PHYSIOLOGY AND MANAGEMENT

Cation Concentrations in Fluid from the Oviduct Ampulla and Isthmus of Cows During the Estrous Cycle

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ABSTRACT

To detect variations in oviduct fluid cation concentrations, Ca++, Mg++, K+, and Na+ were determined for daily samples of blood serum and bovine oviduct fluid collected from indwelling isthmic and ampullary catheters. Isthmic oviduct fluid Ca++ concentration was significantly greater than that in ampullary fluid, particularly around estrus and ovulation. Maximum Ca++ concentrations found in isthmic oviduct fluid at estrus (2.57 ± .22 mM) and at ovulation (2.50 ± .29 mM) were similar to those of medium used for in vitro capacitation of bovine sperm. Concentrations of Mg++ in oviduct fluid differed significantly by estrous cycle stage, but not by oviduct region, and were consistently lower than those detected in serum. No relationships were found for K+ or Na+ with respect to region or stage, but K+ was generally higher in oviduct fluid than in serum. The concentration of K+ averaged over stage and region (4.46 ± .13 mM) and the K+:Na+ ratio (.032 ± .002) were similar to those reported in bovine in vitro capacitating and fertilizing media. Concentrations of Ca++ and Na+ from peritoneal fluid from nonstaged cows were similar to those of oviduct fluid or serum. The Mg++ concentration was greater, and K+ concentration was less, in peritoneal than in oviduct fluid.

(Key words: cations, bovine, oviduct fluid)

INTRODUCTION

Ions serve a variety of important biochemical functions in many biological systems. In vitro studies have demonstrated that several cations are vital for sperm capacitation, the acrosome reaction, motility, and fertilization. Extracellular Ca++ was recently shown to be necessary for in vitro capacitation of bovine sperm with glycosaminoglycans (11). Studies have demonstrated that Ca++ was required for capacitation of mouse (8), hamster (30), and human sperm (28) and that extracellular Ca++, Na+, and K+ were needed for the zona-induced acrosome reaction in guinea pig sperm (32). Studies have also shown that extracellular K+ was necessary for the acrosome reaction (32) and fertilization (2, 9) in hamster and mouse and for fertilization in the guinea pig (24). The presence of Ca++ caused significant motility changes in mouse sperm (20) and was necessary for binding of mouse sperm to the zona pellucida prior to egg penetration (26) and for sperm-egg fusion in the hamster, guinea pig, and human (29).

The oviduct provides the microenvironment for gametes, supporting the events leading up to and including fertilization. In the bovine oviduct, the isthmic region serves as a sperm reservoir for as long as 18 to 20 h (13). Sperm capacitation likely occurs in the oviduct isthmus (6), and the acrosome reaction takes place in the ampulla (12). Sperm hyperactivation also may be triggered in the isthmus, whereas fertilization generally occurs in the ampulla. Cations and other compounds present in regional oviduct fluid may play important roles in gamete physiology and function.

Modification of surgical cannulation procedures developed in our laboratory (15, 16) has enabled us to collect oviduct fluid from the isthmic and ampullary regions of the same bovine oviduct. The objective of the present
study was to analyze samples of oviduct fluid collected daily for Ca++, Mg++, K+, and Na+ to determine whether the cation concentrations varied with oviduct region or with stage of the estrous cycle. These results were compared with cation concentration profiles of bovine serum and peritoneal fluid, two physiological fluids that may influence oviduct fluid composition.

MATERIALS AND METHODS

Oviduct Fluid Collection

Six reproductively normal dairy cows were used. They ranged in age from 3 to 10 yr and included one Ayrshire, one Guernsey, and four Holsteins.

Standing laparotomies were performed for oviduct cannulations as described previously (15, 16). Briefly, the uterotubal and ampullary-isthmic junctions were ligated, and a cannula composed of polyethylene tubing encased in silastic tubing (number 602-205 Dow Corning, Midland, MI) was inserted into the ampullary lumen. The ampullary end was ligated, and a small slit was made in the ampullary-isthmic junction, where a similar isthmic cannula was secured. Both cannulas were exteriorized through puncture wounds made in the flank. Animals were allowed to recover for at least one estrous cycle prior to use of oviduct fluid. Experiments testing the Na+ permeability of the polyethylene-silastic oviduct cannulas (15) showed no appreciable diffusion of Na+ ion through the tubing.

