THE EFFECT OF OXYTOCIN AND ADRENALINE ON THE SEMEN OUTPUT OF RAMS

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Summary. An intravenous injection of 10 i.u. oxytocin into rams 10 min before ejaculation increased the number of spermatozoa per ejaculate by 45%. The optimum dose was between 5 and 10 i.u. oxytocin and the optimum interval between the injection of oxytocin and ejaculation was 5 to 10 min. The increase in the number of spermatozoa per ejaculate was accompanied by increases in semen volume, and/or sperm concentration. The increase in the semen volume and total fructose per ejaculate suggested that oxytocin stimulated the accessory sex glands. Adrenaline injections had no effect on the output of semen.

INTRODUCTION

In flocks of sheep in which large numbers of spermatozoa must be collected from a few rams over a short period for artificial insemination (A.I.), a substantial increase in the output of spermatozoa per ejaculate per ram would be an advantage. Milovanov, Bereznev & Gorohov (1962) increased the number of spermatozoa per ejaculate from bulls used in an A.I. centre by intravenous injection of 3 to 18 i.u. oxytocin just before ejaculation. The optimum dose for the bull was 6 i.u. oxytocin but the ejaculate had to be collected within 5 min of the injection. In rams, oxytocin has also been found to increase the number of spermatozoa per ejaculate (Knight & Lindsay, 1970) but the dose of oxytocin and the conditions for a maximum response have not been determined. The work by Ewy, Bielański & Zapletal (1963) on the output of semen from the cannulated ductus deferens of a ram following oxytocin injection suggests that the optimum time for collection would be 10 to 40 min after the injection of oxytocin.

The contractions of the epididymis and ductus deferens which cause the emission of semen during ejaculation are stimulated by catecholamines released from the sympathetic nerve endings associated with the muscular layers (Cross & Glover, 1958; Sjöstrand, 1965). It might, therefore, be expected that exogenous adrenaline would also increase the output of spermatozoa.

Two experiments were designed to determine the dose of oxytocin and the interval between the injection of oxytocin and ejaculation which would produce the maximum output of semen from rams. A third experiment determined the maximum increase in the output of spermatozoa following injections

of oxytocin and the effect of injecting oxytocin before one of the two ejaculations. The possibility of increasing the output of semen with injections of adrenaline was considered in a fourth experiment. To test the hypothesis that the difference between rams in the output of semen may be due to differences in the endogenous release of oxytocin or adrenaline, the experiments were performed on a group of rams with a high number of spermatozoa per ejaculate and a second group of rams with a low number of spermatozoa per ejaculate.

MATERIALS AND METHODS

Four collections of semen were made from each of eight rams using an artificial vagina. On the basis of the mean number of spermatozoa per ejaculate, the rams were allocated to a high-yielding group (Group 1) or a low-yielding group (Group 2). Each group consisted of four rams.

Two semen collections, 90 min apart, were made from each ram every 3rd day. The volume of the semen was measured and the concentration of spermatozoa in the semen was determined colorimetrically, using a Gilford 2000 spectrophotometer by the method of Knight (1972). The concentration of fructose in the semen was measured by the method of Mann (1948), which was adapted for use on an autoanalyser (Knight, 1972).

All the injections were given into the jugular vein. The oxytocin used was Pitocin (Parke Davis). The adrenaline was made up each day by dissolving 1 mg adrenaline (BDH) in 10 ml sterilized distilled water, to which a small quantity of ascorbic acid was added to prevent oxidation of the adrenaline.

The experiments were a series of paired 4×4 Latin squares on the two groups of rams so that each ram received each treatment once during the experimental period. Four consecutive experiments were performed on the rams.

Experiment 1. This experiment compared the effect of different doses of oxytocin on the output of semen. The maximum interval between the injection of oxytocin and collection of the ejaculate was $1\frac{1}{2}$ min.

The treatments were: (1) no oxytocin—1 ml sterilized saline; (2) 5 i.u. oxytocin; (3) 10 i.u. oxytocin; (4) 15 i.u. oxytocin.

Experiment 2. This experiment compared the effect of collecting the ejaculate at different intervals after the injection of 10 i.u. oxytocin.

The intervals of time were: (1) a maximum of $1\frac{1}{2}$ min; (2) 5 min after the injection; (3) 10 min after the injection; (4) 20 min after the injection.

Experiment 3. This experiment compared the response to 10 i.u. oxytocin injected intravenously 10 min before each ejaculation, and before either the first or the second ejaculation. The experiment was duplicated to increase the precision.

