Influence of oxygen tension on function of isolated spermatozoa from ejaculates of oligozoospermic patients and normozoospermic fertile donors*

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Oxygen radical generation is known to be detrimental to sperm function. An example of a reactive oxygen species-associated male pathology is oligozoospermia in which fertilization and pregnancy rates are low in in-vitro fertilization (IVF) programmes. As the extent of the modifications induced by reactive oxygen species (ROS) depends on several factors, notably from oxygen tension in the incubation medium, the aim of this study was to examine the influence of a low (5%) rather than atmospheric (20%) oxygen tension in the incubator gas phase on the function of Percoll-selected spermatozoa from ejaculates of oligozoospermic patients and normozoospermic fertile donors. After incubation for several hours in a gas phase of either 5% CO2/90% N2/5% O2 or 5% CO2/95% air (20% O2), none of the parameters investigated, e.g. movement characteristics, potential of spermatozoa to acquire hyperactivated motility, to undergo the acrosome reaction when challenged with a calcium ionophore and to fuse with zona-free hamster oocytes, was significantly different between the two oxygen tensions in fertile donors. In contrast, among oligozoospermic patients, the motility parameters, the percentage of hyperactivated motility and of induced-acrosome reaction were significantly improved under a gas phase of 5% O2 compared with those observed under an atmosphere of 20% O2 (P < 0.05). Exposure to 5% rather than 20% oxygen tension also induced a significant increase in the percentage of penetration of zona-free hamster eggs after capacitation for 17 h, but no difference was found in the mean number of bound spermatozoa per oocyte. After incubation for 24 h, a significantly higher survival rate was observed under 5% compared with 20% oxygen tension. These results show that the use of a low oxygen tension rather than air might improve spermatozoan competence of oligozoospermic patients during IVF programmes.

Key words: oligozoospermia/oxygen tension/reactive oxygen species/spermatozoa

Introduction

A new development in the field of human male infertility was the realization, a decade ago, that excessive generation of reactive oxygen species (ROS) in the ejaculate could compromise the functional competence of human spermatozoa (for review see Griveau and Le Lannou, 1997a). Reactive oxygen species are detected in 25–40% of the semen of infertile men, and in up to 96% of the semen of patients with spinal cord injury (Iwasaki and Gagnon, 1992; De Lamirande et al., 1995). Due to their high content of polyunsaturated fatty acids, human spermatozoa are particularly sensitive to lipid peroxidation, although all cellular components are potential targets for ROS (Jones et al., 1979; Aitken et al., 1993; Alvarez and Storey, 1995; Griveau et al., 1995a; Storey, 1997). The production of ROS in semen has been associated with a reduction in sperm motility, a decreased ability for spermatozoa–oocyte fusion and a diminished fertility in vitro, as well as in vivo (Aitken et al., 1991; Iwasaki and Gagnon, 1992; De Lamirande et al., 1995). The two main sources of ROS in the human ejaculate are the spermatozoa themselves (Aitken and Clarkson, 1987; Alvarez et al., 1987; Iwasaki and Gagnon, 1992) and infiltrated leukocytes (Aitken and West, 1990; Kessopoulou et al., 1993; Aitken et al., 1994a,b). The detection of ROS in semen appears to be due to an increased ROS production by spermatozoa or leukocytes rather than to a decrease of the scavenging systems (Ziù et al., 1993). Two generating systems have been proposed to be at the origin of ROS produced by spermatozoa, an NADPH oxidase-like system located at the sperm plasma membrane (Aitken et al., 1992a), and a sperm diaphorase (an NADH-dependent oxidoreductase) located in the middle piece and integrated into the mitochondrial respiratory system (Gavella and Lipovic, 1992).

A typical example of a free radical-associated male pathology is oligozoospermia in which fertilization and pregnancy rates are low in in-vitro fertilization (IVF) programmes. Indeed, half of the cases are associated with generation rates of ROS above the normal fertile range (Aitken et al., 1989a, 1992b). Furthermore, although Percoll-washed spermatozoa produced a much lower level of ROS than conventionally washed spermatozoa (Iwasaki and Gagnon, 1992), Percoll-selected spermatozoa from the ejaculates of patients exhibiting oligozoospermia are characterized by generation rates of ROS that exceed considerably those obtained for the normal fertile population (Aitken et al., 1989a, 1992b; Zalata et al., 1995).