Fluid was collected daily from cannula receptacles, which were contained in a canvas pouch mounted with adhesive on the flank of the cow. After measuring volume and osmolality, oviduct fluid was frozen and stored in liquid nitrogen until analysis. Daily oviduct fluid volumes ranged from 0 to 3.1 ml in the six cows used in this study. Ampulla oviduct fluid volumes were significantly greater than isthmus volumes by a factor of approximately 2. Fluid volumes recovered during the nonluteal stages (average ampulla volume = 1.0 ml; average isthmus volume = .5 ml) were significantly higher than during the luteal stage in both regions (ampulla = .6 ml; isthmus = .3 ml).

Hormonal profiles at the time of oviduct fluid collections were assessed by radioimmunoassay for progesterone (CIBA Corning Diagnostics, Medfield, MA) on daily serum samples using methods described by Killian et al. (17). The presence of cannulas precluded observation of behavioral estrus. The luteal stage of the cycle was assigned to days when serum progesterone concentrations were ≥1.5 ng/ml and nonluteal to serum progesterone concentrations <1.5 ng/ml. The nonluteal phase was further divided into the nonluteal 1 phase, consisting of the 2 to 5 d prior to estrus when serum progesterone concentrations were <1.5 ng/ml. Estrus was estimated to be 4 d prior to the initial rise in progesterone. The nonluteal 2 phase lasted 4 d (estrus to the initial rise in progesterone) and presumably included ovulation on the day after estrus (Figure 1).

Peritoneal Fluid Collection

Peritoneal fluid was collected from four randomly chosen, mature, nonlactating dairy cows that were not assessed for estrous cycle stage. The region between the right fore udder and flank fold was clipped and scrubbed. A posterior paramedian tap was performed by inserting an 18-gauge needle into the peritoneal cavity and allowing the fluid to collect in a sterile 15-ml conical centrifuge tube. The fluid, averaging 1 to 5 ml from each cow, was centrifuged at 1000 × g for 30 min to remove any red blood cells prior to cation analysis. Difficulties associated with the recovery of peritoneal fluid precluded daily collections from oviduct-cannulated cows.

Sample Preparation for Ion Analyses

The first experiment was undertaken to measure Ca++, Mg++, K+, and Na+ concentrations in daily samples of bovine oviduct fluid. Frozen oviduct fluid collected daily during one cycle for each of three cows was thawed, and aliquots of each sample were measured into acid-washed plastic vials for atomic absorption spectrophotometry analysis.

Results from this experiment showed that Ca++ concentrations peaked on the days of estrus and ovulation. To validate this finding, aliquots of oviduct fluid from the daily samples of three additional cows (total n = 6) were
Figure 1. The Ca++ concentrations in regional oviduct fluid of three cows. Fluid was collected daily during each estrous cycle from the ampullary and isthmic regions of the oviduct. The cycle was divided into nonluteal 1 (NL1), nonluteal 2 (NL2), and luteal (L) phases based on daily serum progesterone concentrations with estimated days of estrus (E) and ovulation (O) noted. Missing data points are due to negligible oviduct fluid production on that date. O = cow 1, △ = cow 2, ○ = cow 3.

An aliquot from each daily serum sample was pooled with respect to cycle stage, in a manner identical to the oviduct fluid pools for each cow, prepared as the oviduct fluid samples just described, and analyzed for Ca++, Mg++, and Na+ concentrations.

A third experiment was conducted to determine cation concentrations in peritoneal fluid. For this study, a single sample of peritoneal fluid was obtained from each of four different cows as described. An aliquot of the peritoneal fluid from each cow was measured into acid-washed vials and analyzed identically to oviduct fluid and serum samples by atomic absorption spectrophotometry. The oviduct, residing in the peritoneum, is bathed in peritoneal fluid. Exchange of cations between peritoneal and oviduct fluids was therefore questioned. Also, the preampulla may be open to the peritoneum, allowing peritoneal fluid to enter the oviduct. Thus, this study was designed to identify any difference between cation concentrations in oviduct fluid and two physiological fluids that may influence its composition.

