Unexpected low oxygen tension of intravaginal culture

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The goal of this study was to assess whether the metabolic activity of gametes or the local environment had a greater influence on the pH, pO2 and pCO2 of the culture medium in the intravaginal culture (IVC) technique. The pH, pO2 and pCO2 of the culture medium in four groups of intravaginal cryotubes with or without spermatozoa and oocytes, together with the pO2 and pCO2 of vaginal epithelium, were measured before and after 48 h of IVC. Hermetically closed cryotubes sealed within a cryoflex envelope were used throughout. Similar results were obtained from all four groups. The pH and pCO2 were unchanged but pO2 significantly decreased during IVC, presumably because of equilibration with the low pO2 (5 mmHg) and pCO2 (49 mmHg) present in the vaginal epithelium. A second series of experiments was then performed with standard culture conditions using culture medium with or without motile spermatozoa in cryotubes covered with cryoflex maintained in air supplemented with 5% CO2. The pH, pO2 and pCO2 were all unchanged in all samples. When the samples were maintained in air only, the pH increased, pO2 remained unchanged and pCO2 decreased, presumably because of equilibration with the low pCO2 (0.3 mmHg) present in the air. However, when the samples were cultured under venous blood, the pH and pO2 decreased and pCO2 increased, presumably because of the high pCO2 and low pH of venous blood. Thus the pO2 and pCO2 of the culture medium were able to equilibrate with the local environmental gas milieu owing to the permeability of O2 and CO2 through the plastic material. IVC results in a constant pH due to an identical pCO2 in the vaginal epithelium but in a reduced pO2 concentration due to the lower pO2 in the vaginal epithelium.

Key words: cryotube/intravaginal culture/pH/pO2/pCO2

Introduction

In-vitro fertilization (IVF) of human oocytes was first reported by Steptoe and Edwards in 1978. A new IVF technique, intravaginal culture (IVC) using a cryotube, was later developed by Ranoux et al. (1988a). Early reports of the IVC technique demonstrated similar pregnancy rates to those of conventional IVF. The aim of the present study was to compare changes in pH, pO2 and pCO2 in medium incubated using either the IVC technique or standard IVF conditions and to find an explanation for the mechanisms which maintain the pH almost constant during the 48 h of IVC.

Materials and methods

All women in this study gave informed consent before participating. The pH, pO2 (mmHg) and pCO2 (mmHg) of culture medium were measured with a blood gas analyser (238 PH/Blood Gas Analyzer; Ciba Corning, Tokyo, Japan) just before the closure and immediately after the opening of identical polypropylene cryotubes (Nunc, Kamstrup, Denmark) after a 48 h interval. All of the cryotubes were hermetically closed and sealed within a polyethylene cryoflex envelope (Nunc). The culture medium (Méfnozo B2; CCD, Paris, France) placed in the cryotubes was equilibrated with 5% CO2 in air at the start of the procedure. Four experiments (I, II, III and IV) assessing changes in pH, pO2 and pCO2 were performed.

Experiment I (condition: intravaginal culture)

The IVC technique was performed as previously described (Ranoux et al., 1988a). Up to five oocytes were placed with 100 000 motile sperms in a cryotube filled with pure B2 medium. Semen preparation had been performed using the two-layered Percoll method (55 and 80%) described by Ziebe and Yding Andersen (1993). All the cryotubes were incubated in the vagina for 48 h. Four groups undergoing IVC were studied: group I (n = 18), the tubes contained culture medium only, without spermatozoa or an oocyte; group 2 (n = 13), culture medium with motile spermatozoa (100 000/tube); group 3 (n = 46), culture medium with motile spermatozoa (100 000/tube) and one oocyte from women who did not become pregnant; group 4 (n = 12), culture medium with motile spermatozoa and one oocyte from women who became pregnant. Oocytes from groups 3 and 4 were retrieved from women undergoing clomiphene citrate stimulated cycles or oocytes from women during unstimulated (Ranoux et al., 1988b) or clomiphene citrate-stimulated cycles. Only cycles in which a single oocyte was retrieved were studied. The pO2 and pCO2 of vaginal epithelium were measured in 18 patients in group 1 using a transcutaneous pO2/pCO2 gas monitor (PO-750; Sumitomo, Tokyo, Japan).

Experiment II (condition: incubator with 5% CO2 in air)

The following experiment was performed using two study groups: group 1 (n = 8), culture medium only; group 2 (n = 8), culture medium with 100 000 motile spermatozoa per tube. The cryotubes were maintained at 37°C in an incubator with 5% CO2 in air without exposure to light. Measurements were made of the pH, pO2 and pCO2 of the culture medium. The pO2 and pCO2 of the gas flow of 5% CO2 in air through the incubator was also measured, using a transcutaneous gas monitor.
Table I. The pH, pO\(_2\) (mmHg) and pCO\(_2\) (mmHg) of culture medium in cryotubes before and after intravaginal culture (IVC) of four groups and also the pO\(_2\) and pCO\(_2\) of vaginal epithelium in experiment I. The data are given as means ± SD. The interval between the measurements was 48 h.