The treatments were: (1) no oxytocin—1 ml sterilized saline; (2) 10 i.u. oxytocin before the first and the second ejaculation; (3) 10 i.u. oxytocin before the first but not the second ejaculation; (4) 10 i.u. oxytocin before the second but not the first ejaculation.

Experiment 4. This experiment determined the effect of adrenaline on the output of semen. The maximum time from the injection to the collection of the ejaculate was $1\frac{1}{2}$ min.

The treatments were: (1) no adrenaline—1 ml sterilized saline; (2) 25 μ g adrenaline; (3) 50 μ g adrenaline; (4) 100 μ g adrenaline.

The results were analysed by split plot analysis of variance. Interactions with a mean square less than the error mean square were pooled with the error term. Orthogonal comparisons were made between the treatments.

RESULTS

General

The rams in Group 1 maintained a significantly larger number of spermatozoa per ejaculate, volume of semen and total fructose per ejaculate than the rams in Group 2 throughout the four experiments (Tables 1, 2, 4 and 6). While the sperm concentration was significantly higher for the rams in Group 1 than for those in Group 2 in the first three experiments, there was no difference in the fourth experiment. The relationship between the two groups of rams with respect to the concentration of fructose in the semen varied during the four experiments (Tables 1, 2, 4 and 6).

Experiment 1

While oxytocin injections produced a mean increase of 22.9% in the number of spermatozoa per ejaculate ($P < 0.05$), there were no significant differences between the three doses of oxytocin (Table 1). This increase in the number of spermatozoa per ejaculate was associated with a mean increase of 11.8% in the sperm concentration ($P < 0.05$) and a mean increase of 10.5% in the volume of semen (N.S.). The response was similar in the two groups of rams.

Table 1. The means of the semen characteristics of rams in Exp. 1 for different doses of oxytocin and different groups

Semen characteristic	i.u. Oxytocin				Groups	
	0	5	10	15	1	2
Semen volume (ml)	0.63	0.72	0.69	0.67	0.89 ^{a3}	0.46 ^{b3}
Sperm conc. ($\times 10^9$ /ml)	3.83 ^{a1}	4.06 ^{b1}	4.44 ^{b1}	4.33 ^{b1}	4.90 ^{a3}	3.44 ^{b3}
No. of spermatozoa/ejaculate ($\times 10^9$)	2.57 ^{a1}	3.12 ^{b1}	3.30 ^{b1}	3.06 ^{b1}	4.44 ^{a3}	1.58 ^{b3}
Fructose conc. in semen (mg/ml)	4.38 ^{a2}	3.90 ^{b2}	3.54 ^{b2}	3.81 ^{b2}	3.80	4.02
Total fructose/ejaculate (mg)	2.66	2.93	2.43	2.52	3.36 ^{a3}	1.91 ^{b3}

Means with the same alphabetic superscripts are not significantly different. The superscript numbers designate the level of significance. 1 = $P < 0.05$; 2 = $P < 0.01$; 3 = $P < 0.001$.

Although the concentration of fructose in the semen was significantly less ($P < 0.01$) after oxytocin injections, there was no effect on the total fructose per ejaculate (Table 1).

Experiment 2

When the interval between the injection of oxytocin and ejaculation was greater than 1½ min, there were mean increases of 35.2% in the number of

Table 2. The means of the semen characteristics of rams in Exp. 2 for different time intervals from oxytocin injection to ejaculation and different groups

Semen characteristics	Interval from intravenous injection of 10 i.u. oxytocin to ejaculation (min)				Groups	
	1-1½	5	10	20	1	2
Semen volume (ml)	0.64 ^{a1}	0.73 ^{b1}	0.79 ^{b1}	0.76 ^{b1}	0.98 ^{a3}	0.49 ^{b3}
Sperm conc. ($\times 10^9$ /ml)	3.71	3.88	3.82	3.80	4.65 ^{a3}	2.96 ^{b3}
No of spermatozoa/ejaculate ($\times 10^9$)	2.37 ^{a2}	3.10 ^{b2}	3.24 ^{b2}	3.27 ^{b2}	4.58 ^{a3}	1.41 ^{b3}
Fructose conc. in semen (mg/ml)	3.63	3.38	3.57	4.04	3.38 ^{a1}	3.95 ^{b1}
Total fructose/ejaculate (mg)	2.55	2.41	2.67	2.90	3.23 ^{a3}	2.03 ^{b3}

Means with the same alphabetic superscripts are not significantly different. The superscript numbers designate the level of significance. 1 = $P < 0.05$; 2 = $P < 0.01$; 3 = $P < 0.001$.

spermatozoa per ejaculate ($P < 0.01$), 18.8% in the volume of semen ($P < 0.05$) and 3.3% (N.S.) in the sperm concentration (Table 2). There was no significant effect of increasing the interval between the injection of oxytocin to ejaculation from 5 min to 10 or 20 min. The response was almost totally accounted for by the rams in Group 1 but the difference in the response of the two groups of rams was only significant ($P < 0.01$) for the volume of semen (Table 3).