Ion Analyses

A flame atomization atomic absorption spectrophotometer (Instrument Laboratories aa/ae Spectrophotometer 551, Instrument Laboratories, Inc., Wilmington, MA) equipped with an air-acetylene gas source was used to determine Ca++, Mg++, K+, and Na+ concentrations. To reduce interferences with other ions, La solution (.5%, wt/vol) was used as diluent for Ca++ and Mg++ analyses; Ce solution (.1%, wt/vol) was used for K+ analyses; and K solution (.2%, wt/vol) was used for Na+ determinations.

We also measured Zn++ in undiluted oviduct fluid but found only negligible concentrations (<1 μM).

Statistical Analysis

The effect (P < .05) of estrous cycle stage and anatomical region on ion concentrations in oviduct fluid and serum from six individual cows was determined using a general linear models analysis of variance (27). The model was Ca++ Mg++ K+ Na+ = cow stage region stage × region. Stage levels were nonluteal 1, nonluteal 2, and luteal; region levels were isth-
mic oviduct fluid, ampullary oviduct fluid, and serum. Comparisons were made using Fisher's protected least significant difference test.

Significant factor effects ($P < .05$) for comparisons of peritoneal fluid with oviduct fluid or with serum were determined using the model $Ca^{++}$ $Mg^{++}$ $K^+$ $Na^+$ = region. For this model, region levels were isthmic oviduct fluid, ampullary oviduct fluid, serum, and peritoneal fluid. Estrous cycle stage was not considered in this analysis. Comparisons were made using Fisher's protected least significant difference test.

**RESULTS**

**Experiment 1: Ion Concentrations of Oviduct Fluid Collected Daily**

The concentrations of $Ca^{++}$, $Mg^{++}$, $K^+$, and $Na^+$ in oviduct fluid collected daily throughout the estrous cycles of three cows were plotted. Figure 1 shows the results of $Ca^{++}$ concentration of all three cows. Daily $Ca^{++}$ concentrations in isthmic oviduct fluid were approximately 1.5 times that of ampullary fluid (Figure 1). Although daily samples of ampullary oviduct fluid demonstrated somewhat variable trends, all three cows showed striking similarities in $Ca^{++}$ concentration patterns in isthmic oviduct fluid collected daily, particularly during the nonluteal stages. Maximum $Ca^{++}$ concentrations in isthmic fluid ($n = 6$) were found on the days of estrus ($2.57 \pm .22$ mM) and of ovulation ($2.50 \pm .29$ mM). The $Ca^{++}$ concentrations in ampullary fluid were $1.85 \pm .29$ for estrus and $1.78 \pm .25$ for ovulation. Few general trends for $Mg^{++}$, $K^+$, and $Na^+$ concentrations in oviduct fluid collected daily were observed for the three cows examined (data not shown).

**Experiment 2: Ion Concentrations of Stage-Pooled Oviduct Fluid Samples**

Analysis of ion concentrations in oviduct fluid pooled by cycle stage showed that over all stages of the estrous cycle, $Ca^{++}$ concentration (Figure 2a) in isthmic oviduct fluid was significantly lower in oviduct fluid collected during the nonluteal 2 stage than during the luteal ($P = .006$) or the nonluteal 1 stages ($P = .036$). No significant region or stage effects were noted for $K^+$ or $Na^+$ concentrations in oviduct fluid (Figure 2, c and d).

**Relationship of Ion Concentrations in Oviduct Fluid to Serum**

Serum transudation may be an important source of oviduct fluid constituent molecules (18). The ratio of each ion in ampullary or isthmic oviduct fluid to serum values for the same cycle phase was plotted in Figure 3 to
Figure 3. Ratios of cation concentrations of bovine ampullary (solid bar) and isthmic (open bar) oviduct fluid (ODF) to serum cation concentrations. Fluid collected daily was pooled for each cow by estrous cycle stage into nonluteal 1 (NL1), nonluteal 2 (NL2), and luteal (L) samples based on serum progesterone concentrations (see Materials and Methods). Dashed line (ratio = 1) signifies equivalent ion concentrations in oviduct fluid and serum.

show the differences between cation concentrations in serum versus oviduct fluid. Statistical analyses were performed on cation concentrations, not on the ratios presented in this figure.

