To the Graduate Council:

I am submiting herewith a thesis written by Cindy S. Armstrong entitled "The Effect of Exogenous Oxytocin on the Output of Sperm and on the Uterotonic Effect of the Bovine Ejaculate." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree Master of Science, with a major in Animal Science.

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THE EFFECT OF EXOGENOUS OXYTOCIN ON THE OUTPUT OF SPERM AND ON THE UTEROTONIC EFFECT OF THE BOVINE EJACULATE

A Thesis

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ABSTRACT

Exogenous oxytocin has been found to increase sperm output in several species of animals when given prior to mating. However, the effect of oxytocin given to bulls prior to electrojaculation was not found in the literature review. Also, it has been documented that small molecules of different chemical nature pass from the blood into the ejaculate. The objectives of this study were to investigate if oxytocin, given prior to electroejaculation would increase sperm output and to find if oxytocin is transferred into the electrojaculate by measuring the uterotonic effect of semen from bulls injected with oxytocin.

In experiment I, five Angus bulls were injected (IM) with 0, 2, 20, or 200 IU of synthetic oxytocin and electroejaculated at 15 and 75 minutes post injection. Collections were kept on ice and sperm were counted in the laboratory using a hemocytometer. Results were expressed as total output of sperm.

In experiment II, four bulls were used. Bulls were injected with 400 IU oxytocin (IV) and 200 IU oxytocin (IM). Semen was collected by electroejaculation at 0, 5, 15, 30, and 60 minutes post injection. Blood was taken from an indwelling jugular catheter at 0, 15, and 60 minutes The uterotonic effect of semen and plasma samples was tested by using the rat uterus preparation bioassay. Thioglycolate was used to break the disulfide bonds in the oxytocin molecule to test if oxytocin was responsible for the uterotonic effect of the blood and semen samples.

The results of experiment one showed that oxytocin, given prior to electroejaculation increased the total sperm output of the group up to 415

percent as compared to the non-injected bulls. However, the mean difference between groups was not statistically significant, probably due to individual variation.

Experiment two indicated that the uterotonic effect of the ejaculate was not affected by the injection of oxytocin prior to electroejaculation. Therefore, if there is release of oxytocin in the male during mating, it is doubtful oxytocin is passed into the ejaculate in significant concentrations.

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CHAPTER I

INTRODUCTION

Statement of the Problem

Release of oxytocin has been related to mating in the male. It has been postulated that circulating oxytocin could be part of the ejaculatory mechanism by activating reproductive tract smooth muscles, which are in charge of semen propulsion during ejaculation. Moreover, exogenous oxytocin has been shown to increase the sperm output when given prior to mating in several species of animals. However, the effect of oxytocin given prior to electroejaculation in bulls was not found in our literature search. On the other hand, the passage of small molecules of different chemical nature into the ejaculate from blood have been documented. In the present study, oxytocin, a sulfur containing peptide hormone of 1000 Daltons was tested. Because of the chemical configuration of oxytocin (octapeptide), it was expected to be transferred into the ejaculate after systemic injection. If this transfer of oxytocin occurs, in significant amounts, the uterotonic effect of the semen should be increased.

The objective of this research was to investigate whether or not oxytocin administered prior to electroejaculation will increase the sperm output in bulls. Further, the possibility that oxytocin may have been transferred into the ejaculate was investigated by measuring the uterotonic effect of the electroejaculate obtained from bulls injected with oxytocin.

Review of the Literature

The literature review has been divided into two parts. Part one was concerned with the effect of oxytocin on the sperm output when using electroejaculation. Part two deals with the uterotonic effect of semen of bulls injected with exogenous oxytocin.

Part One: Effect of Oxytocin on Sperm Output in the Electroejaculate. Although the physiological role of oxytocin in the male remains obscure today, it has been studied by various researchers through the years. Cross (1955) found that stimulation of the hypothalamus in the male rabbit released neurohypophyseal hormones. Later, Cross and Clover (1958) tested the effect of oxytocin and vasopressin on the seminal vesicle of sheep and found that they failed to elicit a contractile response in that organ. Cross (1959) stated:
If oxytocin is discharged from the neurohypophysis at coitus in the male, it is difficult to see what function it serves in the conveyance of sperms to the tail of the epididymis or in the emission of semen into the urethra.