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Since considerable deleterious effects on sperm functions by ROS have been well documented (Aitken et al., 1989b; De Lamirande and Gagnon, 1992a,b; Griveau et al., 1995a), it is of importance in clinical terms to prevent the generation of ROS in sperm preparations in order to prevent the potential oxidative stress undergone by spermatozoa during the course of IVF. As it has been shown previously that the oxygen tension in the incubation medium greatly influences the amount of ROS involved and consequently the extent of the modifications induced by these ROS (Alvarez and Storey, 1983; Griveau and Le Lannou, 1997b), the purpose of this work was to study the influence of a low (5%) rather than atmospheric (20%) oxygen tension in the incubator gas phase on the function of Percoll-selected spermatozoa from ejaculates of oligozoospermic patients and normozoospermic fertile donors.

Materials and methods

Sperm preparation

A total of 60 men attending the Unit of Biology of Reproduction were included in the study. Semen samples were produced by masturbation after a recommended sexual abstinence of 2–3 days; after a 15 min liquefaction at 37°C, conventional semen analysis was performed according to the World Health Organization recommendations (WHO, 1992). The outcome of this analysis is presented in Table I. The studied population comprised a group of 32 patients whose spermatozoa were classified as oligozoospermic (<2×10^6 spermatozoa/ml) and a group of 28 normozoospermic men of proven fertility. In the group of oligozoospermic men, 23 presented an associated asthenoteratozoospermia and three a teratozoospermia. Only six out of 32 presented an isolated oligozoospermia. When round cells were seen in the ejaculate, the differentiation between white blood cells (WBC) and spermatogenic cells was performed by peroxidase staining (Leucoscreen; FertiPro, Aalter, Belgium) and white blood cells (WBC) and spermatogenic cells was performed by peroxidase staining (Leucoscreen; FertiPro, Aalter, Belgium). WBC and spermatozoa were separated by Percoll gradient (47% and 90%) (PerWash; FertiPro). After centrifugation at 500 g for 20 min at room temperature, spermatozoa from the 90% Percoll fraction were collected, diluted with 5 ml of FertiCult-IVF medium (FertiPro) and centrifuged at 500 g for 5 min at room temperature. The supernatant was discarded and spermatozoa were resuspended at a concentration of 10^9/ml in FertiCult-IVF medium and then incubated at 37°C either under a gas phase of 5% CO₂/90% N₂/5% O₂, or 5% CO₂/95% air (20% O₂).

Motility analysis

Movement characteristics were analysed using the ATS 20 motility analyser (J.C. Diffusion International, La Ferte Frensel, France) in a 20 μm-deep chamber (CellVision, Heerhugowaard, The Netherlands) at 37°C. System parameter settings for this analysis were 25 frames/s recorded, 12 consecutive frames minimum analysed, spermatozoan minimum size of 8 pixels, spermatozoan maximum size of 20 pixels, 20 μm/s threshold velocity and 300 μm/s maximum velocity. A minimum of 150 trajectories were analysed for each sample. The movement characteristics analysed included percentage motility, curvilinear velocity (VCL, μm/s), straight line velocity (VSL, μm/s), amplitude of lateral head displacement (ALH, μm), average path velocity (VAP, μm/s) and linearity (LIN, %); the percentage of hyperactivated spermatozoa represented cells exhibiting a VCL >90 μm/s, a LIN <65% and an ALH >3.5 μm.

Acrosome reaction

Acrosomal reactions (AR) were evaluated after 3 h of incubation. The acrosome reaction was chemically induced for 45 min at 37°C with 10 μM of A23187 calcium ionophore (Sigma, Saint Louis, MO, USA) (stored at 10⁻³ M in ethanol at −20°C before use) in FertiCult-IVF medium supplemented with FITC-conjugated GB 24 antibody (Theramex, Monaco, France) (1 μg/100 μl), a mouse monoclonal antibody that recognizes an inner acrosomal membrane-associated protein. At 5 min before the end of the incubation, the vital stain propidium iodide (Sigma) (10⁻⁹ M final concentration) was added to the sperm suspension for 5 min. Spermatozoa were then washed by centrifugation at 500 g for 5 min with phosphate-buffered saline–bovine serum albumin (PBS–BSA) and immediately observed with fluorescence microscopy. Two hundred spermatozoa were evaluated for each sample. Only those devoid of red fluorescence (given by propidium iodide) and exhibiting a green homogeneous fluorescence pattern distributed over the entire acrosome cap were scored as positive (living acrosome-reacted spermatozoa). The results were expressed as percentages of positive spermatozoa.