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\text{Experiment I} & \text{Before IVC} & \text{After IVC} \\
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1. Culture medium only (n = 18) & & \\
\text{pH} & 7.30 ± 0.03 & 7.30 ± 0.04 \\
pO\(_2\) & 159.8 ± 15.0* & 90.8 ± 26.1* \\
pCO\(_2\) & 50.4 ± 3.5 & 50.9 ± 4.6 \\
2. Culture medium + spermatozoa (n = 13) & & \\
pH & 7.33 ± 0.04 & 7.32 ± 0.03 \\
pO\(_2\) & 162.8 ± 14.3* & 95.7 ± 14.4* \\
pCO\(_2\) & 50.5 ± 7.8 & 52.8 ± 6.0 \\
3. Culture medium + spermatozoa + oocyte, not pregnant (n = 46) & & \\
pH & 7.33 ± 0.03 & 7.30 ± 0.04 \\
pO\(_2\) & 163.5 ± 11.8* & 94.7 ± 11.6* \\
pCO\(_2\) & 49.3 ± 5.9 & 51.9 ± 6.1 \\
4. Culture medium + spermatozoa + oocyte, pregnant (n = 12) & & \\
pH & 7.33 ± 0.03 & 7.31 ± 0.03 \\
pO\(_2\) & 160.7 ± 18.8* & 94.5 ± 12.8* \\
pCO\(_2\) & 49.5 ± 5.2 & 51.3 ± 4.9 \\
Vaginal epithelium (n = 18) & & \\
pO\(_2\) & 4.0 ± 8.3 & 5.3 ± 7.7 \\
pCO\(_2\) & 49.3 ± 8.6 & 48.8 ± 9.8 \\
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\*P < 0.001.

Table II. pH, pO\(_2\) (mmHg) and pCO\(_2\) (mmHg) of venous blood and culture medium of cryotubes before and after culture in experiments II, III and IV. The pO\(_2\) and pCO\(_2\) of 5% CO\(_2\) in air, air and venous blood are also presented. The interval between the measurements was 48 h. The data are given as means ± SD.

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\begin{array}{|c|c|c|}
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\text{Experiment II (incubator with 5\% CO\(_2\) in air)} & \text{Before culture} & \text{After culture} \\
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1. Culture medium only (n = 8) & & \\
pH & 7.30 ± 0.03 & 7.33 ± 0.02 \\
pO\(_2\) & 159.5 ± 14.0 & 155.7 ± 16.0 \\
pCO\(_2\) & 50.3 ± 3.3 & 49.0 ± 4.0 \\
2. Culture medium + spermatozoa (n = 8) & & \\
pH & 7.31 ± 0.03 & 7.30 ± 0.03 \\
pO\(_2\) & 161.0 ± 16.0 & 156.5 ± 15.0 \\
pCO\(_2\) & 50.5 ± 3.8 & 52.4 ± 3.7 \\
5% CO\(_2\) in air (n = 8) & & \\
pO\(_2\) & 148.6 ± 6.7 & 149.2 ± 7.6 \\
pCO\(_2\) & 49.0 ± 10.0 & 48.2 ± 9.5 \\
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\text{Experiment III (incubator with air only)} & & \\
1. Culture medium only (n = 8) & & \\
pH & 7.29 ± 0.03* & 7.60 ± 0.03* \\
pO\(_2\) & 160.1 ± 16.0 & 172.8 ± 12.0 \\
pCO\(_2\) & 50.1 ± 5.1* & 25.8 ± 1.0* \\
2. Culture medium + spermatozoa (n = 8) & & \\
pH & 7.31 ± 0.03* & 7.59 ± 0.04* \\
pO\(_2\) & 160.0 ± 15.0 & 171.6 ± 14.0 \\
pCO\(_2\) & 50.3 ± 4.5* & 26.9 ± 2.3* \\
Air (n = 8) & & \\
pO\(_2\) & 160.7 ± 3.0 & 160.3 ± 2.9 \\
pCO\(_2\) & 0.5 ± 0.5 & 0.3 ± 0.5 \\
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\text{Experiment IV (in venous blood)} & & \\
1. Culture medium only (n = 8) & & \\
pH & 7.30 ± 0.03* & 7.05 ± 0.06* \\
pO\(_2\) & 158.7 ± 14.0* & 90.3 ± 17.4* \\
pCO\(_2\) & 49.5 ± 4.3* & 97.1 ± 16.0* \\
2. Culture medium + spermatozoa (n = 8) & & \\
pH & 7.31 ± 0.03* & 7.04 ± 0.07* \\
pO\(_2\) & 159.3 ± 15.0* & 91.5 ± 16.8* \\
pCO\(_2\) & 50.2 ± 4.5* & 98.2 ± 18.4* \\
Venous blood (n = 8) & & \\
pH & 7.33 ± 0.04* & 6.73 ± 0.13* \\
pO\(_2\) & 22.6 ± 7.6 & 10.0 ± 6.5 \\
pCO\(_2\) & 47.4 ± 6.1* & 178.8 ± 42.2* \\
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\*P < 0.001.

