Alteration of the anterior acrosome of motile bovine spermatozoa by fructose and hydrogen ion concentration*

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Summary. Storing cauda epididymal spermatozoa in seminal plasma or in defined media at $1 \times 10^9$ spermatozoa/ml for 24 h at 4°C caused swelling of the apical ridge on motile spermatozoa (SAR) provided concentrations of fructose in the range normally found in seminal plasma or comparable levels of glucose were present. Evaluation of these conditions indicated that, with glycolysable sugars in the media, pH dropped from 6-6-6-7 to 5-7-6-0. Most of the pH decrease occurred during the first 2 h of slow cooling from 37 to 4°C. pH decrease was undoubtedly due to sperm organic acid production which overwhelmed the relatively weak buffering capacity of the defined media and/or seminal plasma. Inducing pH decreases with HCl in fructose-free conditions, and using NaOH to prevent a pH decrease when fructose was included in media, demonstrated that exposing spermatozoa to pH values of 5-7-6-0 and not a specific response to fructose was the major cause of SAR.

Introduction

Viable spermatozoa undergo the true acrosome reaction in association with fertilization. Degenerative changes of the acrosome associated with irreversible cell injury, death and cytolysis have been termed the false acrosome reaction (Bedford, 1970) and occur in immotile spermatozoa. For bovine spermatozoa, the false reaction as described by Saacke & Marshall (1968), specifically the disappearance of the apical ridge, has provided the basis for an objective viability test for bovine semen (Robbins et al., 1976; Aalseth et al., 1978; Landa et al., 1980), having good relation to fertility of cryopreserved bovine spermatozoa after artificial insemination (Saacke & White, 1972; Saacke et al., 1980).

Aalseth & Saacke (1985) have described an acrosomal swelling on motile bovine spermatozoa (SAR) that represented a marked departure from either the false or true acrosomal reaction. Using differential interference contrast and electron microscopy, the apical ridge was observed as swollen and the anterior acrosomal matrix was extended into complex folds and projections without loss of acrosomal or plasma membrane integrity. Because this property is without biological explanation and interferes with interpretation of the false acrosome reaction, further study was undertaken. Swelling of the anterior acrosome similar to SAR has also been reported for cryopreserved bovine spermatozoa (Jones & Stewart, 1979; Pace et al., 1981), but the relationship of this alteration to fertility was unclear.

SAR, as described by Aalseth & Saacke (1985), was associated with slow cooling and storage of spermatozoa in seminal plasma or egg-yolk-Tris-fructose media but not in egg-yolk-citrate extender. They postulated that SAR was due to fructose and/or amines (Tris-hydroxymethylaminomethane is a primary amine) which are unique to both seminal plasma and egg-yolk-Tris-fructose. The present

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study was undertaken to determine whether fructose and/or primary amines (Tris) were responsible for SAR and how these medium component(s) cause SAR.

Materials and Methods

Preparation of media. Egg-yolk-based media contained 20% (v/v) egg yolk (*Gallus domesticus*) and 80% (v/v) of appropriate buffer solutions (defined in each experiment). Media contained 1000 i.u. penicillin and 1000 µg dihydrostreptomycin/ml. pH was adjusted to 6.8 with citric acid (monohydrate). Osmolarities were 288 ± 11 (range) mosmol. Media were centrifuged at 15 000 g for 10 min and passed through a 0.45 µm pore size Millipore filter to remove egg-yolk particles.

Seminal plasma and epididymal samples. Spermatozoa recovered from the cauda epididymidis immediately after slaughter were used in these experiments instead of ejaculated spermatozoa because of their lack of exposure to seminal vesicle secretions that contain fructose. Just before slaughter (<30 min), an ejaculate was collected with an artificial vagina and centrifuged (3900 g, 5 min) to provide seminal plasma. Seminal plasma was held on ice until needed the same day. Epididymids recovered from each bull were flushed (retrogradely) at 34°C with media (without antibiotics) for each experimental replication. Epididymal flush solutions appropriate for each experiment were 101 mM-sodium citrate (Exp. 1), egg-yolk–Tris–based on 172 mM-Tris osmotically adjusted with NaCl (Exp. 2) and 150 mM-NaCl (Exp. 3). Sperm concentration was determined with a Coulter Counter and adjusted to 3.5 × 10⁹ spermatozoa/ml with the flush medium. Penicillin and dihydrostreptomycin were added at 1000 i.u. and µg/ml, respectively.