Zona-free hamster egg penetration test

The zona-free hamster egg penetration test (HEPT) was performed according to the protocol described by Yanagimachi et al. (1976).

Sexually mature golden hamsters were superovulated on the morning of oestrus by intraperitoneal injection of 30 IU pregnant mare’s serum (Intervet SA, Pireaux, France) followed 60 h later by 30 IU human chorionic gonadotrophin (HCG; Organon Pharmaceuticals, Pireaux, France). At 14 h after HCG injection, animals were killed and their eggs recovered and treated with hyaluronidase (Sigma) and trypsin (Sigma) to remove the cumulus cells and the zona pellucida. About 40 zona-free hamster eggs were inseminated with 2×10⁶ capacitated spermatozoa in a final volume of 200 μl of Biggers, Whitten and Whittingham medium (BBW) containing 3% human serum albumin (HSA, fraction V; Sigma) and incubated at 37°C in 5% CO₂ in air for 3 h. Spermatozoa were capacitated in Ferticult-IVF medium for 17 h at 37°C under a gas phase of either 5% CO₂/90% N₂/5% O₂ or 5% CO₂/95% air (20% O₂). Thereafter, the eggs were washed in BBW containing 3% HSA, mounted under a coverslip, and examined at ×400 magnification with a phase-contrast microscope. The presence of a decondensing sperm head with attached or closely associated sperm tail in the cytoplasm was considered indicative of positive penetration. The penetration rate was defined as the number of ova with positive penetration divided by the total

### Table I. Semen profiles of the populations studied

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Oligozoospermia (n = 32)</th>
<th>Normozoospermia (n = 28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration (10⁶/ml)</td>
<td>11 ± 1 (1–20)</td>
<td>117 ± 15 (65–160)</td>
</tr>
<tr>
<td>Motility (%)</td>
<td>29 ± 3 (8–63)</td>
<td>55 ± 5 (43–80)</td>
</tr>
<tr>
<td>Normal morphology (%)</td>
<td>40 ± 3 (19–71)</td>
<td>65 ± 4 (52–77)</td>
</tr>
<tr>
<td>VCL (μm/s)</td>
<td>56 ± 2 (34–81)</td>
<td>64 ± 3 (50–72)</td>
</tr>
<tr>
<td>VSL (μm/s)</td>
<td>39 ± 2 (26–58)</td>
<td>42 ± 2 (34–58)</td>
</tr>
<tr>
<td>LIN (%)</td>
<td>69 ± 1 (58–80)</td>
<td>65 ± 2 (56–79)</td>
</tr>
<tr>
<td>VAP (μm/s)</td>
<td>45 ± 2 (28–65)</td>
<td>53 ± 3 (41–72)</td>
</tr>
<tr>
<td>tALH (μm)</td>
<td>1.8 ± 0.1 (1.0–3.5)</td>
<td>1.7 ± 0.1 (1.1–3.2)</td>
</tr>
</tbody>
</table>

Values are mean ± SE (range). Significant differences between groups, aP < 0.01, bP < 0.001. ALH = amplitude of lateral head displacement; LIN = linearity; VAP = average path velocity; VCL = curvilinear velocity; VSL = straight-line velocity.