Debackere, Peeters and Tuyttens (1961) performed an experiment using cross circulation between a ram and a ewe. They observed a sharp increase in the intramammary pressure in the ewe after massage of the seminal vesicles and ampulla in the ram which suggested the release of oxytocin into the blood stream of the ram. They postulated that the release of oxytocin from the ram during coitus played an important physiological role in the reproductive physiology of the male.

Sharma and Hays (1973) found that the oxytocic activity of the blood plasma of bulls increased before and after copulation, ejaculation into an artificial vagina, during massage of the seminal vesicles and ampulla and by

stimulation using an electroejaculator. However, these workers did not measure plasma concentrations of oxytocin. Similarly, Bereznev (1963) detected the presence of oxytocin in the blood of bulls immediately before and immediately after service.

By this time, researchers accepted the fact that oxytocin was released in the male during sexual activity. Studies were then done to test the effect of exogenous oxytocin on the male. Milovanov et al., (1962) injected male rabbits with oxytocin and found that the volume of the ejaculate was increased by 17 percent over saline injected controls. In similar experiments using bulls, oxytocin was found to increase ejaculate volume, spermatozoal concentrations and total number of spermatozoa when bulls were collected within 5 minutes of the intravenous injection. They found that 6 IU oxytocin was the optimal dosage.

The effect of exogenous oxytocin on male rabbits was determined by Fjellstrom et al. (1968). They found that exogenous oxytocin significantly increased the volume of the first ejaculate. The total number of sperm cells also increased but concentration did not change. This is probably due to the increased amount of secretions from the accessory glands (Fjellstrom et al., 1968).

In rams, the injection of exogenous oxytocin immediately before collection of semen with an artificial vagina resulted in an increase in the volume of semen and number of spermatozoa per ejaculate (Knight and Lindsay, 1970; Knight, 1974). They found oxytocin to have an immediate stimulating effect on the ejection of spermatozoa and seminal plasma, but a long term adverse effect on spermatogenesis.

Voglmayr (1975) looked at the output of spermatozoa and epididymal fluid

in rams by catheterizing the rete testis. He found an increase in the flow of epididymal fluid and sperm concentration in response to exogenous oxytocin. This experiment suggested that oxytocin was effective at the testicular level.

In contrast to the work by Fjellstrom et al. (1968), oxytocin was found to have no overall effect on the number of spermatozoa ejaculated in the rabbit (Agmo, 1975). Discrepancies may have been due to the different dosages of oxytocin used in the studies. Agmo (1975) also found that physiological doses of vasopressin increased the number of ejaculated spermatozoa by more than 100 percent in rabbits. The effect of vasopressin was predominant when compared to that of oxytocin.

By using radioimmunoassay to determine the plasma concentrations of oxytocin in cattle, Schams et al. (1982) found that electroejaculation caused an increase in oxytocin concentration in the blood. However, electroejaculation cannot be regarded as normal ejaculation. Oxytocin release may have been caused by the electrical stimulus rather than by ejaculation itself. The positive effect of oxytocin release after ejaculation has also been reported in rams (Sharma et al., 1972) and boars (Ewy et al., 1972). No report on the effect of oxytocin on the sperm output in the bovine electroejaculate was found in this literature search.

Part Two: Uterotonic Effect of Semen Obtained from Oxytocin Injected Bulls. The passage of oxytocin into the ejaculate has not been investigated. If oxytocin is released into the blood circulation during coitus, it seems reasonable to expect the passage of oxytocin into the ejaculate.

Recently, the passage of different substances from the blood into the ejaculate has been investigated. Exogenous estrogen (Eiler and Graves, 1977),

exogenous cortisol (Graves and Eiler, 1979), and exogenous testosterone and progesterone (Eiler and Graves, 1981), have been found to be readily transferred from blood into the semen of the bull at ejaculation. In all cases, the transfer was immediate and occurred within 20 minutes of the injection of the exogenous substance.

