Estradiol Metabolism and Its Inhibition in Bull Semen

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Abstract

Bull semen was examined to determine if estradiol and testosterone were oxidized. Spermatozoa converted $^{14}C$-17$\beta$-estradiol into a single product identified as estrone. The reverse reaction did not take place, nor was testosterone metabolized by the sperm. The reaction with 17$\beta$-estradiol was completely inhibited by seminal plasma, so that spermatozoa had to be washed and resuspended in Ringer's solution for the conversion to take place. The inhibition was not due to a heat-labile constituent of seminal plasma nor, apparently, to pyridine nucleotide nucleosidase in semen. Added cofactors (NAD or NADP) did not enhance the reaction in whole sperm, but did restore activity to sonified sperm. Dilute suspensions of spermatozoa were several times more active than dense suspensions. Ejaculates collected from the same bull over a 14-month period showed wide variations in ability to convert 17$\beta$-estradiol, with complete lack of activity occurring in some specimens.

The in vitro metabolism of steroid hormones in semen has been observed in a number of divergent species ranging from an oyster to man, with attention directed primarily towards the interconversions of testosterone and androstenedione in domestic animals. Scott, Baggett, and White (16) reported conversion of testosterone to androstenedione in semen of bull, cock, ram, and dog. Seamark and White (17) observed the conversion of testosterone to androstenedione in spermatozoa-rich seminal fractions of bull, boar, and stallion, but reported negative results with ram, cock, and dog. Mann (11) has reviewed much of this information and other results that indicate extremely low levels of endogenous steroids in semen of the mammalian species so far examined. The metabolism of steroids has also been studied in semen of fishes (8, 12, 19), where endogenous levels in one species are appreciable (19), and in invertebrates (7). Since other work on steroid metabolism in bull semen (16, 17) had been primarily concerned with testosterone, and because previous studies of ours (6-8) with other species showed preferential oxidation of estradiol over testosterone, it seemed of interest to test bull semen again. Under the conditions of these tests the oxidation of testosterone was minimal or zero, while considerable amounts of 17$\beta$-estradiol were converted to estrone. Unexpectedly, seminal plasma was inhibitory to these reactions.

Experimental Procedures

Semen was collected with the aid of an artificial vagina from three Holstein-Friesian dairy bulls with good records of reproduction. Experiments over a period of 14 months utilized 22 ejaculates in tests that involved the incubation of seminal constituents with exogenous radioactive steroids. Each ejaculate provided enough material for from 6 to 12 incubations, so that the effects of variables could be tested on a single sample of semen. Each datum in this paper is from an individual incubation and does not represent an average or maximum value, except where the method of quantitation was checked for precision (see below). All ejaculates had good to excellent wave motion.
after collection, but further evaluations of sperm motility were not made in a systematic way. As might be expected, sperm incubated at unnatural pH levels were immotile. Spermatozoa in aliquots of each sample were counted eight times with the aid of a hemocytometer, and the average sperm count is reported in this paper. All critical results were checked with semen of at least two bulls. Incubation of seminal preparations with steroids was at 37 C, with air as the gas phase, for specific periods up to three hours. A shaking incubator was not usually available, so an experiment was performed to test for conversion of estradiol with and without shaking. The results were essentially identical, so shaking was regarded as unnecessary. A large surface-to-volume ratio for the exchange of respiratory gases was provided in the incubation vessel, which was a round-bottomed 50-ml centrifuge tube with a glass stopper that also served as an extraction vessel. The reaction was initiated by bringing the complete incubation mixture to 37 C, and was stopped by adding 10 ml of chloroform in preparation for extraction of steroids. An incubation mixture consisted of the following, except for variations specifically mentioned in the text or noted in tables:

0.025 ml ethanolic solution of 1.4 to 278 ngamoles of steroid, labeled with 0.1 μC C-14
1.0 ml buffered seminal preparation, pH ca. 9.4 (or buffer alone in control incubates)
0.1 ml 20 mM NAD in aqueous solution.

