SHORT COMMUNICATION

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The effects of IVF aspiration on the temperature, dissolved oxygen levels, and pH of follicular fluid

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Purpose: To investigate the effects of IVF aspiration on the temperature, pH, and dissolved oxygen of bovine follicular fluid.

Methods: The temperature was monitored at various positions in an aspiration kit. Dissolved oxygen and pH were measured before and after aspiration.

Results: The temperature of follicular fluid dropped by $7.7 \pm 1.3^\circ\text{C}$ upon aspiration. Dissolved oxygen levels rose by 5 ± 2 vol.%. The pH increased by 0.04 ± 0.01 .

Conclusions: The temperature change was attributed mainly to evaporation of fluid in the collection tube. Changes in dissolved oxygen levels and pH were due to contact with air. Standard vacuum-based aspiration may induce changes in follicular fluid, which could be detrimental to oocyte health and affect attempts to correlate chemical characteristics with oocyte quality.

KEY WORDS: Aspiration; follicular fluid; oxygen; pH; temperature.

In-vitro fertilization (IVF) seeks to maximize the chances of an infertile couple having a successful pregnancy. The highest quality oocytes and embryos must be selected. If the procedure employed from aspiration to transfer is suboptimal then the developmental capacity of even the highest quality oocytes and embryos may be reduced.

The physical impact of oocyte aspiration on developmental competence has received some attention. The effects of needle diameter, length, and sharpness upon the oocyte have been investigated (1–3), as have the magnitude of the applied vacuum, and method of vacuum application (syringe vs. vacuum pump) (3, 4), but the effect of aspiration on follicu-

lar fluid properties has not been reported. The effect of follicle aspiration on the temperature, dissolved oxygen levels, and pH of bovine follicular fluid (used as a model fluid) is reported here.

Follicular fluid was collected from the ovaries of cows killed at a local abattoir. Aspiration kits were set up so that thermocouples, oxygen, and pH electrodes could be used to monitor changes in fluid as it was drawn through the kits (Fig. 1). Sections of the kits were variously heated and insulated so that heat transfer could be estimated. Aspiration was performed using a vacuum pump at flow-rates between 8 and 22 mL/min. For measurement of pH, samples were equilibrated with 5 vol.% carbon dioxide before aspiration.

When aspirated, fluid initially at $37 \pm 0.5^\circ\text{C}$, dropped in temperature by an average of 7.7°C from source to collection tube (TI to TV; Fig. 2). Of this drop, 6.4°C occurred after the fluid fell from the tubing exit to the bottom of the tube (TIV to TV). The drop was relatively independent of flow rate, except between TIV and TV where it increases with increasing flowrate.

Table 1 shows the temperature drops from TIV to TV when various combinations of collection tube heating and insulation were employed. From these, it was estimated that of the heat lost, 82% was due to internal heat losses, 15% to heating collection tube walls (tubes not prewarmed), and 2% to convective heat loss to the environment. The internal heat losses are thought to be due principally to evaporation of water in the collection tube under the conditions of reduced vapor pressure.

Aspiration caused significant rises in the dissolved oxygen content of follicular fluid particularly at lower initial levels ($p < 0.01$) (Fig. 3). When fluid was sampled from the middle of the tubing line there was no significant change in oxygen levels ($p = 0.307$) (Fig. 3), indicating that the cause of change in oxygen was mixing with air in the collection tube.

When fluid was aspirated into the collection tube, the pH rose an average of 0.04 ± 0.01 pH units ($\pm\text{SD}$, $n = 15$) ($p < 0.01$). When fluid was sampled directly from the tubing line no significant change in pH was observed ($p = 0.15$).

The physical properties of bovine follicular fluid, which govern the observed heat and mass transport properties are not expected to be significantly different from those of human fluid. The changes in the temperature, dissolved oxygen levels, and pH are of greater importance than the absolute values

Fig. 1. Diagram of the aspiration kit (Follicle Aspiration Sets, Swedmed Lab International AB, Sweden) showing positions at which oxygen, temperature, and pH of bovine follicular fluid was measured.

Fig. 2. The mean temperatures at each of the thermocouple positions as follicular fluid was aspirated.

Table I. Mean ($\pm$ SE; $n = 10$) Temperature Drops ($^{\circ}$ C) from Thermocouple IV to V Under Different Heating Conditions ($^{\circ}$ C).

ΔT_1	ΔT_2	ΔT_3
6.4 $\pm$ 0.1	5.3 $\pm$ 0.1	6.2 $\pm$ 0.1

Note. ΔT_1 : aspiration performed with fluid at 37 $^{\circ}$ C, collection tube at ambient temperature; ΔT_2 : collection tube heated to 37 $^{\circ}$ C, and then insulated prior to aspiration; ΔT_3 : collection tube insulated (not heated) prior to aspiration. Flow-rate = 14 ml min.⁻¹

of these parameters as they illustrate the underlying heat and mass transport phenomena.

The large and rapid temperature drops observed here are unlike any oocyte will have experienced in its previous history. In a clinic, the retrieval process would take the environment of the oocyte from about 37 $^{\circ}$ C preaspiration to approximately 29 $^{\circ}$ C immediately postaspiration, before returning to 37 $^{\circ}$ C.

Research suggests that human oocytes exposed to room temperature can have disrupted spindles and chromosomal dispersal (5). While these effects can be reversed on re-warming (6), research on pig oocytes indicated that recovery could be incomplete (7) leading to reduced pregnancy rates.

Evaporation and an associated temperature drop will occur regardless of how quickly the oocyte is transferred. The magnitude of the drop will vary depending on the particular aspiration kits employed, the magnitude of the applied vacuum, and the degree of heating. Differences in clinical operating practices with regard to fluid temperature control may contribute to the documented variation in pregnancy rates among fertilization centers (8).

The magnitude of the changes in dissolved oxygen will vary from system to system, but will be present if aspiration allows for significant gas-liquid

Fig. 3. Comparison of the dissolved oxygen in follicular fluid before aspiration and in the collection tube, or when sampled from tubing line before the collection tube (see position III, Fig. 1). Symbols: ◆—fluid in collection tube; △—fluid from position III; straight line is $y = x$.

contact. Fluid cannot simply be aspirated into the bulk collection tube and analyzed for oxygen. The large variation (7–17 vol.%) in reported values of dissolved oxygen in follicular fluid (9–13) suggests that either the populations are different, or that analytical errors are present. Inadequate attention to avoidance of oxygen level changes during fluid sampling may underlie the variation observed in these reports.

The small increase in pH of the fluid, which was eliminated when fluid was sampled from the tubing line and air–fluid contact in the collection tube avoided, was most likely caused by a reduction in the CO_2 levels in the fluid as it mixed with air in the collection tube which would have had relatively low CO_2 levels. The rise in pH is unlikely to have major physiological significance but may have important implications if very accurate determinations of follicular fluid pH are required.

The changes highlight the need for preanalytical precautions when analyzing follicular fluid samples and to consider whether the method of aspiration could affect the health of oocytes.

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