Because the Ca++ concentration was greater in isthmic than in ampullary oviduct fluid, the ratio of isthmic oviduct fluid Ca++ concentration to serum Ca++ concentration was somewhat higher than the corresponding ratio of ampullary fluid to serum (Figure 3). During the luteal stage, the Ca++ concentration in ampullary fluid was significantly lower than that in serum ($P = .0027$).

During every stage of the estrous cycle, Mg++ in oviduct fluid was significantly ($P \leq .001$) less than Mg++ in serum (Figure 3). Unlike Mg++, K+ concentration in oviduct fluid was greater than K+ concentration in serum (Figure 3). Significance was noted comparing both isthmic and ampullary fluid K+ concentration with serum K+ during the luteal phase ($P \leq .012$) and for ampullary fluid during nonluteal 1 ($P = .028$). Oviduct fluid Na+ concentration, however, was not significantly different from serum Na+ concentration (Figure 3).

**Experiment 3: Relationship of Ion Concentrations in Peritoneal Fluid to Oviduct Fluid and Serum**

The concentration of Ca++ in peritoneal fluid ($2.12 \pm .21 \text{ mM}$) did not differ significantly from that of oviduct fluid or serum. However, Mg++ concentration in peritoneal fluid ($1.32 \pm .19 \text{ mM}$) was significantly greater than Mg++ concentration in oviduct fluid and serum ($P \leq .04$). The concentration of K+ in peritoneal fluid ($3.44 \pm .12 \text{ mM}$) was significantly less than that of oviduct fluid ($P \leq .01$) but did not differ from serum K+. The concentration of Na+ in peritoneal fluid was not significantly different from Na+ in oviduct fluid or serum.

**DISCUSSION**

Cation concentrations determined in this study were similar to those reported for ewe regional oviduct fluids and bovine oviduct fluid (Table 1) with the exception of K+. Bovine K+ concentration that we detected was 13 times lower than that found by Olds and VanDemark (21). However, K+ concentrations in human, rabbit, and sheep oviduct fluids (3, 4, 14, 23) were only two- to eightfold greater

<table>
<thead>
<tr>
<th>Species</th>
<th>Ca++</th>
<th>Mg++</th>
<th>K+</th>
<th>Na+</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine</td>
<td>Whole</td>
<td>3.19</td>
<td>. .</td>
<td>65.7</td>
<td>86.1</td>
</tr>
<tr>
<td></td>
<td>Ampulla</td>
<td>1.83 ± .25</td>
<td>.662 ± .172</td>
<td>4.53 ± .42</td>
<td>140.9 ± 12.9</td>
</tr>
<tr>
<td></td>
<td>Isthmus</td>
<td>2.46 ± .25</td>
<td>.685 ± .170</td>
<td>4.24 ± .21</td>
<td>159.3 ± 16.3</td>
</tr>
<tr>
<td>Ovine</td>
<td>Ampulla</td>
<td>7.60</td>
<td>1.18</td>
<td>8.12</td>
<td>135</td>
</tr>
<tr>
<td></td>
<td>Isthmus</td>
<td>5.96</td>
<td>1.08</td>
<td>6.90</td>
<td>141</td>
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</tbody>
</table>

CATIONS IN OVIDUCT FLUID

TABLE 2. Optimal concentrations of Ca++ relating to sperm function and fertilization for several species.

<table>
<thead>
<tr>
<th>Ca++ Concentration</th>
<th>Function</th>
<th>Species</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 mM</td>
<td>Capacitation in vitro</td>
<td>Bovine</td>
<td>(22)</td>
</tr>
<tr>
<td></td>
<td>Increase percentage of heparin-induced capacitation</td>
<td>Guinea pig</td>
<td>(7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hamster</td>
<td>(7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mouse</td>
<td>(8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bovine</td>
<td>(11)</td>
</tr>
<tr>
<td>1.8 mM</td>
<td>Capacitation in vitro; optimize acrosome reaction</td>
<td>Human</td>
<td>(28)</td>
</tr>
<tr>
<td></td>
<td>Optimize hyperactivation; zona penetration; fertilization of zona-intact eggs</td>
<td>Mouse</td>
<td>(10)</td>
</tr>
<tr>
<td>0.9 mM</td>
<td>Acrosome reaction; gamete fusion</td>
<td>Mouse</td>
<td>(10)</td>
</tr>
</tbody>
</table>

than the bovine K+ concentration that we report.

