EFFECTS OF PILOCARPINE AND ATROPINE ON COPULATORY BEHAVIOUR, EJACULATION AND SEMEN COMPOSITION IN THE BULL

R. D. BAKER,* N. L. VANDEMARK,† C. N. GRAVES and H. W. NORTON*

Department of Dairy Science, University of Illinois,
Urbana, U.S.A.

(Received 19th March 1964)

Summary. To determine the effects of parasympathetic-influencing drugs on reproductive phenomena in bulls, four ejaculates were collected at 15-min intervals from each of six bulls beginning 30 min after a subcutaneous injection of 400 mg pilocarpine, 200 mg atropine, or physiological saline solution. Each treatment was administered twice, so that a total of 144 ejaculates was collected. Pilocarpine significantly ($P<0.01$) decreased the concentration of spermatozoa and increased the time required to mount, the duration of ejaculation, the volume of semen, the number of spermatozoa per ejaculate and the concentration of chloride in the semen. Atropine had the reverse effect on these characteristics and significantly ($P<0.01$) increased the concentration of fructose in the semen. These results demonstrate that atropine and pilocarpine, which are known to influence the parasympathetic system, alter the reaction time of the bulls, the secretion of one or more accessory sex glands, the passage of spermatozoa through the male reproductive tract, and the emission of semen during ejaculation.

INTRODUCTION

The role of the autonomic nervous system in regulating the reproductive processes in the male is not well understood. Histochemical studies summarized by Gerbetzoff (1959) demonstrated the presence of cholinergic nerve fibres in the vas deferens, seminal vesicles, ampulla, ejaculatory duct and urethra of the guinea-pig and rat. Risley & Skrepetos (1964) have identified acetylcholinesterase in the vas deferens and cauda epididymidis, but not in the testis, rete testis, ductuli efferentis and upper caput epididymidis of the rat. The presence of cholinergic nerve fibres in the proximal vas deferens and cauda epididymidis, where spermatozoa are stored prior to emission, suggests that the parasympathetic system might be involved in ejaculation. However, earlier investigators (Bacq, 1931; Gunn, 1936; Semans & Langworthy, 1938) reported that

* Present address: Department of Animal Science, University of Illinois, Urbana, Illinois, U.S.A.
† Present address: Department of Dairy Science, Ohio State University, Columbus 10, Ohio, U.S.A.
ejaculation is controlled by the sympathetic system and erection is predominantly under the influence of the parasympathetic system.

The parasympathetic nerves which supply the external genitalia of the bull arise from the sacral segments of the spinal cord and are carried in the ventral roots of spinal segments 2, 3 and 4 (Larson & Kitchell, 1958). These parasympathetic fibres are distributed, as in other species, via the pelvic nerve and pelvic plexus to all the genital organs except perhaps the testes.

Since erection and ejaculation appear to be under the control of the autonomic nervous system, one would expect them to be affected by drugs which are known to influence this system. Epinephrine has been found to reduce both the semen volume and spermatozoan concentration of the bull ejaculate (VanDemark & Baker, 1953). Carbachol, a powerful parasympathomimetic agent, and dibenamine, which effectively prevents the excitatory responses of smooth muscle and exocrine gland cells to adrenergic stimuli, have no effect on copulatory behaviour in rabbits (Dziuk & Norton, 1962). Atropine reduces the total volume of semen and increases the spermatozoan concentration of the boar (Dziuk & Norton, 1962; Dziuk & Mann, 1963) and bull ejaculates (Signoret, 1962). Thus, some drugs which are known to affect the autonomic nervous system can be used to alter the ejaculatory process and semen composition of the male.

The purpose of the present study was to determine the effects of parasympathetic-influencing drugs on the copulatory behaviour, the ejaculatory process, and the semen composition of bulls. Pilocarpine, which is especially effective on secretory glands, was selected as the parasympathomimetic drug, while atropine was used as the parasympathetic-inhibiting drug.

**MATERIALS AND METHODS**

Six Holstein-Friesian bulls weighing from 1600 to 1900 lb and ranging from 3 to 7 years of age were injected with 4 ml physiological saline solution, alone or containing either 200 mg of atropine sulphate or 400 mg of pilocarpine hydrochloride. The injections were given subcutaneously into the bull’s neck.

Beginning 30 min after the injection, four ejaculates were collected with the artificial vagina with 15-min intervals between collections. The time required for the bulls to mount the teaser cow was called the reaction time, and the time-lapse between mounting and the ejaculatory thrust was called the ejaculation time.

The volume of the ejaculate was noted immediately after collection. Semen samples were examined under the microscope for both the percentage of motile spermatozoa and rate of sperm motility. The concentration of spermatozoa was determined with a photometer. When available, approximately 3 ml of semen from each ejaculate was placed on ice to cool and was frozen for storage.