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<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VA (µm/s)</td>
<td>56.3</td>
</tr>
<tr>
<td>VCL (µm/s)</td>
<td>33.4</td>
</tr>
<tr>
<td>LIN (%)</td>
<td>64.5</td>
</tr>
<tr>
<td>Minimum size (µm)</td>
<td>0.6</td>
</tr>
<tr>
<td>Maximum size (µm)</td>
<td>20.0</td>
</tr>
<tr>
<td>Minimum velocity</td>
<td>2.0</td>
</tr>
<tr>
<td>Maximum velocity</td>
<td>10.0</td>
</tr>
<tr>
<td>Average path velocity</td>
<td>5.0</td>
</tr>
</tbody>
</table>

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20% O\textsubscript{2}, none of the parameters investigated, e.g. movement characteristics (Table II), potential of spermatozoa to acquire capacitated under an atmosphere of 20% O\textsubscript{2}, whereas the LIN was significantly reduced (\(P < 0.05\)). After incubation for 24 h, the survival rate was even increased 2-fold (44.2 ± 10.1% versus 86.2 ± 9.3%, \(P < 0.001\)) (Figure 3). The influence of oxygen tension on the unfolding of sperm capacitation was assessed by measurement of the hyperactivated motility (Figure 1A) and the capacity for spermatozoa to acrosome react when challenged with a calcium ionophore (Figure 1B). These two parameters were increased dramatically (\(P < 0.01\)) under a gas phase of 5% O\textsubscript{2} (12.7 ± 2.2% and 20.2 ± 1.1%, respectively) compared with those observed under a gas phase of 20% O\textsubscript{2} (6.1 ± 2.2% and 12.3 ± 2.1%, respectively). Consequently to the observed increase in the sperm capacitation process under a gas phase of 5% O\textsubscript{2}, differences in the levels of spermatozoa–oocyte fusion were observed between oxygen tensions (Figure 2). The percentage of zona-free hamster eggs penetrated was significantly higher when spermatozoa were capacitated under 5% rather than 20% oxygen tension (16.5 ± 5.2% versus 9.2 ± 3.0%), but no difference was found in the mean number of bound spermatozoa per oocyte (6.4 ± 2.8 versus 4.0 ± 1.6). 

**Discussion**

This study has demonstrated that, in cases of oligozoospermia, there appears to be advantage in the use of an atmosphere of 5% CO\textsubscript{2}/90% N\textsubscript{2}/5% O\textsubscript{2} rather than 5% CO\textsubscript{2}/95% air (20% O\textsubscript{2}) in the incubator gas phase to improve sperm function.

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**Table II. Movement characteristics after incubation for 3 h in a gas phase of either 20% O\textsubscript{2} or 5% O\textsubscript{2}**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normozoospermia</th>
<th>Oligozoospermia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gas phase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20% O\textsubscript{2}</td>
<td>5% O\textsubscript{2}</td>
<td>20% O\textsubscript{2}</td>
</tr>
<tr>
<td>Motility (%)</td>
<td>82 ± 3 (73–92)</td>
<td>83 ± 4 (72–96)</td>
</tr>
<tr>
<td>VCL (µm/s)</td>
<td>78 ± 3 (68–92)</td>
<td>80 ± 2 (69–91)</td>
</tr>
<tr>
<td>VSL (µm/s)</td>
<td>45 ± 2 (33–65)</td>
<td>49 ± 2 (35–68)</td>
</tr>
<tr>
<td>LIN (%)</td>
<td>64 ± 3 (51–78)</td>
<td>65 ± 3 (50–80)</td>
</tr>
<tr>
<td>VAP (µm/s)</td>
<td>57 ± 2 (53–69)</td>
<td>62 ± 3 (52–75)</td>
</tr>
<tr>
<td>ALH (µm)</td>
<td>2.6 ± 0.1 (2.1–3.5)</td>
<td>2.8 ± 0.1 (2.2–3.9)</td>
</tr>
</tbody>
</table>

Values are mean ± SE (range); \(n = 25\).
Significant difference between oligozoospermic subgroups. \(\ast P < 0.05\), \(\ast\ast P < 0.005\).
For abbreviations, see Table I.