At the end of the incubation period, 50 μg each of unlabeled substrate and expected product were added as carriers to diminish losses of radioisotope during subsequent procedures. The steroids were immediately extracted three times, using 10 ml of chloroform each time. The average recovery of radioactivity by this method was 90 ± 6.5% when checked by liquid scintillation counting of extracts of eight identical incubation mixtures. Liquid scintillation counting was not routinely available for checking recoveries in all of the incubations, so the figure of 90% recovery was used in evaluating these data. Interpretations from the data take into account the large standard deviation. The chloroform extract was evaporated at 40 C under a jet of air, and the residue chromatographed to the front on Whatman no. 1 paper impregnated with formamide. Identification of the product of conversion as estrone was confirmed by recrystallization to constant specific radioactivity with authentic estrone. To do this, material in the product peak was eluted with 10 ml methanol and dried. The residue usually contained debris from the paper, so the steroid was transferred to another tube with ten 1-ml volumes of methylene chloride, which removed the steroid but left the debris. To this was added 10 mg of authentic estrone, and the mixture of product and known steroid was recrystallized from four solvents. After each crystallization a portion of the crystalline material was removed, weighed on an electrobalance, and counted by liquid scintillation.

The developed and dried chromatograms were assayed for radioactivity on a chromatogram scanner, giving a tracing of radioactive peaks on chart paper from which the Rf values and amounts of radioactivity in the peaks were measured. In controls where no conversion occurred a single peak representing substrate was observed. If conversion of substrate occurred, two peaks of radioactivity were separated in these systems. The radioactivity represented by the peaks was regarded as 90% of the steroid present (based upon average recovery), and the percentage conversion of original steroid was calculated on the basis of the relative area of the two peaks as measured by triangulation. In tests of the precision of this method three groups of eight identical incubations each were measured for conversion of estradiol. Crude extract of mature rat testes was used as a source of 17-hydroxysteroid dehydrogenase, and in other respects the incubation mixtures were as stated. The amount of extract and the time of incubation were varied, so that each group had a different level of conversion. The mean values for conversion and standard deviations for each of the three groups were 9.6 ± 1.9%, 24.3 ± 1.4%, and 44.6 ± 2.9%. All other conversion results in this paper are values for individual incubations.

Further analysis of steroids in the extracts was carried out by eluting the steroids from the first chromatograms and rechromatographing them in the systems already mentioned, or with chloroform as the mobile phase over paper impregnated with formamide. Identification of the product of conversion as estrone was confirmed by recrystallization to constant specific radioactivity with authentic estrone. To do this, material in the product peak was eluted with 10 ml methanol and dried. The residue usually contained debris from the paper, so the steroid was transferred to another tube with ten 1-ml volumes of methylene chloride, which removed the steroid but left the debris. To this was added 10 mg of authentic estrone, and the mixture of product and known steroid was recrystallized from four solvents. After each crystallization a portion of the crystalline material was removed, weighed on an electrobalance, and counted by liquid scintillation.

Labeled steroids utilized in this study ("C-4-

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samples were left to cool gradually to room temperature (19°C). When whole semen was to be incubated it was mixed with buffer and measured for pH. Seminal plasma was prepared by centrifuging the semen for 5 min at 5,000 × g in an angle head, and withdrawing the supernatant plasma. Buffer was added to the plasma and the pH measured. When spermatozoa were to be incubated free of seminal plasma (i.e., washed), the semen was diluted with 35 ml of calcium-free Ringer's solution and centrifuged 5 min at 5,000 × g. The sedimented spermatozoa were resuspended in 40 ml of Ringer's solution and recentrifuged. The sedimented spermatozoa were then resuspended in 13 to 15 ml of Ringer's solution, to which was added one-tenth volume of 0.1 M buffer. Glycine buffer was used for pH values over 9, tris for values between 8 and 9, and phosphate buffer for values below 8. Final buffer concentrations were 0.01 M, except that adequate buffering of seminal plasma required 0.025 M.

Sonification was performed for 10 min with a Branson Model S-75, with care taken to avoid heating the mixture over 20°C.

Results

The analysis of paper chromatographs of extracts from incubates of spermatozoa with 14C-17β-estradiol revealed two radioactive peaks with Rf values of 0.26 and 0.60 in the benzene/formamide system. The first peak was always the larger, and upon sequential chromatography behaved as a single substance with Rf values of 0.07 and 0.55 corresponding to estradiol in the hexane-benzene/formamide and chloroform/formamide systems, respectively. The second peak usually comprised less than 20% of the recovered radioactivity and also behaved as a single substance with Rf values of 0.31 and 0.73 corresponding to estrone in the hexane-benzene/formamide and chloroform/formamide systems, respectively. The material in the second peak, representing the product of conversion, was eluted from a representative chromatogram and recrystallized four successive times with authentic estrone. The specific radioactivity of the crystals remained near a constant value during the four recrystallizations (Table 1). On this basis the product is judged to be estrone and the reaction a dehydrogenation of the 17β-hydroxy group of estradiol. In subsequent experiments reported below, analyses consisted of measuring the areas of chromatograph peaks as described. and did not include further recrystallizations.