The penetration of various drugs into the ejaculate is important in the treatment of infectious diseases of reproductive organs such as in accessory gland disease. Antibiotics such as metronidazole (Eliasson and Dornbusch, 1980) and pentoxifyllin (Schramm and Hinze, 1980) have been reported to pass into the ejaculate of humans. Metronidazole was found in the seminal plasma 2-3 hours after oral dosage.

Swanson and workers (1978) established that d-methadone (a narcotic drug) accumulates in the reproductive organs of the rabbit achieving high concentrations in the semen in relation to levels in the blood. This agrees with Gerber and Lynn (1976) who found that the methadone concentration in human semen was 1.8 times the drug level in the blood.

Similarly, naltrexone, a narcotic antagonist, was found to pass readily into the semen of rabbits. Naltrexone was found in higher concentrations in the semen than concurrent concentrations in the plasma (Taylor et al., 1980).

Armstrong, Smalling and Eiler (1982) found that exogenous thyroxine, an iodine containing amino acid hormone, was passed from the blood into the ejaculate of the bovine at 120 minutes of the thyroxine injection.

A protein hormone, prolactin, was also demonstrated to be present in the semen in concentrations of three to five fold higher than serum levels (Sheth et al., 1975). Factors controlling levels of prolactin in the semen are not known.

Prolactin concentration in the serum and semen were found to increase in monkeys treated with chlorpromazine, with the concentration of prolactin being much higher in the seminal plasma than in the serum (Sheth et al., 1979). The maximum levels of prolactin were found in the serum six hours after treatment, whereas, semen prolactin did not peak until day eight. This suggests that there is a lag period between increased serum prolactin concentrations and the time prolactin is highest in the seminal plasma. Chlorpromazine was assumed to cause the release of prolactin from the anterior pituitary. Whether the prolactin from the blood is passed into the semen, or it is produced there, has not been determined.

The presence of pituitary glycoprotein hormones in the seminal plasma has also been investigated. The concentration of lutenizing hormone (LH) (DeAloysio et al., 1974) and LH and follicle stimulating hormone (FSH) (Shoenfeld et al., 1978) was found to be similar in the blood serum and seminal plasma of humans. Conversely, Sheth et al. (1976) reported the concentration of LH to be significantly higher in seminal plasma than in blood serum of humans. On work carried out in the bovine, Sairam et al. (1980) determined that the concentration of FSH is 100 times higher in seminal plasma than in circulation. Just the opposite was found with LH. Seminal plasma LH levels were low, and the blood levels of LH were high. This is in conflict with the work of Razdan et al. (1977) who found LH levels in the seminal plasma ofcross-bred bulls to be higher than blood serum LH levels. So far, the homeostatic mechanism keeping the spermatozoa environment constant in the ejaculate seems to be of little efficiency. Foreign and endogenous compounds can readily pass into the ejaculate from blood.

Besides the passage of organic compounds, the passage of Hepatitis B virus into the semen has been reported by Ayoola et al. (1980), Scott et al. (1980) and Alter et al. (1977). Carriers of the Hepatitis B surface antigen can be infectious causing transmission of the virus through the semen.

The chemical structure of the substances that transfer from the blood into the ejaculate show a variety of chemical configurations. The steroids are lipid derived ring structures with differing substituents and a molecular weight close to 300. Although they are similar in chemical structure, there was a difference in their rate of transfer between steroids. Cortisol was found to pass more readily from the blood to the semen. This could be due to the hydroxyl groups on the cortisol molecule (Eiler and Graves, 1981).

The amino acid derived hormone, thyroxine, has a molecular weight around 800. Protein (prolactin) and glycoprotein (FSH and LH) hormones have a large molecular weight from 20,000 to 30,000. The antibiotics mentioned previously had a molecular weight of 171 and 278. Since substances with such diverse molecular weights (200 to 30,000) are found in the ejaculate, it would be improbable that transfer of substances is specifically limited by the size of the molecule under 30,000 Daltons. The critical size for kidney capsule filtrate is approximately 62,000 Daltons. This is suggestive that the critical molecular weight, if any, for the male reproductive tract, may be larger than 30,000.