**Table III. Movement characteristics after incubation for 24 h in a gas phase of either 20% O\textsubscript{2} or 5% O\textsubscript{2}**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Oligozoospermia</th>
<th>Normozoospermia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gas phase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20% O\textsubscript{2}</td>
<td>5% O\textsubscript{2}</td>
<td>20% O\textsubscript{2}</td>
</tr>
<tr>
<td>Motility (%)</td>
<td>37 ± 6 (15–51)</td>
<td>69 ± 5 (57–87)</td>
</tr>
<tr>
<td>VCL (µm/s)</td>
<td>38 ± 5 (20–53)</td>
<td>59 ± 3 (51–64)</td>
</tr>
<tr>
<td>VSL (µm/s)</td>
<td>22 ± 4 (10–37)</td>
<td>39 ± 3 (29–49)</td>
</tr>
<tr>
<td>LIN (%)</td>
<td>52 ± 6 (30–68)</td>
<td>64 ± 3 (58–73)</td>
</tr>
<tr>
<td>VAP (µm/s)</td>
<td>28 ± 4 (15–41)</td>
<td>44 ± 3 (34–53)</td>
</tr>
<tr>
<td>ALH (µm)</td>
<td>1.8 ± 0.1 (1.5–2.0)</td>
<td>2.2 ± 0.1 (1.9–2.5)</td>
</tr>
</tbody>
</table>

Values are mean ± SE (range).
Significant difference between oligozoospermic and normozoospermic subgroups. \(\ast P < 0.05\), \(\ast\ast P < 0.001\).
For abbreviations, see Table I.

The number of ova examined×100. The spermatozoa bound per egg were also counted.

**Statistical analysis**

Differences between groups were assessed using the Wilcoxon signed rank test (non-parametric test) for paired data. Differences with a \(P\) value of < 0.05 were considered to be statistically significant.

**Results**

After incubation for 3 h in a gas phase of either 5% O\textsubscript{2} or 20% O\textsubscript{2}, none of the parameters investigated, e.g. movement characteristics (Table II), potential of spermatozoa to acquire the hyperactivated motility (9.1 ± 1.1% versus 9.2 ± 1.1%; Figure 1A) and to undergo the acrosome reaction when challenged with a calcium ionophore (24.1 ± 2.2% versus 23.0 ± 2.1%; Figure 1B) was significantly different between the two oxygen tensions in the group of normozoospermic fertile donors. The capacity of spermatozoa for spermatozoa–oocyte fusion assessed after 17 h incubation (50.0 ± 5.3% versus 43.3 ± 4.1%; Figure 2) and their survival rate (82.3 ± 8.3% versus 81.1 ± 9.2%; Figure 3) recorded after incubation for 24 h were also unaffected by the oxygen tension.

In contrast, in the group of oligozoospermic patients, the VCL and the ALH (Table II) of the spermatozoa capacitated under a gas phase of 5% O\textsubscript{2} were significantly increased (\(P < 0.005\)) compared with the VCL and ALH of spermatozoa capacitated under an atmosphere of 20% O\textsubscript{2}, whereas the LIN
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Figure 1. (A) Hyperactivated motility (%) and (B) acrosome reaction (%) of spermatozoa from normozoospermic or oligozoospermic men incubated for 3 h at 37°C under low (5% O\textsubscript{2}) or ambient (20% O\textsubscript{2}) oxygen tension. Values are mean ± SE, \( n = 25 \). *\( P < 0.01 \) compared with 20% O\textsubscript{2} in the same group.

Indeed, marked differences in the motility parameters, acrosome reaction, capacity of spermatozoa for spermatozoa-oocyte fusion and survival rates were observed whether the spermatozoa were incubated under 5% or 20% oxygen tension. This improvement in sperm function observed when incubated under low oxygen tension could be explained by a lower production of ROS by spermatozoa than under an ambient oxygen tension and consequently by less peroxidative damage to the sperm plasma membrane. Indeed, spermatozoa from oligozoospermic patients are very susceptible to lipid peroxidation, since half of the cases of oligozoospermia are associated with generation rates of ROS above the normal fertile range in seminal plasma (Aitken \textit{et al.}, 1989a, 1992b; Zalata \textit{et al.}, 1995). It has been shown that the oxygen tension in the incubation medium greatly influences the amount of ROS involved and consequently the extent of the modifications induced by these ROS (Alvarez and Storey, 1983; Griveau and Le Lannou, 1997b). We have demonstrated in a previous study that, under standardized conditions, spermatozoa incubated in the presence of 20 mM NADPH, generated 8-fold more superoxide anion in the incubation medium when incubated under 20% oxygen tension than when incubated under 5% oxygen tension (Griveau and Le Lannou, 1997b).