Conversion of 17β-estradiol to estrone appears to depend upon the activity of enzymes, because no conversion occurred when the enzyme inhibitor cetyl trimethylammonium bromide (2 mM) was included, or when sperm suspensions were preheated 1 min at 95°C, or when buffer without sperm was incubated. Conversion of estradiol was greatest in a pH range around 9, as would be consistent for the activity of a dehydrogenase. Conversion values were 2.2, 3.8, 4.4, 4.7, and 0 μmoles/109 sperm at pH levels of 6.8, 7.8, 8.8, 9.4, and 11, respectively, with an initial substrate concentration of 18.4 μmoles/flask, and 8 × 108 sperm/flask. Since the sensitivity of the method for detecting conversion was greatest around pH 9, most tests were performed in that range.

Flasks containing the largest quantity of sperm usually had the highest percentage conversion, but if the results are expressed as millimicromoles (10-8 moles) of estrone produced per 109 sperm, it is apparent that individual cells were more active in the less dense sperm suspensions (Table 2). This conclusion would be valid, whether or not the values for per cent conversion in Table 2 are significantly different. If the percentages were assumed to be identical, the differences in

<table>
<thead>
<tr>
<th>Sperm concentration (cells/flask)</th>
<th>Estradiol converted to estrone (μmoles/109 sperm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.7 × 10^8</td>
<td>7.5</td>
</tr>
<tr>
<td>3.3 × 10^8</td>
<td>8.1</td>
</tr>
<tr>
<td>5.4 × 10^8</td>
<td>10</td>
</tr>
<tr>
<td>6.5 × 10^8</td>
<td>12</td>
</tr>
</tbody>
</table>

All results are from a single sample of semen from Bull 3. Spermatozoa were washed free of seminal plasma and resuspended in calcium-free Ringer's solution, as described in the text.

mumoles/10^9 sperm would be even greater than given in the table. This was examined more closely in semen of two bulls (Fig. 1) where, during the first hour of the reaction, there was a fourfold and 1.8-fold increase in estrone produced per 10^9 sperm in the two samples as a result of fourfold dilution of the sperm suspension. It should be noted that the sperm concentration in the sample from Bull 3 was initially low as compared to Bull 2.

As would be expected, increased substrate concentration resulted in greater conversion up to a point, with maximum conversion taking place with 180 mumoles of estradiol per flask (Table 3). The solubility of 17β-estradiol in 0.16 M NaCl solution at 5 C, pH 7.0, is 9.9 mumoles/ml (15), so there is little doubt that solutions used for this experiment were saturated with estradiol. Regardless, the withdrawal of estradiol from solution by adsorption to sperm and conversion to estrone resulted in up to 16 mumoles being utilized as substrate over the three-hour period of incubation. Apparently, the saturation of enzymes with estradiol did not occur until comparatively large amounts of the steroid (180 mumoles) were available (Table 3). In all likelihood the adsorption of steroid to the cells increased the amount of steroid dissolving in the incubate beyond the solubility in buffer alone.

The presence or absence of added coenzymes, NAD or NADP, had no significant effect on the conversion of substrate by whole sperm; however, conversion by sonically disrupted sperm was markedly less in the absence of added coenzyme (Table 4). Addition of either coenzyme to the disrupted sperm resulted in conversion equal to that seen with intact sperm.

Although washed sperm would convert 17β-estradiol to estrone, whole semen from the same ejaculate would not make this conversion (Table 5). It might be expected that the basis for this inhibition of estradiol dehydrogenation would be the destruction of NAD or NADP by the pyridine nucleotide nucleosidase in bull seminal plasma (9), but seminal plasma was still inhibitory when the nucleosidase was inactivated by preheating for 1 min at 95 C or when NAD was protected with 0.1 M nicotinamide in the incubation flask (1). It appears that bull seminal plasma contains something besides nucleosidase that inhibits the conversion of estradiol. This inhibition was observed in ejaculates from three bulls at various substrate and sperm concentrations. The results in Table 5 reflect the greater activity per 10^9 sperm in the more dilute sperm suspensions as discussed before (cf. Table 2).