There were no effects of the time from oxytocin injection to semen collection on total fructose per ejaculate or concentration of fructose in the semen (Table 2).

Experiment 3

The injection of 10 i.u. oxytocin 10 min before the collection of an ejaculate significantly increased ($P < 0.001$) the number of spermatozoa per ejaculate compared with the rams not receiving oxytocin (Table 4). The number of spermatozoa per ejaculate was 45.5% greater with oxytocin injections before each of the two ejaculations but only 27.5% greater with a single injection of oxytocin before either the first or the second ejaculation. Two injections of

Table 3. The means of the number of spermatozoa per ejaculate, volume of semen and sperm concentration for the group $\times$ treatment interactions for rams in Exp. 2

Semen characteristics	Groups	Interval from intravenous injection of 10 i.u. oxytocin to ejaculation (min)			
		1-1½	5	10	20
No. of spermatozoa/ejaculate ($\times 10^9$)	1	3.40	4.82	4.92	5.17
	2	1.34	1.38	1.56	1.37
Semen volume (ml)	1	0.76	1.03	1.03	1.10
	2	0.53	0.44	0.55	0.43
Sperm conc. ($\times 10^9$ /ml)	1	4.50	4.67	4.79	4.65
	2	2.92	3.09	2.86	2.96

Table 4. The means of the semen characteristics of rams in Exp. 3 for the oxytocin treatment and different groups

Semen characteristics	10 i.u. Oxytocin before ejaculation				Groups	
	0	1+2	1	2	1	2
Semen volume (ml)	0.58 ^{a2}	0.77 ^{b2}	0.70 ^{b2}	0.70 ^{b2}	0.84 ^{a3}	0.54 ^{b3}
Sperm conc. ($\times 10^9$ /ml)	3.80	4.02	3.91	3.82	4.33 ^{a3}	3.44 ^{b3}
No. of spermatozoa/ ejaculate ($\times 10^9$)	2.23 ^{a3}	3.24 ^{b2}	2.80 ^{c2}	2.88 ^{c2}	3.68 ^{a3}	1.90 ^{b3}
Fructose conc. in semen (mg/ml)	3.69	3.77	3.65	3.52	3.83 ^{a1}	3.47 ^{b1}
Total fructose/ejaculate (mg)	2.11 ^{a1}	3.02 ^{b1}	2.57 ^{b1}	2.41 ^{b1}	3.15 ^{a3}	1.91 ^{b3}

Means with the same alphabetic superscripts are not significantly different. The superscript numbers designate the level of significance. 1 = $P < 0.05$; 2 = $P < 0.01$; 3 = $P < 0.001$.

Table 5. The means of the number of spermatozoa per ejaculate, volume of semen and sperm concentration for the group treatment interactions of rams in Exp. 3

Semen characteristics	Groups	10 i.u. Oxytocin before ejaculation			
		0	1+2	1	2
No. of spermatozoa per ejaculate ($\times 10^9$)	1	2.84	4.55	3.47	3.84
	2	1.61	1.93	2.13	1.92
Semen volume (ml)	1	0.69	0.97	0.79	0.87
	2	0.47	0.54	0.61	0.53
Sperm conc. ($\times 10^9$ /ml)	1	4.18	4.59	4.28	4.29
	2	3.43	3.44	3.55	3.36

Table 6. The means of the semen characteristics of rams in Exp. 4 for different doses of adrenaline and different groups

Semen characteristics	Adrenaline (μ g)				Groups	
	0	25	50	100	1	2
Semen volume (ml)	0.76	0.68	0.71	0.69	0.84 ^{a3}	0.53 ^{b3}
Sperm conc. ($\times 10^9$ /ml)	3.51	3.56	3.43	3.70	3.56	3.54
No. of spermatozoa/ ejaculate ($\times 10^9$)	2.67	2.42	2.48	2.53	2.97 ^{a2}	2.07 ^{b2}
Fructose conc. in semen (mg/ml)	4.80	4.70	4.97	4.41	5.69 ^{a3}	3.75 ^{b3}
Total fructose/ejaculate (mg)	3.85	3.36	3.63	3.26	4.82 ^{a3}	2.23 ^{b3}

Means with the same alphabetic superscripts are not significantly different. The superscript numbers designate the level of significance. 2 = $P < 0.01$; 3 = $P < 0.001$.

oxytocin were more effective than one ($P < 0.01$), but there was no difference in the effectiveness of an injection before the first or the second ejaculation.