Frozen semen samples were thawed, and analysed for fructose, chloride, sodium, calcium and potassium content. Fructose concentration was determined by the Roe colorimetric method modified for semen (1934). Chloride was determined by a modification of the Volhard procedure (Volhard, 1874). The sodium, calcium and potassium levels of the semen were determined by flame photometry (Cragle, Salisbury & VanDemark, 1958).
Drug effects on bull copulation and semen

RESULTS

The first detectable effects of the drugs were changes in the secretory activity of various glands. Pilocarpine stimulated salivation, lacrymation, and dripping of fluid from the sheath within 20 min after injection, and atropine inhibited the secretion of saliva in all the bulls. These drugs had no apparent effect on urination, defaecation or bloat. Signoret (1962) reported that 800 mg of atropine caused definite reactions of excitement and bloat which persisted for 24 hr. In this study, the drug effects disappeared within 6 hr.

Copulatory behaviour

The drugs also altered the copulatory behaviour of the bulls. Atropine significantly \( (P<0.05) \) decreased the reaction time, whereas pilocarpine significantly \( (P<0.01) \) increased it (Text-fig. 1). In the pilocarpine-treated group, there was a significant linear increase in reaction time from the first to the fourth ejaculation.

![Text-fig. 1](image)

The time required for ejaculation was prolonged by pilocarpine, the average time of 6.0 sec being significantly \( (P<0.01) \) longer than 2.5 sec for controls and 3.5 sec for atropinized bulls. Although the reaction time and the duration of ejaculation were altered by the drug treatment, no change was detected in the bull’s ability to erect the penis.

Characteristics of the semen

Both the quantity and quality of semen collected in this study were markedly altered by the drug treatment. Pilocarpine significantly increased the volume of semen and the number of spermatozoa per ejaculate and decreased the
concentration of spermatozoa (Table 1). Atropine reduced the volume of semen and, in fact, completely inhibited the emission of any semen in thirteen of forty-eight attempted ejaculations. When semen was obtained in the atropine-treated group, the concentration of spermatozoa was greater than in the semen of control bulls, but the number of spermatozoa per ejaculate was significantly reduced. Both the percentage and rate of sperm motility were significantly positively correlated with the concentration of spermatozoa.

### Table 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Treatment</th>
<th>Pilocarpine</th>
<th>Control</th>
<th>Atropine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (ml)</td>
<td></td>
<td>4.56*</td>
<td>2.53</td>
<td>0.85*</td>
</tr>
<tr>
<td>Conc. of sperm (×10^6)</td>
<td></td>
<td>866*</td>
<td>1074</td>
<td>1523*</td>
</tr>
<tr>
<td>Sperm/ejaculate (×10^9)</td>
<td></td>
<td>5.4*</td>
<td>2.8</td>
<td>1.8*</td>
</tr>
</tbody>
</table>

* Significantly (P<0.01) different from control.
† Mean of thirty-five observations rather than forty-eight.

### Table 2

<table>
<thead>
<tr>
<th>Composition</th>
<th>Treatment</th>
<th>Pilocarpine</th>
<th>Control</th>
<th>Atropine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructose (mg/ml)</td>
<td></td>
<td>4.35 (48)</td>
<td>4.27 (46)</td>
<td>6.28* (30)</td>
</tr>
<tr>
<td>Chloride (µg/ml)</td>
<td></td>
<td>230* (48)</td>
<td>192 (46)</td>
<td>58* (30)</td>
</tr>
<tr>
<td>Potassium (µg/ml)</td>
<td></td>
<td>205 (44)</td>
<td>123 (47)</td>
<td>166 (29)</td>
</tr>
<tr>
<td>Sodium (µg/ml)</td>
<td></td>
<td>256 (44)</td>
<td>262 (47)</td>
<td>228 (29)</td>
</tr>
<tr>
<td>Calcium (µg/ml)</td>
<td></td>
<td>20.6 (44)</td>
<td>23.2 (47)</td>
<td>22.1 (29)</td>
</tr>
</tbody>
</table>

Numbers in parentheses refer to number of observations.
* Significantly (P<0.01) different from control.

The bulls produced significantly different volumes of semen, but the number and concentration of spermatozoa did not vary significantly between bulls within treatments. No significant trend in semen characteristics was detected in the period covered by the four collections.

**Chemical composition of the semen**

Atropine significantly increased the concentration of fructose in the semen (Table 2) but reduced the total content of fructose in the ejaculate to one-half that of the control semen. Pilocarpine, however, had no significant influence on the concentration of fructose, but increased the total content of fructose to twice that of the control.

Neither fructose nor spermatozoa were detected in a sample of the pre-ejaculatory fluid which dripped from the sheath of one of the pilocarpine-
treated bulls. This sample contained a relatively large amount of chloride (430 μg/ml).