Of 16 ejaculates collected from Bull 1 on different days over a 14-month period, washed sperm from six ejaculates showed no detectable conversion of estradiol. Washed sperm from all of six ejaculates from Bulls 2 and 3 (three ejaculates each) were active in making the conversion. Since conditions of incubation were similar in all of these experiments, it is clear that there is considerable variation between

\[
\begin{array}{cccc}
\text{Initial estradiol concentration} & \text{Estradiol converted to estrone} \\
\text{mumoles} & \frac{\text{mumoles}}{\text{flask}} & \frac{\text{mumoles}}{10^9 \text{sperm}} & \%
\end{array}
\]

\[
\begin{array}{cccc}
18 & 14 & 2.5 & 3.8 \\
22 & 12 & 11 & 17 \\
180 & 9.0 & 16 & 25 \\
270 & 5.7 & 15 & 23 \\
\end{array}
\]

Semen was from single ejaculate of Bull 3. Spermatozoa were washed free of seminal plasma and resuspended in calcium-free Ringer's solution, as described in the text.

\[
\begin{array}{cccc}
\text{HOURS} & \text{mumoles/10^9 sperm} & \text{mumoles/10^9 sperm}
\end{array}
\]

\[
\begin{array}{cccc}
1 & 1.3 \times 10^9 & 1.3 \times 10^9 \\
2 & 5.5 \times 10^8 & 5.5 \times 10^8 \\
3 & 4.4 \times 10^8 & 4.4 \times 10^8 \\
\end{array}
\]

\[
\begin{array}{cccc}
\text{Bull 3} & \text{Bull 2}
\end{array}
\]

Fig. 1. Conversion of estradiol by dense and dilute suspensions of sperm measured hourly for three hours, 37 C, pH 9.4. Initial estradiol concentration 4.6 mumoles/flask.

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TABLE 4
Effect of added coenzymes on the conversion of 17β-estradiol to estrone by whole and sonically disrupted bull sperm. Initial estradiol concentration was 1.4 mmoles/flask, and the sperm concentration was $7.1 \times 10^9$/flask, pH 9.4, 3 hours, 37 C

<table>
<thead>
<tr>
<th>Coenzyme added</th>
<th>Whole sperm</th>
<th>Sonically disrupted sperm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estradiol converted to estrone</td>
<td>Estradiol converted to estrone</td>
</tr>
<tr>
<td></td>
<td>(%) (mmoles)</td>
<td>mmoles (10^9 sperm) (%) (mmoles)</td>
</tr>
<tr>
<td>None</td>
<td>17</td>
<td>0.24</td>
</tr>
<tr>
<td>NAD</td>
<td>19</td>
<td>0.27</td>
</tr>
<tr>
<td>NADP</td>
<td>19</td>
<td>0.27</td>
</tr>
<tr>
<td>NAD &amp; NADP</td>
<td>15</td>
<td>0.21</td>
</tr>
</tbody>
</table>

ejaculates with regard to the ability of sperm to dehydrogenate 17β-estradiol.

Washed sperm that readily converted estradiol to estrone were virtually inactive towards testosterone. Several ejaculates from all three of the bulls were tested under a variety of conditions (Table 6), but detectable conversion of testosterone occurred in only one case. In this test 1% of 18 mmoles of testosterone was converted to a product that had properties similar to androstenedione in the hexane-benzene/formamide chromatographic system. No further identification procedures were carried out on this product. Incubations were at various pH's, with the positive result occurring at 9.4. Other tests in this pH range, however, failed to demonstrate conversion of testosterone. In all of these experiments concurrent incubations showed the conversion of 17β-estradiol by the same preparation of sperm. The solubility of testosterone is 125 mmoles/ml at pH 7.3 in phosphate buffer or in Locke's physiological buffer at 37 C (4). If this solubility is roughly valid for the incubation mixtures used here, then the testosterone was entirely dissolved in all the experiments and the lack of conversion is not due to insolubility of the substrate.

The ability of washed sperm to convert estrone to estradiol was tested in three samples from the same bull at various pH's, in the presence of NADH or NADPH, but the results were always negative (Table 7). Control incubations showed that the same sperm converted 17β-estradiol.

The difficult question arises as to whether bacteria in semen might account for the conversion of steroids. Seamark and White (17) have discussed this problem. If bacteria were involved, it might be expected that cellular multiplication during incubation would result in an increase in the activity of the preparation. No increase in activity was observed when washed sperm suspension was premixed with seminal plasma.