CHAPTER II

EXPERIMENT I—EFFECT OF OXYTOCIN ON SPERM OUTPUT IN ELECTROEJACULATED BOVINE SEMEN; DOSE-RESPONSE RELATIONSHIP

Introduction

Exogenous oxytocin has been reported to increase sperm output and ejaculate volume in rams subjected to natural mating (Knight, 1973). Similarly, in the rabbit, when oxytocin was injected shortly before mating, the sperm output was also increased (Fjellstrom, et al., 1968). Exogenous vasopressin has been reported to increase the number of spermatoza in the rabbit ejaculate (Agmo, 1975). Conversely, no conclusive results have been found when exogenous oxytocin has been given prior to electroejaculation in the ram. The positive effect of exogenous oxytocin on the sperm output in natural mating in several species has been documented as mentioned above. The release of oxytocin during natural mating has not been found to occur in the bull (Schams, et al., 1982), as investigated by RIA procedure, regardless of increased oxytocin activity, as measured by bioassay procedure, of plasma found in bulls subjected to mating stimulation (Sharma and Hays, 1968 and 1973). It has been shown that exogenous oxytocin increased the outflow of sperm in the seminiferous tubules of the ram (Voglmayr, 1975). It is known that the concentration of sperm in the electroejaculate is smaller than in natural mating (Dziuk et al., 1954; Marden, 1954) probably due to the overstimulation of the accessory glands secretions. It seems reasonable to expect that oxytocin given prior to electrojaculation may increase the spermatozoa output in bulls collected by electroejaculation. This would be of practical interest, when efficiency of electrojaculation needs to be

increased. The objective of this research was to determine the effect of oxytocin on the sperm output in bulls subject to electroejaculation.

Materials and Methods

Five eighteen-month old Angus bulls were used in this study. Bulls were injected at random with either 2, 20, or 200 IU synthetic oxytocin (W.A. Butler Co., Columbus, Ohio) and electroejaculated (Plectron Electroejaculator, Overton, Nebraska) at 15 and 75 minutes post oxytocin injection. The volume of the electroejaculate was standardized at 9 to 10 ml per collection. Each collection consisted of two consecutive fractions of 4 to 5 ml each. These two fractions were pooled and referred to as a collection. Each collection was completed in approximately a 4 minute period. The bulls were allowed at least three days of rest between testing the effect of different dosages of oxytocin.

Five experimental groups were used in this study (Figure 1.). Bull groups one and two were electroejaculated before any oxytocin was injected. Group one bulls were electroejaculated, rested 60 minutes, then electroejaculated again. Group two bulls were electroejaculated, rested 60 minutes, injected with 200 IU oxytocin (IM), then electroejaculated 15 minutes after injection. Bull groups three, four, and five received oxytocin injection (IM) before electroejaculation was attempted. Group three bulls were injected with 2 IU, group four bulls were injected with 20 IU, and group five bulls were injected with 200 IU oxytocin. The bulls were electroejaculated at 15 and at 75 minutes after injection.

Collections were kept on ice and sperm were counted in the laboratory using a hemocytometer. Results were expressed as total output of sperm. Total output of sperm was equal to sperm cell concentration times volume of collection.