The volume of semen increased by 33% when oxytocin was injected before each ejaculation and 20% when oxytocin was injected before either the first or the second ejaculation (Table 4). Although these increases in the volume of semen were significant ($P < 0.01$), the difference between the responses to one or two injections of oxytocin was not significant. The 3% increase in the concentration of spermatozoa with injections of oxytocin was not significant.

While the injection of oxytocin increased the number of spermatozoa per ejaculate and the volume of semen in both groups of rams, the response was larger in Group 1 than in Group 2 and two injections of oxytocin were better than one injection in Group 1, but not in Group 2 (Table 5). These differences in the response between two groups of rams only reached significance ($P < 0.001$) for the number of spermatozoa per ejaculate.

Total fructose per ejaculate was increased ($P < 0.05$) by the injection of oxytocin, but there was no significant effect on the concentration of fructose in the semen (Table 4).

Experiment 4

The injection of adrenaline had no significant effect on any of the parameters measured (Table 6).

DISCUSSION

The results of Exp. 1 indicated that the dose of oxytocin which produced the maximum number of spermatozoa per ejaculate was between 5 and 10 i.u. oxytocin or 0.09 to 0.18 i.u. oxytocin/kg body weight. The method of collection of semen does not seem to be important since Knight & Lindsay (1970) found that when using an electroejaculator, the optimum dose of oxytocin was also between 5 and 10 i.u. oxytocin. Similarly, the optimum dose of oxytocin in the bull was 6 i.u. (Milovanov *et al.*, 1962) and Kihlström & Melin (1963) found that the optimum response to oxytocin in the rabbit was obtained with an intravenous dose of 0.15 i.u. oxytocin/kg body weight.

The number of spermatozoa per ejaculate increased as the interval between the injection of oxytocin and ejaculation increased from 1½ min to 20 min. The optimum interval between the injection of oxytocin and ejaculation would appear to be 5 to 10 min. This contrasts with the bull for which ejaculates must be collected within 5 min of an intravenous injection of oxytocin (Milovanov *et al.*, 1962). When the interval between ejaculations was 90 min, there was no carry-over effect of oxytocin from the first to the second ejaculation and the injection of oxytocin was equally effective before either the first or the second ejaculation.

The intravenous injection of 10 i.u. oxytocin 10 min before each of two ejaculations increased the number of spermatozoa per ejaculate from 2.23×10^9 to 3.24×10^9 , or approximately by 45%. Generally, an increase of this magnitude would not be large enough to warrant the cost and effort involved in injecting the rams with oxytocin when collecting semen for A.I.

Following oxytocin, the increases in the number of spermatozoa per ejaculate were the result of increases in both the volume of semen and/or the sperm concentration. In Exp. 1, the major effect of oxytocin was to increase the concentration of spermatozoa with a resulting decrease in the concentration of fructose in the semen. By contrast, the major effect of oxytocin in Exp. 3 was to increase the volume of semen. This difference in the response to oxytocin may be associated with the different intervals between oxytocin injection and ejaculation in the two experiments. Increasing the interval between oxytocin injection and ejaculation in Exp. 2 increased the volume of semen while producing only a small change in the sperm concentration per ejaculate.

In Exp. 3, the increase both in the volume of semen and in the total fructose per ejaculate suggests that oxytocin had stimulated either the formation of seminal plasma or its expulsion from the seminal vesicles. Kihlström & Melin (1963) and Fjellström, Kihlström & Melin (1968), working with rabbits, also obtained indirect evidence that oxytocin stimulated the accessory sex glands. Oxytocin has been shown to stimulate contractions of the epididymides in the ram (Knight, 1972) and the epididymides and ductus deferens of the bull and rabbit (Milovanov *et al.*, 1962; Bereznev, 1964; Melin, 1970) but there is no direct evidence that oxytocin will stimulate contractions or secretion in the male accessory sex glands. Cross & Glover (1958) and Melin (1970) found that oxytocin did not increase the muscular activity of the seminal vesicles of the rabbit.

Intravenous adrenaline had no effect on the output of semen when the ejaculate was collected immediately after the injection. Although VanDemark & Baker (1953) found that intravenous injection of adrenaline into bulls decreased the volume of semen and sperm concentration, they did not state the length of the interval between injection and collection. The lack of response to exogenous adrenaline is possibly because adequate amounts of endogenous adrenaline are already released near the organs.

The rams with the high output of semen responded more regularly to the injection of oxytocin and the responses were larger than for those with the low output of semen. The large difference in the output of semen by the two groups of rams cannot be explained either by a lack of the release of endogenous oxytocin or by adrenaline.

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