The concentration and total content of chloride per ejaculate was increased by pilocarpine and reduced by atropine. The semen from atropine-treated bulls contained only one-tenth as much chloride per ejaculate as the control semen.

The drugs had no significant effect on the concentration of the bulk cations: sodium, potassium and calcium. However, the total amounts of sodium, of potassium and of calcium were increased by pilocarpine and decreased by atropine in proportion to their influence on the volume of semen ejaculated.

DISCUSSION

In therapeutic dosage, atropine blocks the transmission of the post-ganglionic cholinergic impulses to the effector cells; pilocarpine mimics the effect of acetylcholine by direct stimulation of the effector cells which are innervated by the cholinergic nerves (Jones, 1954). The effects of these drugs on various secretions of the bull in this study indicated that the doses were effective in altering the activity of the parasympathetic system.

Copulatory behaviour

When an adequate sexual stimulation prior to copulation is not provided, the sex drive of the bull is reduced and the vigour of the ejaculatory reflex diminished, resulting in the production of poor semen and a decreased conception rate (Kerruish, 1955). Bereznev (1963) reported that the quality of semen from bulls was improved by injecting carbocholine or prozerine 30 to 40 min before semen collection and oxytocin 1 to 1.5 min before collection. Oxytocin also reduced the reaction time in the rabbit and increased the number of ejaculates collected within a 30-min period (Melin & Kihlström, 1963). These authors concluded that the increased sexual drive may reflect an activation of the receptors in the genital organs.

In this study the effect of these drugs on the bull’s reaction time seemed to be an indirect result of the changes in the number of spermatozoa and volume of semen ejaculated during the previous collection of the series. The bulls under the influence of atropine required the least amount of time to mount and were the most difficult to restrain, especially after a collection in which semen emission was completely inhibited. The pilocarpine-treated bulls showed a significant linear increase in reaction time from the first to the fourth collection (Text-fig. 1). This decrease in sexual drive followed the production of increased numbers of spermatozoa and volume of semen at the previous collection under the influence of pilocarpine.

Characteristics and chemical composition of semen

Since Mann (1946) proved that the seminal vesicles were the primary site of fructose synthesis, the concentration of fructose in the semen has been used as a measure of the relative activity of the vesicular glands. The fluid from these glands was almost totally lacking in chloride, a phenomenon infrequently
encountered in other body fluids (Mann, 1954). Therefore, the concentration of chloride in the semen has been used as an indicator of activity for urethral glands which produce a chloride-rich fluid (Dziuk & Mann, 1963).

In the present study, pilocarpine stimulated the secretion of seminal fluids from the accessory glands and promoted the passage of spermatozoa through the vas deferens and epididymis. The fact that pilocarpine had no effect on the concentration of fructose but doubled the total content of fructose in the ejaculate indicates that the seminal vesicles were stimulated equally with the other glands. The decrease in the concentration of spermatozoa and increase in concentration of chloride suggests that the mineral-producing glands such as the prostate, urethral and bulbo-urethral glands were the primary targets of pilocarpine. Huggins (1947) found that the isolated canine prostate gland secreted as much as four times its weight of fluid (60 ml) following pilocarpine treatment.

The complete lack of detectable fructose, and the high concentration of chloride, in the samples of pre-ejaculatory fluids indicate that these secretions were of prostatic and urethral origin. Injection of large amounts of pilocarpine may be a method for obtaining seminal fluids void of spermatozoa and vesicular secretion.

The decreased concentration of chloride and increased concentration of fructose in the semen of atropinized bulls (Table 2) agree with the results reported by Dziuk & Mann (1963) on the fructose concentration of semen from atropinized boars. These results provide additional support for the hypothesis that the parasympathetic system has a more dramatic effect on the mineral-producing accessory glands than on the seminal vesicles. However, Desjardins & Hafis (1963) reported that atropine did prevent the contraction of rat seminal vesicles after in-vitro perfusion with acetylcholine or epinephrine. The contraction was also blocked by dibenamine, but more slowly.

The results of this study are in contrast to reports that erection is predominantly controlled by the parasympathetic system and that semen emission is actively dependent on the sympathetic system. However, Rose (1953) reported that the sympathetic system did seem to facilitate ejaculation by closing the internal sphincter to prevent semen from entering the bladder. Loewe & Puttuck (1953) found that drug-induced ejaculation was abolished by dibenamine, a sympathetic drug, in mice, and that atropine interfered with the emission of semen.

Changes in the volume and composition of semen ejaculated under the influence of pilocarpine and atropine demonstrate that the parasympathetic system plays an active role in the secretion and excretion of seminal fluids from the male accessory glands and the reproductive tract, as well as affecting the movement of spermatozoa through the vas deferens and epididymis during ejaculation.

REFERENCES
Drug effects on bull copulation and semen


