

Unexpected low oxygen tension of intravaginal culture

Misao Fukuda^{1,3}, Kiyomi Fukuda¹ and Claude Ranoux²

¹Fukuda Ladies Clinic, 30–9 Kariya, Ako, Hyogo 678–02, Japan and ²Boston Regional Center for Reproductive Medicine, Medical Office Building Suite 321, Three Woodland Road, Stoneham, MA 02180, USA

³To whom correspondence should be addressed

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 48 h after IVC. Hermetically closed cryotubes sealed in 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 (Ménézo 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 sperm 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 1 (*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 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, pO₂ (mmHg) and pCO₂ (mmHg) of culture medium in cryotubes before and after intravaginal culture (IVC) of four groups and also the pO₂ and pCO₂ of vaginal epithelium in experiment I. The data are given as means ± SD. The interval between the measurements was 48 h

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

^a*P* < 0.001.**Experiment III (condition: incubator with air only)**

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 cryotube. Both groups were placed in an incubator which had air injection only. The pH, pO₂ and pCO₂ of the medium and the air flow were measured.

Experiment IV (condition: in venous blood)

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 covered with heparinized venous blood in an air-free glass tube. The pH, pO₂ and pCO₂ were measured in the medium and the venous blood.

Statistical analysis

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

Results**Experiment I (intravaginal culture)**

The pH, pO₂ and pCO₂ of the culture medium before and after the IVC for the four groups are summarized in Table I. The pH and pCO₂ did not change during the culture period but the pO₂ significantly (*P* < 0.001) decreased from 160 to 90–95 mmHg in all four groups. The values of pH and pCO₂ and the reduction in pO₂ are quite similar in the four groups, including that with culture medium alone. The pO₂ and pCO₂ of the vaginal epithelium were consistently ~5 and 49 mmHg respectively, as shown in Table I. The pCO₂ of IVC and vaginal epithelium remained constant at 50 mmHg, maintaining a constant pH of the IVC medium of 7.30.

Table II. pH, pO₂ (mmHg) and pCO₂ (mmHg) of venous blood and culture medium of cryotubes before and after culture in experiments II, III and IV. The pO₂ and pCO₂ of 5% CO₂ in air, air and venous blood are also presented. The interval between the measurements was 48 h. The data are given as means ± SD

	Before culture	After culture
Experiment II (incubator with 5% CO₂ in air)		
1. Culture medium only (<i>n</i> = 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 (<i>n</i> = 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 (<i>n</i> = 8)		
pO ₂	148.6 ± 6.7	149.2 ± 7.6
pCO ₂	49.0 ± 10.0	48.2 ± 9.5
Experiment III (incubator with air only)		
1. Culture medium only (<i>n</i> = 8)		
pH	7.29 ± 0.03 ^a	7.60 ± 0.03 ^a
pO ₂	160.1 ± 16.0	172.8 ± 12.0
pCO ₂	50.1 ± 5.1 ^a	25.8 ± 1.0 ^a
2. Culture medium + spermatozoa (<i>n</i> = 8)		
pH	7.31 ± 0.03 ^a	7.59 ± 0.04 ^a
pO ₂	160.0 ± 15.0	171.6 ± 14.0
pCO ₂	50.3 ± 4.5 ^a	26.9 ± 2.3 ^a
Air (<i>n</i> = 8)		
pO ₂	160.7 ± 3.0	160.3 ± 2.9
pCO ₂	0.5 ± 0.5	0.3 ± 0.5
Experiment IV (in venous blood)		
1. Culture medium only (<i>n</i> = 8)		
pH	7.30 ± 0.03 ^a	7.05 ± 0.06 ^a
pO ₂	158.7 ± 14.0 ^a	90.3 ± 17.4 ^a
pCO ₂	49.5 ± 4.3 ^a	97.1 ± 16.0 ^a
2. Culture medium + spermatozoa (<i>n</i> = 8)		
pH	7.31 ± 0.03 ^a	7.04 ± 0.07 ^a
pO ₂	159.3 ± 15.0 ^a	91.5 ± 16.8 ^a
pCO ₂	50.2 ± 4.5 ^a	98.2 ± 18.4 ^a
Venous blood (<i>n</i> = 8)		
pH	7.33 ± 0.04 ^a	6.73 ± 0.13 ^a
pO ₂	22.6 ± 7.6	10.0 ± 6.5
pCO ₂	47.4 ± 6.1 ^a	170.8 ± 42.2 ^a

^a*P* < 0.001.**Experiment II (incubator with 5% CO₂ in air)**

Culture medium alone and culture medium containing spermatozoa had pO₂ values of 155–160 mmHg both before and after culture. The gas flow (5% CO₂ in air) had a similar pO₂ value of 149 mmHg. The pCO₂ of the culture medium and gas flow remained unchanged (~48–52 mmHg), thereby maintaining a constant pH.

Experiment III (incubator with air only)

Culture medium alone and culture medium containing spermatozoa showed similar pO₂ values of 160–172 mmHg both before and after culture. However, the pCO₂ of the air flow was consistently very low (0.3–0.5 mmHg). Therefore, the pCO₂ of the culture medium decreased significantly from 50 to 25 mmHg and the pH increased significantly from 7.30 to 7.60 after 48 h of culture.

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 $p\text{CO}_2$ 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 $p\text{CO}_2$ significantly increased from 49 to 97 mmHg during the culture period.

No significant differences were observed between the pH, $p\text{O}_2$ and $p\text{CO}_2$ 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, $p\text{O}_2$ and $p\text{CO}_2$ 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 $p\text{O}_2$ might decrease and the $p\text{CO}_2$ increase during culture. However, similar results were obtained from all four groups: a distinct reduction of the $p\text{O}_2$ was observed with no change in $p\text{CO}_2$. It appears that the intravaginal conditions had the capacity to buffer the medium containing metabolically active cells. The low $p\text{O}_2$ found in the vaginal epithelium (experiment I) probably explains the low $p\text{O}_2$ 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 $p\text{O}_2$ and $p\text{CO}_2$ 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 $p\text{CO}_2$ of the flowing air was very low (0.3–0.5 mmHg), leading to a reduction in the $p\text{CO}_2$ in the culture medium in the cryotubes to 25 mmHg and an increase in pH to 7.60.

In experiment IV, the high $p\text{CO}_2$ (170 mmHg) and low pH (6.73) of the covering venous blood led to an increase in $p\text{CO}_2$ 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 $p\text{O}_2$ and $p\text{CO}_2$ 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 $p\text{CO}_2$ by equilibration with the vaginal epithelium gas milieu, but the $p\text{O}_2$ of the medium is reduced because of the lower $p\text{O}_2$ in vaginal epithelium. Thus, in this system the vagina works as a CO_2 generator and an O_2 reducer.

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