Statistical analysis
Statistical evaluation was performed using Student’s t-test. Results are presented as means ± SD.

Results

Experiment I (intravaginal culture)
The pH, pO\(_2\) and pCO\(_2\) of the culture medium before and after the IVC for the four groups are summarized in Table I. The pH and pCO\(_2\) did not change during the culture period but the pO\(_2\) significantly (\(P < 0.001\)) decreased from 160 to 90–95 mmHg in all four groups. The values of pH and pCO\(_2\) and the reduction in pO\(_2\) are quite similar in the four groups, including that with culture medium alone. The pO\(_2\) and pCO\(_2\) of the vaginal epithelium were consistently ~5 and 49 mmHg respectively, as shown in Table I. The pCO\(_2\) of IVC and vaginal epithelium remained constant at 50 mmHg, maintaining a constant pH of the IVC medium of 7.30.

Experiment III (incubator with air only)
Culture medium alone and culture medium containing spermatozoa had pO\(_2\) values of 155–160 mmHg both before and after culture. The gas flow (5% CO\(_2\) in air) had a similar pO\(_2\) value of 149 mmHg. The pCO\(_2\) of the culture medium and gas flow remained unchanged (±48–52 mmHg), thereby maintaining a constant pH.

Experiment IV (in venous blood)
A significant decrease in the pH of the covering layer of venous blood from 7.33 to 6.73 and a significant increase of
the pCO$_2$ from 47 to 170 mmHg were noted after 48 h of culture. Therefore, the pH of the culture medium alone and culture medium containing spermatozoa significantly decreased from 7.30 to 7.05 and the pCO$_2$ significantly increased from 49 to 97 mmHg during the culture period.

No significant differences were observed between the pH, pO$_2$ and pCO$_2$ in culture medium alone and in culture medium containing motile spermatozoa in experiments II, III and IV. The results are summarized in Table II.

Discussion

This study was performed to elucidate changes in pH, pO$_2$ and pCO$_2$ in medium incubated using the IVC technique.

In experiment I, culture medium alone was used in group 1 cryotubes, but motile spermatozoa with or without an oocyte were added to the culture medium in groups 2, 3 and 4. Spermatozoa and/or oocytes consume O$_2$ and accumulate CO$_2$. Thus, it would be expected that the pO$_2$ might decrease and the pCO$_2$ increase during culture. However, similar results were obtained from all four groups: a distinct reduction of the pO$_2$ was observed with no change in pCO$_2$. It appears that the intravaginal conditions had the capacity to buffer the medium containing metabolically active cells. The low pO$_2$ found in the vaginal epithelium (experiment I) probably explains the low pO$_2$ in the medium from all of the IVC.

In experiment II, the culture medium alone and culture medium containing motile spermatozoa maintained in cryotubes had similar pO$_2$ and pCO$_2$ values to those of the atmosphere (5% CO$_2$ in air) of the incubator in which they were stored. Thus, no difference was seen in these values before or after culture.

In experiment III, using an incubator with only air injection, the pCO$_2$ of the flowing air was very low (0.3–0.5 mmHg), leading to a reduction in the pCO$_2$ in the culture medium in the cryotubes to 25 mmHg and an increase in pH to 7.60.

In experiment IV, the high pCO$_2$ (170 mmHg) and low pH (6.73) of the covering venous blood led to an increase in pCO$_2$ in the culture medium in the cryotubes from 49 to 97 mmHg and a decrease in pH from 7.30 to 7.05 after 48 h of culture. It is known that O$_2$ and CO$_2$ can pass through certain plastic materials, including polypropylene (cryotube) and polyethylene (cryoflex). Thus the pO$_2$ and pCO$_2$ of culture medium in an air-free cryotube completely covered with cryoflex is probably influenced by the local environmental gas milieu because of this permeability. O$_2$ and CO$_2$ are able to pass through the plastic material, but bacteria-containing vaginal secretions are not able to pass through.

In conclusion, IVC maintains medium pH and pCO$_2$ by equilibration with the vaginal epithelium gas milieu, but the pO$_2$ of the medium is reduced because of the lower pO$_2$ in vaginal epithelium. Thus, in this system the vagina works as a CO$_2$ generator and an O$_2$ reducer.

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References


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