Experiment 1. This experiment was designed to evaluate the contribution of amines, fructose or glucose, to the development of SAR by observing spermatozoa stored at 4°C. Using 5 replicates and a split-sample technique, epididymal samples were extended at 1 × 10⁹ spermatozoa/ml in seminal plasma, conventional egg-yolk–citrater (98-6 mM, sodium citrate buffer solution), egg-yolk–citrater containing 54 mM-glucose or fructose (78-2 mM, sodium citrate buffer solution), conventional egg-yolk–Tris without fructose (295 mM, Tris buffer solution) or with 54 mM-fructose (235 mM, Tris buffer solution). Aliquots were cooled slowly from 37 to 5°C over 10 h. After storage at 4°C for an additional 24 h, aliquants were further extended to 50 × 10⁹ spermatozoa/ml in egg-yolk–Tris (no fructose) and incubated at 37°C. Evaluation for percentage motile spermatozoa and proportion of motile spermatozoa exhibiting SAR was carried out after 5 min and 2 and 4 h of incubation.

Experiment 2. Results of Exp. 1 demonstrated that the presence of fructose or glucose, but not amines, was important to SAR. Experiment 2 was therefore designed to define this relationship more clearly by exposing spermatozoa to increasing concentrations of fructose during storage at 4°C. Also, pH of the medium at the end of storage was measured to evaluate the possibility that low pH due to organic acid accumulation from sperm metabolism contributed to SAR. Using a split-sample technique and 5 replicates, cauda epididymal samples were extended to 1 × 10⁹ spermatozoa/ml in seminal plasma (control) or egg-yolk–Tris (172 mM, Tris buffer solution) containing 0, 6.8, 13.5, 27.0, 54.0 or 108.0 mM-fructose and sufficient NaCl to adjust final osmolarity of each treatment to 290 mosmol. Fructose concentrations after addition of epididymal samples were 0, 3.9, 7.7, 15.4, 30.8, and 61.7 mM respectively. Such fructose concentrations were physiologically since the average fructose concentration of bovine seminal plasma is 30-8 mM (Mann, 1964). Egg yolk contains ~0.7% (w/v) free glucose (Romanoff & Romanoff, 1949), thus contributing about 8 mM-glucose to all egg-yolk–Tris Treatments. Aliquants were cooled slowly from 37 to 5°C over 10 h. After storage at 4°C for an additional 24 h, 2 aliquants (for duplicate evaluation) of each storage medium were further extended to 50 × 10⁹ spermatozoa/ml in egg-yolk–Tris (172 mM, Tris buffer solution) and incubated at 37°C. Semen was evaluated for proportion of motile and live (unstained) spermatozoa exhibiting SAR.

Experiment 3. Results of Exp. 2 strongly suggested that SAR was caused by the reduced pH (5.7–6.0) resulting from the metabolism of a glycosylable sugar. To verify this, the effect of pH was studied independently from the presence of fructose. In 5 replicates, cauda epididymal samples were split and extended to 1 × 10⁹ spermatozoa/ml in the presence or absence of fructose (54 mM) in egg-yolk–Tris (172 mM, Tris buffer solution) or egg-yolk–citrater (78-2 mM, sodium citrate buffer solution) as well as in seminal plasma. Extender osmolarity was appropriately adjusted with NaCl to 290 mosmol. The 5 extended samples were cooled in duplicate preparations from 37 to 5°C over 10 h and stored for 24 h at 4°C. One preparation of each duplicate was allowed to undergo spontaneous pH change (uncontrolled). Spermatozoa in seminal plasma and media with fructose were expected to attain pH values of 5.7–6.0 during cooling and storage at 4°C. In contrast, the pH of the medium for spermatozoa stored without fructose was expected to remain at 6.6–6.8 (based on Exp. 2). The other preparation of the duplicates was not permitted to undergo spontaneous pH change (controlled). Fructose-containing media and seminal plasma aliquants were maintained at a pH comparable to those of media without fructose (uncontrolled pH change) by periodic addition of µl quantities of 1 M-NaOH. For media containing no fructose, the pH was reduced to 5.7–6.0 at a rate simulating that of fructose-containing samples by addition of µl quantities of 1 M-HCl. After extension of epididymal spermatozoa, pH measurements and adjustments were carried out immediately (0 h) and at 0.2, 0.4, 0.7, 1, 2, 3, 6, 12, 24 and 34 h after the start of cooling. At the end of storage, two aliquants (duplicate evaluation) of each of the 10 storage media treatments were diluted in egg-yolk–Tris (172 mM, Tris buffer solution, no fructose) to 50 × 10⁹ spermatozoa/ml and incubated in a 37°C water bath. Evaluations of each treatment for the proportion of motile spermatozoa with SAR, percentage
motile spermatozoa, percentage live (unstained) spermatozoa with SAR and percentage live spermatozoa were made after 5 min and 4 h of incubation.

