Effect of Oxygen Concentration in the Gas Atmosphere during In Vitro Insemination of Bovine Oocytes on the Subsequent Embryonic Development In Vitro

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ABSTRACT. In vitro matured bovine oocytes were co-incubated with sperm for 18 hr in a droplet of fertilization medium under a gas atmosphere of 5% CO₂ with 5 or 20% O₂. After removing the cumulus cells, they were fixed to examine their fertilization rate, or cultured for another 154 hr in a chemically defined medium under 5% O₂ to determine their development to the blastocyst stage. There was no difference between the 5 and 20% O₂ groups in the fertilization rate. However, the percentage of inseminated oocytes which developed to the blastocyst stage was higher when in vitro insemination was conducted under 5% O₂ compared with that under 20% O₂ (34.4 vs. 24.7%, P<0.05). — KEY WORDS: fertilization, oocyte, oxygen.


Oxygen tension in the female reproductive tract is lower than that in the atmospheric gas phase [5]. It is well known that oxygen at excessive tension gives an oxidative stress to mammalian cells, including embryos [1]. An oxygen concentration of 5 to 10% in the gas atmosphere has been reported to be optimal for the development of bovine embryos when they are cultured in vitro without feeder cells [4, 6, 8, 14–16, 20]. When bovine embryos were co-cultured with feeder cells such as oviduct cells, cumulus/granulosa cells, or other somatic cells, the optimal oxygen tension varied according to the cell type, medium and culture system itself [6, 8, 19].

The in vitro insemination of bovine oocytes is usually performed by co-incubating spermatozoa with oocytes surrounded by cumulus cells. It is therefore expected that the optimal oxygen concentration in the gas atmosphere during in vitro insemination may also vary according to the in vitro insemination conditions. However, empirical protocols for the in vitro insemination of bovine oocytes commonly utilize atmospheric gas conditions of 5% CO₂ in air (approximately 20% O₂) with different details (i.e., media, medium volume, oocytes density, oil covering etc.) [10]. Few attempts have been made to determine the effects of the oxygen concentration during in vitro insemination on the fertilization of bovine oocytes [10, 18] or the subsequent embryonic development in vitro [18]. In the present study, the hypothesis that the in vitro insemination of bovine oocytes under a low oxygen concentration (5% O₂), which is similar to the physiological level [5], would improve the fertilization rate and the subsequent embryonic development was tested by co-incubating in vitro matured oocytes with frozen-thawed spermatozoa using our in vitro insemination procedure [12–14].

The in vitro maturation of follicular oocytes was performed as described previously [14]. Briefly, bovine cumulus-oocyte complexes (COCs) aspirated from small antral follicles of slaughterhouse-obtained bovine ovaries, were washed twice with HEPES-buffered Tyrode’s medium (TALP-HEPES) [2]. The oocytes with morphologically normal ooplasm and with an intact cumulus were selected for culture. The in vitro maturation was accomplished in HEPES-buffered TC1999 (Gibco Laboratories, Grand Island, NY, U.S.A.) supplemented with 10% heat-treated fetal calf serum (Gibco), 0.02 units/ml of follicle stimulating hormone (from porcine pituitary, Sigma), 1 µg/ml of estradiol-17β (Sigma), 0.2 mM sodium pyruvate, and 50 µg/ml of gentamicin sulfate. After being washed twice with the maturation medium, selected COCs were transferred to 50-µl drops of maturation medium (10–13 COCs per drop) under paraffin oil in a 60-mm plastic dish (Falcon 1007, Becton Dickinson Labware, Lincoln Park, NJ, U.S.A.). They were then cultured for 22 hr at 39°C in humidified air with 5% CO₂ (in a CO₂ incubator; Napco 5100, Napco Scientific Co., Tualtin, OR, U.S.A.).

The in vitro insemination was conducted according to the procedure described elsewhere [14]. In brief, frozen sperm from a Holstein bull were thawed at 37°C for 30 sec, and subsequently layered onto a Percoll gradient (45 and 90%). The Percoll gradient was centrifuged at 700 g for 20 min. After the top layers were removed, the sperm pellet was resuspended in 6 ml of modified Brackett and Oliphant isotonic medium [3] without bovine serum albumin (BSA), penicillin and streptomycin but with 1.7 µg/ml phenol red (Sigma) and 25 µg/ml gentamicin sulfate, which was designated as mBO medium [12]. They were then washed again by centrifugation at 500 × g for 5 min. After the centrifugation and aspiration of the supernatant, the sperm pellet was resuspended to 10 × 10⁶ cells/ml in mBO medium. Initial in vitro insemination droplets were prepared with 50-µl of mBO medium containing 6 mg/ml of BSA (Fatty acid-free, Sigma) and 5 mM theophylline (Sigma). The droplets were covered with paraffin oil in a 35-mm plastic dish (Falcon 1008), and were kept at 39°C under 5 or 20% O₂ for more than 3 hr before use. Fifty µl of sperm suspension was introduced into the initial insemination droplet to give a final insemination droplet of 5 × 10⁶ cells/ml sperm concentration, 3 mg/ml BSA, and 2.5 mM theophylline. COCs were then immediately put into the
The effect of oxygen concentration in the gas atmosphere on the in vitro fertilization rate

<table>
<thead>
<tr>
<th>Item</th>
<th>Concentration of oxygen (%)</th>
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<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>No. of replicates (zygotes)</td>
<td>5 (150)</td>
</tr>
<tr>
<td>% of cleaved zygotes</td>
<td>79.9 ± 3.7</td>
</tr>
<tr>
<td>% of blastocysts</td>
<td>34.4 ± 6.7a</td>
</tr>
<tr>
<td>Total cell numbers per blastocyst (N)</td>
<td>193.4 ± 67.1 (52)</td>
</tr>
</tbody>
</table>

Values are means ± SD determined at around 172 hr after insemination. a), b) Values with different superscripts denote a significant difference (P<0.05).
shown that the blastocyst formation rate in mice embryos was significantly decreased after a 1-hr exposure of pronuclear embryos to 20% O\textsubscript{2} [9, 17]. It is likely that the present \textit{in vitro} insemination procedure in 5% O\textsubscript{2} might reduce the oxidative stress and/or production of reactive oxygen species (ROS) [1, 7] resulting in an improvement of the subsequent embryonic development as a latent effect. Alternatively, the time course of the \textit{in vitro} fertilization in 5% O\textsubscript{2} might differ from that in 20% O\textsubscript{2}. The delay in the sperm penetration causes the aging of oocytes and reduces the viability of fertilized oocytes [11]. Further experiments on the oxidative stress/production of ROS during \textit{in vitro} insemination and the time course of fertilization are needed to clarify the beneficial effect of \textit{in vitro} insemination under 5% O\textsubscript{2} on fertilization and subsequent embryonic development.

In conclusion, the present results demonstrated that lowering the oxygen concentration from 20 to 5% during \textit{in vitro} insemination does not affect the fertilization rate, but improves the subsequent embryonic development to the blastocyst stage under the present experimental conditions.

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REFERENCES