TABLE 5
Inhibitory effect of bull seminal plasma on conversion of 17β-estradiol to estrone. Incubated for three hours, 37 C, pH 9.4

<table>
<thead>
<tr>
<th>Bull</th>
<th>Sperm conc</th>
<th>Estradiol conc</th>
<th>Whole semen plus 0.1M nicotineamide</th>
<th>Ringer's plus 0.1M nicotineamide</th>
<th>Seminal plasma preheated 1 min 95 C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(cells)</td>
<td>(mmoles) flask</td>
<td>(mmoles) (10^9 sperm)</td>
<td>(mmoles) (10^9 sperm)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.0X10⁶</td>
<td>4.6</td>
<td>0 0 0.19 (8.3%)</td>
<td>0.18 (7.9%)</td>
<td>0 0</td>
</tr>
<tr>
<td>2</td>
<td>6.2X10⁶</td>
<td>4.6</td>
<td>0 0 2.0 (27%)</td>
<td>1.9 (25%)</td>
<td>0 0</td>
</tr>
<tr>
<td>2</td>
<td>7.2X10⁶</td>
<td>92</td>
<td>0 0 13.0 (10%)</td>
<td>1.1 (12%)</td>
<td>0 0</td>
</tr>
<tr>
<td>3</td>
<td>5.0X10⁶</td>
<td>4.6</td>
<td>0 0 2.2 (24%)</td>
<td>1.1 (12%)</td>
<td>0 0</td>
</tr>
<tr>
<td>3</td>
<td>6.3X10⁶</td>
<td>92</td>
<td>0 0 14.0 (9.4%)</td>
<td>1.1 (12%)</td>
<td>0 0</td>
</tr>
</tbody>
</table>

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Conditions in which washed bull sperm were incubated with testosterone for three hours at 37 C. In all cases estradiol was converted by sperm from the same ejaculate

<table>
<thead>
<tr>
<th>Bull no.</th>
<th>Sperm conc. (cells flask)</th>
<th>Initial substrate concentration (mumoles flask)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.2×10^9</td>
<td>4.6</td>
<td>9.2</td>
</tr>
<tr>
<td>1</td>
<td>6.7×10^9</td>
<td>18</td>
<td>6.8, 9.4^a</td>
</tr>
<tr>
<td>1</td>
<td>6.6×10^9</td>
<td>18</td>
<td>7.5, 8.5, 9.4</td>
</tr>
<tr>
<td>2</td>
<td>6.2×10^9</td>
<td>4.6</td>
<td>9.5</td>
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<tr>
<td>2</td>
<td>7.2×10^9</td>
<td>92</td>
<td>8.6</td>
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<tr>
<td>3</td>
<td>5.0×10^9</td>
<td>4.6</td>
<td>9.5</td>
</tr>
<tr>
<td>3</td>
<td>5.3×10^9</td>
<td>4.6</td>
<td>9.5</td>
</tr>
<tr>
<td>3</td>
<td>6.3×10^9</td>
<td>92</td>
<td>8.6</td>
</tr>
</tbody>
</table>

^a Conversion took place in this incubate to the extent of about 1% as described in the text. In all other cases there was no detectable conversion of testosterone.

TABLE 7
Conditions under which sperm from Bull 1 failed to convert estrone after three hours at 37 C. In all cases estradiol was converted by sperm from the same ejaculates

<table>
<thead>
<tr>
<th>Sperm conc. (cells flask)</th>
<th>Initial substrate concentration (mumoles flask)</th>
<th>pH</th>
<th>Coenzyme^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.7×10^9</td>
<td>18</td>
<td>6.8</td>
<td>NADPH^a</td>
</tr>
<tr>
<td>6.6×10^9</td>
<td>18</td>
<td>5.7, 6.5</td>
<td>NADH</td>
</tr>
<tr>
<td>8.3×10^9</td>
<td>18</td>
<td>6.7, 7.6, 8.6, 9.7</td>
<td>NADH</td>
</tr>
</tbody>
</table>

^a Electron donors NADH or NADPH substituted for electron acceptor NAD used when estradiol or testosterone was the substrate.