**Evaluation of semen.** Percentage of motile spermatozoa was estimated from wet smears using a phase-contrast microscope (×100) equipped with a heated stage (37°C). Proportion of motile spermatozoa with SAR was scored subjectively (0–3) on the same smears using a differential interference-contrast microscope (×1250). The estimated value of scale units assigned to the proportion of motile spermatozoa exhibiting SAR were: 0 = <5%, 1 = 5–25%, 2 = 26–50%, and 3 = >50% (Aalseth & Saacke, 1985). All samples were coded to prevent evaluator bias. In Exp 2 and 3, an objective measure of SAR on fixed vitally stained samples was also utilized (Aalseth & Saacke, 1986). Two vitally stained dried smears were prepared at each evaluation for subsequent observation using differential interference-contrast microscopy. With this technique, percentages of live (unstained) spermatozoa and of live spermatozoa with SAR were quantified. Differential interference-contrast optics allow simultaneous examination of acrosomal morphology and sperm viability (based on degree of eosinophilia) on the same cell: 100 spermatozoa were evaluated from each smear and averaged for each duplicate evaluation.

**Staining procedures.** The stain was fast green FCF (2%, w/v) and Eosin B (0.8%, w/v) prepared according to Mayer et al. (1951). Semen (5 μl) was admixed with 10–15 μl stain at the end of a slide. Using a second slide, the mixture was drawn into a smear and dried in a 62°C jet of hot air (Oster Model 202C Airjet) for 30 sec. Dried smears were protected with resin-mounted coverslips and coded to prevent observer bias.

**Statistical analysis.** All data were transformed for statistical analysis with the following formulas: 2 × arcsin square root for percentage values and the square root of (x + 0.5) for SAR scale values. Analysis of variance was according to Goodnight (1979). Means were compared with Duncan’s new multiple range test (Duncan, 1955).

**Results**

**Experiment 1**

Effects of storage media and incubation interval on SAR are presented in Table 1. Seminal plasma, egg-yolk–citrate–fructose, egg-yolk–citrate–glucose and egg-yolk–Tris–fructose induced a strong SAR response in contrast to both media without sugar (P < 0.01). Although SAR occurred with storage at 4°C, SAR also increased slightly with incubation at 37°C (P < 0.01). Fructose and glucose rather than Tris (primary amine) were responsible for SAR.

**Table 1.** Effect of Tris, fructose and glucose in storage media and duration of incubation on (a) swelling of the apical ridge (SAR) and (b) percentage motility

