilliker AJ, Rouault TA and Phillips JP (2003) Compartment-specific protection of iron-sulfur proteins by superoxide dismutase. *J Biol Chem* **278**, 47365–9.

Morgan HD, Santos F, Green K, Dean W and Reik W (2005) Epigenetic reprogramming in mammals. *Hum Mol Genet* **14**, R47–58.

Nakada T, Kubota Y, Sasagawa I, Yagisawa T, Watanabe M and Ishigooka M (1995) Remarkably suppressed manganese superoxide dismutase activity in malignant pheochromocytoma. *J Urol* **153**, 1787–90.

Ng KYB, Mingels R, Morgan H, Macklon N and Cheong Y (2018) *In vivo* oxygen, temperature and pH dynamics in the female reproductive tract and their importance in human conception: a systematic review. *Hum Reprod Update* **24**, 15–34.

Ottosen LD, Hindkaer J, Husth M, Petersen DE, Kirk J and Ingerslev HJ (2006) Observations on intrauterine oxygen tension measured by fibre-optic microsensors. *Reprod Biomed Online* **13**, 380–5.

Rinaudo PF, Giritharan G, Talbi S, Dobson AT and Schultz RM (2006) Effects of oxygen tension on gene expression in preimplantation mouse embryos. *Fertil Steril* **86**(4 Suppl), 1252–65.

Rodesch F, Simon P, Donner C and Jauniaux E (1992) Oxygen measurements in endometrial and trophoblastic tissues during early pregnancy. *Obstet Gynecol* **80**, 283–5.

Smith GD, Takayama S and Swain JE (2012) Rethinking *in vitro* embryo culture: new developments in culture platforms and potential to improve assisted reproductive technologies. *Biol Reprod* **86**, 62.

Smith GD and Takayama S (2017) Application of microfluidic technologies to human assisted reproduction. *Mol Hum Reprod* **23**, 257–68.

Steptoe PC, Edwards RG and Purdy JM (1971) Human blastocysts grown in culture. *Nature* **29**(5280), 132–3.

Swain, JE, Cabrera, L, Xu, X and Smith, GD (2012) Microdrop preparation factors influence culture-media osmolality, which can impair mouse embryo preimplantation development. *Reprod Biomed Online* **24**, 142–7.

Swain JE, Lai D, Takayama S and Smith GD (2013) Thinking big by thinking small: application of microfluidic technology to improve ART. *Lab Chip* **13**, 1213–24.

Talevi R, Sudhakaran S, Barbato V, Merolla A, Braun S, Di Nardo M, Costanzo V, Ferraro R, Iannantuoni N, Catapano G and Gualtieri R, (2018) Is oxygen availability a limiting factor for *in vitro* folliculogenesis? *PLoS One* **13**, e0192501.

Takahashi MA and Asada K (1983) Superoxide anion permeability of phospholipid membranes and chloroplast thylakoids. *Arch Biochem Biophys* **226**, 558–66.

Takahashi M, Keicho K, Takahashi H, Ogawa H, Schultz RM and Okano A (2000) Effect of oxidative stress on development and DNA damage in in-vitro cultured bovine embryos by comet assay. *Theriogenology* **54**, 137–45.

Thompson JG, Simpson AC, Pugh PA, Donnelly PE and Tervit HR (1990) Effect of oxygen concentration on in-vitro development of preimplantation sheep and cattle embryos. *J Reprod Fertil* **89**, 573–8.

Thompson JG, Partridge RJ, Houghton FD, Cox CI and Leese HJ (1996) Oxygen uptake and carbohydrate metabolism by *in vitro* derived bovine embryos. *J Reprod Fertil* **106**, 299–306.

Thompson JG, Sherman ANM, Allen NW, McGowan LT and Tervit HR (1998) Total protein content and protein synthesis within pre-elongation stage bovine embryos. *Mol Reprod Dev* **50**, 139–45.

Trimarchi JR, Liu L, Porterfield DM, Smith PJ and Keefe DL (2000) Oxidative phosphorylation-dependent and -independent oxygen consumption by individual preimplantation mouse embryos. *Biol Reprod* **62**, 1866–74.

Umaoka Y, Noda Y, Narimoto K and Mori T (1992) Effects of oxygen toxicity on early development of mouse embryos. *Mol Reprod Dev* **31**, 28–33.

Ventura-Juncá P, Irarrázaval I, Rolle AJ, Gutiérrez JI, Moreno RD and Santos MJ (2009) *In vitro* fertilization (IVF) in mammals: epigenetic and developmental alterations. Scientific and bioethical implications for IVF in humans. *Biol Res* **48**, 68.

Waldenström U, Engström AB, Hellberg D and Nilsson S (2009) Low oxygen compared with high-oxygen atmosphere in blastocyst culture, a prospective randomized study. *Fertil Steril* **91**, 2461–5.

Wale PL and Gardner DK (2012) Oxygen regulates amino acid turnover and carbohydrate uptake during the preimplantation period of mouse embryo development. *Biol Reprod* **87**, 24.

Wale PL and Gardner DK (2013) Oxygen affects the ability of mouse blastocysts to regulate ammonium. *Biol Reprod* **89**, 75.

Yang Y, Xu Y, Ding C, Khoudja RY, Lin M, Awonuga AO, Dai J, Puscheck EE, Rappolee DA and Zhou C (2016) Comparison of 2, 5, and 20% O_2 on the development of post-thaw human embryos. *J Assist Reprod Genet* **33**, 919–27.

Yedwab GA, Paz G, Homonnai TZ, David MP and Kraicer PF (1976) The temperature, pH, and partial pressure of oxygen in the cervix and uterus of women and uterus of rats during the cycle. *Fertil Steril* **27**, 304–9.

https://doi.org/10.1017/S0967199419000522 Published online by Cambridge University Press