Results presented here confirm the observations of Seamark and White (17) with regard to the conversion of 17β-estradiol by components of bull semen. Their use of epididymal semen evidently avoided the introduction of seminal inhibitors found in whole semen. Our results are similar also in the failure to observe the reverse reaction, estrone to estradiol, in bull seminal components. Seamark and White and others (16, 17) found conversion of testosterone to androstenedione, but not the reverse reaction, in diluted bull semen and in the separated spermatozoa. They incubated whole semen diluted with 4 vol of Ringer's solution containing 0.1% fructose at pH 7.4 with 0.3 to 80 μg (1.1 to 294 μmole) of testosterone and observed 1-16% conversion to androstenedione in semen from two Dexter bulls and two Friesian bulls, but found no conversion in semen from one Friesian bull. In contrast, it was not possible to observe the conversion of testosterone in the present study with semen from three Friesian bulls under a variety of conditions. Variability between ejaculates of the same and different bulls is indicated in this paper with respect to estradiol oxidation, and similar variability between ejaculates is reported by Seamark and White with respect to testosterone oxidation. Therefore, conflicting reports of steroid metabolism in semen of a species might be attributable to biological variability.

The dehydrogenase activities appear to be associated with spermatozoa, but this does not eliminate the possibility that the enzymes are synthesized in the accessory organs and secreted with the seminal plasma, there to be completely adsorbed to the spermatozoa. Some antigens of seminal plasma are known to adsorb very strongly to the spermatozoa. Some antigens of seminal plasma are known to adsorb very strongly to the spermatozoa (20). Another possibility, that the dehydrogenase activity is present in the plasma fraction of semen, but is not detected there because of inhibition, is consistent with my results but not with those of Seamark and White (17), who observed steroid conversions in diluted whole semen and in epididymal semen (with no inhibition, presumably) but who, nevertheless, found none of the activity in the seminal plasma alone.

The effect of reducing sperm numbers in the incubation flasks was to increase the apparent activity of each cell. At constant levels of estradiol there would be less substrate per cell when more sperm were present, and it might appear that substrate concentration would be limiting, but the data in Fig. 1 indicate that the rate of conversion is low and linear for three hours in the most dense sperm suspension, and that the initial rate of conversion is very high but drops off rapidly in the least dense suspension. From this it would not appear that substrate is limiting the reaction with the dense sperm suspension, but rather...
that some cellular control mechanism is holding metabolism of estradiol to a low but steady level. This control is evidently lost when the sperm are diluted, so the oxidation of estradiol proceeds at a much higher rate until the substrate concentration becomes limiting.

Complete saturation of enzyme or enzymes catalyzing this reaction does not occur until prodigious amounts of steroid are present (Table 3). The higher activity of dilute sperm suspensions is probably related to the phenomenon known as dilution effect, usually recognized as an increase in the motility, respiration, or glycolysis accompanying dilution of semen of a variety of species (5, 13, 14). There is as yet no satisfactory explanation for dilution effect.

The involvement of coenzymes is not apparent in intact sperm, but sonic disruption of the cells reveal at least a partial dependence on either NAD or NADP for maximum conversion. Whole sperm may have sufficient intracellular coenzymes to satisfy the reaction, whereas coenzymes would have to be replaced in the sonified suspension.

Shannon (18) has described a constituent of bull seminal plasma that reduces livability of stored sperm. This constituent, unlike the inhibitory effect described here, is heat-labile.

Sperm inhibitors from the male reproductive tract are of interest because of the possibility that they are in part responsible for the inability of seminal and epididymal spermatozoa to fertilize ova. It has been known for some time that spermatozoa of some animals require a period of capacitation in the female reproductive tract, during which they acquire the capacity to fertilize the ovum. Recent evidence has lent itself to the view that capacitation involves the alteration of inhibitory seminal factors, probably macromolecules, that coat the spermatozoa at the time of ejaculation (2, 3).

Capacitation of sperm has not been thoroughly evaluated in the bovine, but evidence indicates that it is either a very rapid process or that it is not a prerequisite for fertilization. Insemination of heifers at various intervals after ovulation with nonincubated control sperm and sperm exposed 6-14 hours to the uterine environment of estrous heifers indicated no detectable effect of the uterus on the fertilizing capacity of the sperm (10). The presence of inhibitory substances in bull semen suggests, however, that bull spermatozoa, like those of most species so far studied, must undergo some activation or release from inhibition in the female tract before they are able to fertilize the ovum. It is to be hoped that studies of the seminal inhibitors will clarify their role, if any, in the processes leading up to fertilization.

Acknowledgments

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References