<table>
<thead>
<tr>
<th>Incubation time (37°C)</th>
<th>Storage media (4°C)</th>
<th>Egg yolk–citrate</th>
<th>Egg yolk–Tris</th>
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<tbody>
<tr>
<td></td>
<td>Seminal plasma</td>
<td>− Fructose</td>
<td>+ Fructose</td>
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<tr>
<td></td>
<td></td>
<td>2.6 ± 0.4</td>
<td>0.0 ± 0.0</td>
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<td>2.8 ± 0.2</td>
<td>0.6 ± 0.4</td>
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<td></td>
<td>3.0 ± 0.0</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>Overall mean</td>
<td>± S.E.M. (n = 30)</td>
<td>2.8 ± 0.1c</td>
<td>0.4 ± 0.2d</td>
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<td></td>
<td></td>
<td></td>
<td>2.1 ± 0.3c</td>
</tr>
<tr>
<td></td>
<td>− Fructose</td>
<td>0.2 ± 0.2</td>
<td>1.2 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>+ Fructose</td>
<td>0.6 ± 0.2</td>
<td>2.4 ± 0.4</td>
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<tr>
<td></td>
<td>+ Glucose</td>
<td>0.8 ± 0.4</td>
<td>2.6 ± 0.2</td>
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<td></td>
<td>0.5 ± 0.2d</td>
<td>2.1 ± 0.3e</td>
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<td>(b) % motility†</td>
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<td></td>
<td>5 min</td>
<td>58 ± 1</td>
<td>57 ± 1</td>
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<td></td>
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<td>54 ± 2</td>
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<td>57 ± 1</td>
<td>54 ± 2</td>
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<tr>
<td></td>
<td>4 h</td>
<td>53 ± 2</td>
<td>56 ± 2</td>
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<td>56 ± 1</td>
<td>51 ± 2</td>
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<tr>
<td>Overall mean</td>
<td>± S.E.M. (n = 30)</td>
<td>55 ± 1d</td>
<td>58 ± 1d</td>
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<td>52 ± 1</td>
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<td>57 ± 1d</td>
<td>52 ± 1e</td>
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</table>

*Proportion of motile spermatozoa with SAR based on a scale of 0–3 (0 = <5%, 1 = 5–25%, 2 = 26–50%, and 3 = >50%)).
†Mean ± s.e.m. (n = 5).
Means without common superscripts are different (P < 0.01).
Fig. 1. Effect of fructose concentration and seminal plasma (SP) on swelling of the apical ridge (SAR) and storage medium pH (□) after the 24-h storage period at 4°C. Means ± s.e.m. with n = 10 for SAR and 5 for pH. Proportion of motile spermatozoa with SAR was based on a scale of 0–3 (0 = <5%, 1 = 5–25%, 2 = 26–50% and 3 = >50%). Values with different superscript letters are significantly different (P < 0.05).

Fig. 2. Effect of fructose concentration and seminal plasma (SP) on the percentage live (unstained) spermatozoa with swelling of the apical ridge (SAR). Means ± s.e.m., with n = 10. Values with different superscript letters are significantly different (P < 0.05).

Storage media and incubation interval effects on sperm motility are also shown in Table 1. Motility was slightly greater (P < 0.01) for spermatozoa stored in egg-yolk–citrate or egg-yolk–Tris than for either medium containing fructose. Regardless of the occurrence of SAR, motility was well maintained.

Experiment 2

The proportion of motile spermatozoa with SAR increased linearly as fructose concentrations increased to 7.7 mM and pH decreased comparably (Fig. 1, P < 0.01). Storage media containing 7.7, 15.4, 30.8, and 61.7 mM-fructose and seminal plasma induced maximal and comparable SAR responses (P > 0.05). The pH of media giving maximal SAR responses ranged from 5.97 to 5.74.

Based on the objective evaluation of the acrosome on fixed vitally stained spermatozoa, the percentage live spermatozoa with SAR also increased (P < 0.01) with fructose concentration (Fig. 2).
A comparison to the proportion of motile spermatozoa with SAR (Fig. 1) shows that both measures of SAR explain the effects of storage medium fructose concentrations and seminal plasma similarly. These measures of SAR were highly correlated with each other ($r = 0.88$, $P < 0.01$).

Percentage motile spermatozoa was not affected by fructose concentration or seminal plasma and averaged $60 \pm 5\%$ (s.d.) over all treatments. The percentage live spermatozoa ranged from 93.0 to 96.7 for all egg-yolk–Tris treatments. However, unstained live spermatozoa were lower after storage in seminal plasma (79.3%, $P < 0.01$).