It is not possible to compare our results directly with those of Olds and VanDemark (21) because the "oviduct fluid" that they assayed was collected by passing dissected oviducts through a clothes wringer. In the study of the ewe (23), however, ion concentrations in isthmic and ampullary oviduct fluid at estrus were similar to those that we report (Table 1), although they found neither regional nor stage differences.

We report that Ca++ concentrations in bovine oviduct fluid showed a significant region effect, which was most striking during the nonluteal 2 phase. The maximum Ca++ concentration (2.57 ± .22 mM), measured in isthmic oviduct fluid on the day of estrus, was similar to that reported to facilitate sperm capacitation, the acrosome reaction, and hyperactivation in several species (Table 2). The Ca++ concentration found in ampullary fluid, although lower than Ca++ concentration in isthmic fluid, was similar to that required for fertilization in the mouse (Table 2).

The localization of high Ca++ concentrations in isthmic fluid around estrus suggests that a transport mechanism may be responsible for maintaining the concentration differences between the oviduct lumen and the vasculature or peritoneal fluid. Likewise, because the ampullary concentrations of Ca++ were consistently lower than those in serum, movement of Ca++ from vasculature to ampullary fluid appears to be restricted. Because oviduct fluid is a product of active secretion as well as transudation, epithelial cells lining the isthmus may respond to hormonal changes at estrus and shift the equilibrium of Ca++ release toward the isthmic lumen. Also supporting the concept of regulated ion transport by oviduct epithelium were the Mg++ concentrations, which were significantly lower in oviduct fluid than in serum but greater in oviduct fluid than in peritoneal fluid.

The concentration of Mg++ in oviduct fluid was similar to that used for in vitro capacitation or acrosome reaction of rodent sperm (7) and bull sperm (22) and similar to that in rodent gamete fusion studies (31). However, the presence of both Ca++ and Mg++ may be more important than either ion alone, because penetration rates of hamster, guinea pig, and human eggs have been reported to be greatest in media containing both ions (29). Furthermore, ratios of Ca++:Mg++ >1.7 were found to be optimal for the guinea pig sperm acrosome reaction in vitro (25). In the present study, the minimum Ca++:Mg++ ratio (1.8) was found in ampullary nonluteal oviduct fluid, and the maximum (3.6) was in isthmic nonluteal 2 oviduct fluid.

Potassium concentrations in the range that we observed in oviduct fluid have been recognized as important to sperm function. Hamster sperm required 3.0 mM K+ for capacitation, the acrosome reaction, or both, and Na+, K+-ATPase was important to K+ transport (19). Bovine sperm actively maintain intra- and extracellular distribution of both K+ and Na+ (5). Fusion of guinea pig sperm with eggs requires 3 mM extracellular K+ (31) or .27 mM K+ for
fusion of mouse sperm with zona-free eggs (9). Once guinea pig sperm are bound to the zona pellucida, few undergo the acrosome reaction if eggs are transferred to media deficient in Na⁺ or K⁺ (30).

The ratio of K⁺ to Na⁺ in bovine oviduct fluid may be important in regulating fertilization. A K⁺:Na⁺ ratio of .018 was optimal for egg fertilization by mouse sperm, but a high K⁺:Na⁺ ratio (.222) inhibited the acrosome reaction, hyperactivated motility, and fertilization (9). Interestingly, the K⁺:Na⁺ ratio (.02) of media commonly used for bovine and hamster in vitro fertilization (1) approximates the K⁺:Na⁺ ratio (.025 to .032) that we observed in bovine oviduct fluid.

CONCLUSIONS
In summary, the cation concentrations in bovine oviduct fluid that we report clearly fall within the range of concentrations determined to be functionally relevant for in vitro sperm capacitation and fertilization in several species, including bovine. This study also has demonstrated that cation concentrations in oviduct fluid are apparently regulated and that the changes in cation concentration vary with the estrous cycle and oviduct region. Further studies are now needed to evaluate the specific roles of these cation concentrations on bovine gamete function, particularly sperm capacitation and fertilization.

ACKNOWLEDGMENTS
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