If the oxygen tension in the incubator gas phase appears to have no influence on the functional competence of normozoospermatozoa, the situation is different with oligozoospermatozoa. Two ROS-generating systems have been proposed, an NADPH oxidase-like system at the sperm plasma membrane level, and a sperm diaphorase (an NADH-dependent oxidoreductase) located in the middle piece and integrated into the mitochondrial respiratory system. ROS would emanate in part from abnormal spermatozoa, which are characterized by the retention of excess residual cytoplasm as a result of defective spermiogenesis (Aitken \textit{et al.}, 1994b). Membrane lipid peroxidation has been correlated with midpiece morphological defects (Rao \textit{et al.}, 1989) and abnormal morphology (Aitken and Clarkson, 1988). In fact, oligozoospermia is often associated with astheno- and/or teratozoospermia. In our study, 26 of the 32 patients presented oligo- and astheno- and/or teratozoospermia. As a consequence of this enhanced cytoplasmic space, the spermatozoa exhibit high cellular contents of a variety of cytoplasmic enzymes, including lactic acid dehydrogenase, creatine phosphokinase and glucose-6-phosphate dehydrogenase (G-6-PDH), all of which have been associated with defective sperm function (Casano \textit{et al.}, 1991; Aitken \textit{et al.}, 1994b; Huszar and Vigue, 1994). The cellular damage would
be induced by the G-6-PDH which, by stimulating generation of NADPH, fuels excessive production of ROS. Only one-third of the ROS produced by the spermatozoa are released extracellularly (Plante et al., 1994), so the usual ROS scavengers such as catalase and superoxide oxidase, though very effective against ROS produced by an external source such as activated polymorphonuclear leukocytes (Kovalski et al., 1992) or by a ROS generation system such as the association of xanthine and xanthine oxidase (Griveau et al., 1995a), are relatively ineffective when produced intracellularly. In contrast, by reducing the oxygen tension in the incubator gas phase, we directly prevented the formation of ROS, which is probably the best way to prevent the related oxidative stress undergone by the cells, as suggested by the improved sperm function seen under low oxygen tension. It is also probable that in the same way, the toxic oxygen metabolites generated by activated polymorphonuclear leukocytes, which are a major potential source of ROS and whose infiltration into sperm preparations has been associated with marked loss of motility, will be significantly reduced.

From the viewpoint of assisted reproduction, these results can be of importance for the strategies that might be used to reduce oxidative stress experienced by deficient spermatozoa during the course of IVF, since the production of ROS in semen has been associated with a decreased ability of spermatozoa to initiate the membrane fusion events associated with the acrosome reaction and fusion with the oocyte as a consequence of the changes in membrane fluidity and integrity induced by the accumulation of lipid peroxides. Previous reports have suggested that the outcome of IVF cycles has not been substantially influenced whether high (20%) or low (5%) oxygen tensions were used (Dumoulin et al., 1995). One can pose the question as to whether human spermatozoa can be of importance for the strategies that might be used to prevent the related oxidative stress undergone by the embryos can be cultured successfully using a gas phase of 5% oxygen tension was mainly due to the fact that the studies have been mostly performed with normal spermatozoa and were essentially involved with embryonic development. The results we observed with normozoospermatozoa are effectively consistent with the data already published. In fact, ROS production by normal spermatozoa is very low under normal culture conditions and oxygen tension, and even plays a key role in regulating cell function (De Lamirande and Gagnon, 1993; Griveau et al., 1994, 1995b; Aitken, 1997; De Lamirande et al., 1997).

A prospective study should now be undertaken to address the real influence of oxygen tension on the outcome of IVF in cases of oligozoospermia. If many animal studies show the superiority of the use of reduced oxygen tension compared with ambient tension (Lindenauf and Fisher, 1994; Farrell and Foote, 1995), one can pose the question as to whether human embryos can be cultured successfully using a gas phase of 5% CO₂/90% N₂/5% O₂. On this point, several studies have shown that culturing human embryos under low oxygen tension was not detrimental to their development (Dumoulin et al., 1995; Jones et al., 1998). In summary, these results show that the use of low oxygen tension rather than air might improve the spermatozoan competence of oligozoospermic patients in the course of IVF programmes.

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