**Experiment 3**

As expected, the inclusion of 30.8 mM-fructose (egg-yolk–citrate–fructose, egg-yolk–Tris–fructose) in the storage medium or the use of seminal plasma as the storage medium resulted in a rapid (uncontrolled) pH decrease during the first 2 h of cooling from 37 to 25°C (Fig. 3). For egg-yolk–citrate and egg-yolk–Tris media in which pH was uncontrolled, there was a slight increase in pH during cooling and storage. The periodic addition of HCl to egg-yolk–citrate or egg-yolk–Tris storage media reduced pH at a comparable rate to that achieved in the presence of 30.8 mM-fructose (egg-yolk–citrate–fructose, egg-yolk–Tris–fructose). Similarly, by addition of NaOH throughout cooling and storage of spermatozoa in seminal plasma, egg-yolk–citrate–fructose and egg-yolk–Tris–fructose, it was possible to maintain a normal pH comparable to that of egg-yolk–citrate and egg-yolk–Tris treatments in which pH was uncontrolled.

The effect of storage medium, storage medium pH and incubation interval on proportion of spermatozoa with SAR, and percentage motility is presented in Table 2. For SAR, there was an interaction between storage medium and storage medium pH ($P < 0.01$). While a high SAR response was evident after storage at reduced pH regardless of the presence or absence of fructose, moderate SAR occurred in relation to storage at normal pH in seminal plasma (4 h), egg-yolk–citrate and egg-yolk–Tris. Incubation interval did not affect SAR ($P > 0.05$).

For motility, the interaction of all main factors was significant ($P < 0.01$). At the start of incubation, motility was lower for seminal plasma and HCl-controlled egg-yolk–citrate. By 4 h of incubation, motility was depressed after sperm storage at reduced pH in egg-yolk–citrate, egg-yolk–Tris, egg-yolk–Tris + fructose and at reduced and normal pH in seminal plasma. However, reduced motility was not associated with storage conditions resulting in high SAR as noted for egg-yolk–citrate–fructose.

The effect of storage medium pH and incubation interval on proportion of live (unstained) spermatozoa with SAR and percentage live unstained spermatozoa is presented in Table 3. There was no interaction ($P > 0.01$) between storage medium, storage medium pH and incubation interval for percentage live spermatozoa with SAR. Reduced storage medium pH (5.83–5.96) was always associated with pronounced SAR regardless of presence or absence of 30.8 mM-fructose. Maintaining pH in a normal range (6.59–6.86), even with fructose in the storage medium, resulted in minimal SAR after 5 min of incubation. However, by 4 h of 37°C incubation, there was a moderate increase in SAR following storage at normal pH values. Spermatozoa stored at normal pH in seminal plasma, egg-yolk–citrate and egg-yolk–Tris exhibited a greater degree of SAR than those stored in egg-yolk–citrate–fructose and egg-yolk–Tris–fructose. The percentage live spermatozoa with SAR described the effects of pH and fructose comparable to the estimated proportion of motile spermatozoa with SAR. The scale estimation of motile spermatozoa with SAR and the objectively measured proportion of unstained (live) spermatozoa with SAR were correlated ($r = 0.83$; $P < 0.01$).

For percentage live spermatozoa (Table 3), all main factors interacted ($P < 0.01$). Storing spermatozoa in seminal plasma suppressed sperm viability the most. Inclusion of fructose in the storage medium and control of medium pH with NaOH or HCl depressed percentage live spermatozoa slightly. A strong SAR response was associated with reduced storage pH (5.83–5.96), but not necessarily a depression in sperm viability.
Fig. 3. Storage medium pH changes during cooling and storage with or without periodic addition of NaOH or HCl. Means with n = 5 are shown. Standard deviations averaged 0.05 pH units and ranged from 0.01 to 0.19 units. Media temperatures at appropriate cooling and storage intervals are indicated: (a) pH changes for egg-yolk–citrate-based storage medium treatments; (b) pH changes for seminal plasma-based storage medium treatments; (c) pH changes for egg-yolk–Tris-based storage medium treatments.
Table 2. Effect of controlled and uncontrolled pH during storage in seminal plasma and media with and without fructose on (a) swelling of the apical ridge (SAR)* and (b) percentage motility

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<tr>
<td></td>
<td>Un-controlled</td>
<td>Controlled (NaOH)</td>
<td>Un-controlled</td>
<td>Controlled (HCl)</td>
<td>Un-controlled</td>
</tr>
<tr>
<td>(a) 5 min</td>
<td>1.7 ± 0.4</td>
<td>0.3 ± 0.3</td>
<td>1.2 ± 0.4</td>
<td>2.9 ± 0.9</td>
<td>2.2 ± 0.2</td>
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<tr>
<td>4 h</td>
<td>2.6 ± 0.2</td>
<td>1.9 ± 0.3</td>
<td>1.6 ± 0.0</td>
<td>2.9 ± 0.1</td>
<td>2.1 ± 0.2</td>
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<tr>
<td>(b) 5 min</td>
<td>42 ± 3</td>
<td>49 ± 2</td>
<td>60 ± 1</td>
<td>54 ± 1</td>
<td>63 ± 1</td>
</tr>
<tr>
<td>4 h</td>
<td>34 ± 3</td>
<td>42 ± 3</td>
<td>64 ± 1</td>
<td>42 ± 2</td>
<td>60 ± 1</td>
</tr>
<tr>
<td>pH†</td>
<td>5.83</td>
<td>6.69</td>
<td>6.86</td>
<td>5.96</td>
<td>5.95</td>
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</table>

Values are mean ± s.e.m. (n = 10).
*Proportion of motile spermatozoa with SAR based on a scale of 0–3 (0 = <5%, 1 = 5–25%, 2 = 26–50%, and 3 = >50%).
†Mean pH (n = 5) of the storage medium at the end of storage.

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Table 3. Effect of controlled and uncontrolled pH during storage in seminal plasma and media with and without fructose on (a) percentage live spermatozoa (unstained) with swelling of the apical ridge (SAR) and (b) percentage of live spermatozoa (unstained)

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<td></td>
<td>Un-controlled</td>
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<td>Controlled (HCl)</td>
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<tr>
<td>(a) 5 min</td>
<td>54 ± 6</td>
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<td>37 ± 5</td>
<td>53 ± 4</td>
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<td>61 ± 4</td>
</tr>
<tr>
<td>(b) 5 min</td>
<td>55 ± 3</td>
<td>60 ± 1</td>
<td>96 ± 0</td>
<td>89 ± 1</td>
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<td>4 h</td>
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<td>pH*</td>
<td>5.83</td>
<td>6.69</td>
<td>6.86</td>
<td>5.96</td>
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</table>

Values are mean ± s.e.m. (n = 10).
*Mean pH of the storage medium at the end of storage (n = 5).
These experiments show that the presence of seminal plasma concentrations of fructose and/or a like concentration of glycolysable sugar in the medium were responsible for development of SAR by spermatozoa (Exps 1 and 2). The basis for this response was an associated pH reduction (Exps 2 and 3). If pH was controlled or permitted to vary independently of the presence of sugars, SAR developed predominantly in response to reduced pH (Exp. 3). When sperm viability and concentration (1 \times 10^9/ml) are relatively high along with optimum temperatures, a glycolysable substrate and poor buffering capacity (characteristic of seminal plasma, egg-yolk–citrate and egg-yolk–Tris), hydrogen ion concentration increases from abundant production of organic acids (especially lactic), thus reducing pH (Salisbury et al., 1978). The rapidity with which pH drops in an uncontrolled manner when the above conditions prevail is illustrated in Fig. 3. Such was undoubtedly the case in this study and our initial experiments describing SAR and conditions for its development (Aalseth & Saacke, 1985). Neither fructose, glucose nor lactic acid directly caused SAR. Extended exposure to pH in the range of 5.7–6.0 at 4°C was the primary inducer. Babcock et al. (1983) indicate that weak acids and bases in the media will directly influence cytosolic pH of bovine spermatozoa. Such a change may alter acrosomal enzyme activity and/or molecular relationships resulting in SAR.

In the bovine cauda epididymidis, pH values of 5.7–6.0 are normal (Acott & Carr, 1984) and have been shown to depress motility by virtue of the presence of a weak acid (Carr et al., 1985). However, fluids from all regions of the reproductive tract of the cow range from pH 6.8 to pH 8.9 (Lardy et al., 1940; Sergin et al., 1941; Olds & VanDemark, 1957; Akhtar & Singh, 1979). While perhaps unphysiological, there is some evidence that pH values of 5.7–6.0 may have a positive influence on the fertilizing ability of bovine spermatozoa. Holding neat bovine semen for a few to several hours at 37°C has facilitated in-vitro fertilization (Brackett et al., 1980, 1981; Iritani et al., 1984). Based on data reported herein, the pH of the seminal plasma would have dropped rapidly during incubation and potentially been in the range of 5.7–6.0 for 0.5–2.5 h of their incubation periods. In addition, Rajamannan et al. (1971) found that incubation of neat semen for 20 min before cryopreservation (−196°C) for artificial insemination improved the conception rates of low fertility bulls. Therefore, based on conditions needed to induce SAR, it is difficult to discern the negative or positive significance of SAR to fertility.

In all experiments, it was evident that occurrence of a high proportion of spermatozoa with SAR was not necessarily associated with reduced viability. Based on lack of eosinophilia, it would appear that the membranes of spermatozoa with SAR were functional. This supports the membrane structural integrity of the SAR condition based on electron microscopy (Aalseth & Saacke, 1985). From this perspective, SAR represents a departure from the false acrosome reaction as it is currently understood.

While the physiological importance of SAR is not known at this time, it does have impact on use of acrosomal integrity (presence of a normal apical ridge) as an indicator of sperm viability (Saacke & Marshall, 1968). Pace et al. (1981) and Jones & Stewart (1979) have observed bovine spermatozoa with acrosomal morphology similar to SAR after cryopreservation. While Pace et al. (1981) considered such spermatozoa to be injured, neither they nor Jones & Stewart (1979), or Linford et al. (1976), observed any negative effects of such spermatozoa on fertility. However, a similar morphological change has been reported for motile boar spermatozoa (Pursel et al., 1972a) which were not fertile (Pursel et al., 1972b). Refrigeration of ram spermatozoa also induced a swelling of the anterior acrosome that was associated with impaired fertility (Jones & Martin, 1973). Bamba & Cran (1985) described an involved anterior acrosomal swelling on boar spermatozoa with retention of motility. Such was caused by a rapid warming (5 to 37°C). Physiological significance was not investigated. Understanding the importance of SAR should allow more critical interpretation of acrosomal morphology as an indicator of sperm viability.

In Exp. 3, the increase in SAR during incubation of spermatozoa stored at normal pH values was more apparent in stained rather than wet smears (motile spermatozoa). It appeared that the
30–50% increase in SAR was the result of spermatozoa that had very subtle forms of SAR. By arresting motility on stained smears and examination using differential interference-contrast optics (Aalseth & Saacke, 1986), acrosomal change was more likely to be detected. This combination of a differential interference-contrast image of sperm morphology with simultaneous vital stain identification of sperm viability could be useful in further research involving acrosomal changes of viable spermatozoa.

Sperm metabolism of glycolysable substrates to organic acids will cause SAR if buffering capacity of the medium is exceeded such that spermatozoa are exposed to a pH of 5.7–6.0 for several hours. Important considerations to organic acid concentration are sperm concentration and viability, temperature, substrate concentration and buffering capacity of the medium. Some methodologies of industrial processing for bovine semen provide similar conditions and could potentially allow exposure of spermatozoa to such pH values. Holding partly extended semen (1 part semen/1–5 parts extender) at 30–37°C for 0.5–1 h followed by slow cooling (1–3 h) to 4°C for further processing could facilitate an effective increase in organic acids. Consequently, it is very possible that SAR occurs frequently during commercial semen processing.

As spermatozoa with SAR retain motility and membrane integrity, it is unclear whether this phenomenon should be considered a unique form of deterioration or whether such spermatozoa are still quite capable of the true acrosome reaction. Also, the functional life of spermatozoa with SAR in the female tract is not known. Determining in-vitro and in-vivo fertilizing ability is the next step in understanding this acrosomal function and properly characterizing acrosomal morphology as it is used to determine sperm viability. Because low pH is considered injurious to spermatozoa during in-vitro incubation, we suggest that SAR be considered potentially detrimental to fertility at this time.

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