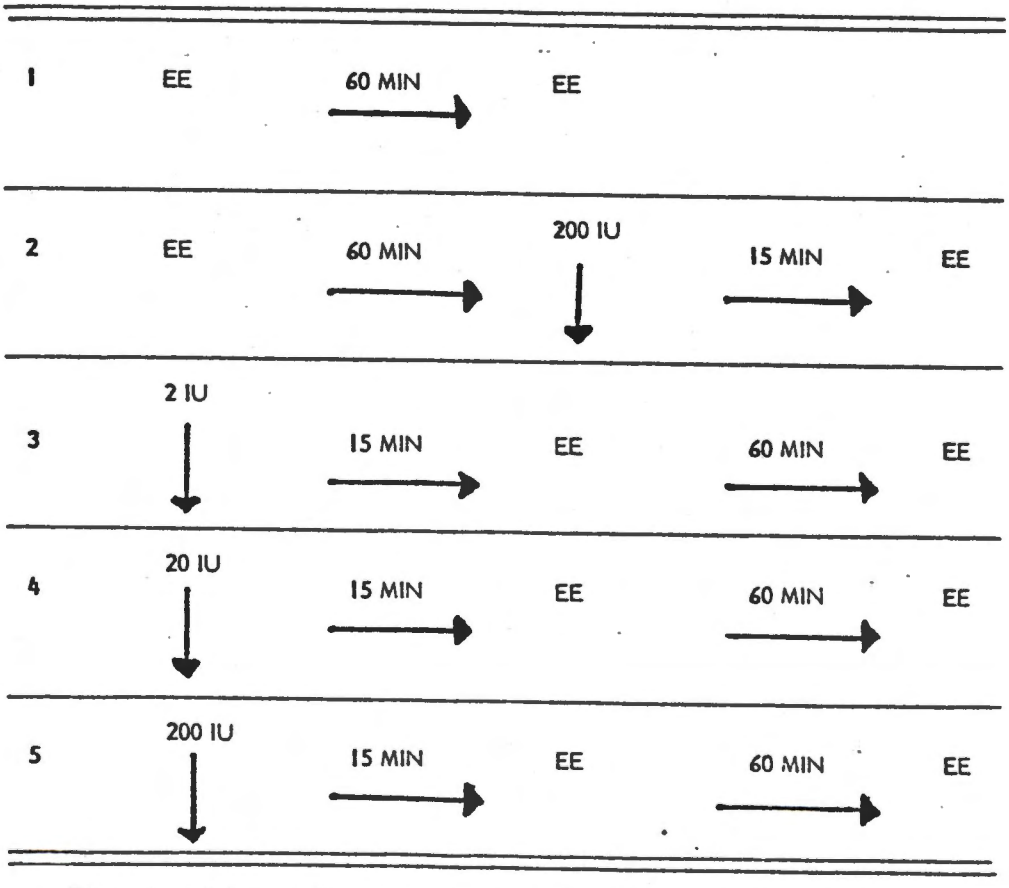


Figure 1. A Schematic Representation of the Procedure used in Experiment 1. EE represents the times the bulls were electroejaculated. Horizonal arrows represent a time lapse between collections. Vertical arrows indicate an injection of oxytocin.

Design and Statistical Analysis

The general design of the experiment was dose-response and time-response relationship. Mean separation was accomplished by Students t-test. Significance was reported at 0.05 level.

Results

The effect of different doses of oxytocin on average sperm numbers collected at 15 and 75 minutes after oxytocin (IM) injection is shown in Table 1.

The average number of sperm per collection of the 15 minute post oxytocin injection was maximal (186 percent) at the 20 IU dose. Differences between the control group and treated group were not statistically significant ($P > 0.05$).

The delta percentage of average sperm numbers at 15 minutes post oxytocin injection was increased over controls by each dosage of oxytocin injected into the bull. Twenty IU of oxytocin produced the maximal increment (186 percent) ($P > 0.05$) in sperm count.

The average number of sperm per collection of the 75 minute post oxytocin injection was maximal at the 200 IU dose. Differences between the control group and treated group were not statistically significant ($P > 0.05$). The delta percentage of average sperm numbers at 75 minutes post oxytocin injection was moderately increased ($P > 0.05$) at 200 IU of oxytocin. However, Delta percentage was decreased at 2 and 20 IU of oxytocin.

The average number of sperm per collection at 15 minutes post oxytocin injection was very similar to that at 75 minutes post oxytocin injection. The average number of sperm per collection in the 75 minute post oxytocin collection were less than at 15 minutes. However, differences between 75 and 15 minute

TABLE I

EFFECT OF DIFFERENT DOSES OF OXYTOCIN ON GROUP AVERAGE SPERM NUMBERS COLLECTED AT 15 AND 75 MINUTES AFTER OXYTOCIN (IM) INJECTION.

Dose of Oxytocin (IU)	15 Minutes Post Oxytocin Injection		75 Minutes Post Oxytocin Injection	
	Average number of sperm per collection	Δ %*	Average number of sperm per collection	Δ %*
0	$3.8 \times 10^8 \pm 2.2 \times 10^{8a}$	0	$4.3 \times 10^8 \pm 2.1 \times 10^{8a}$	0
2	$4.1 \times 10^8 \pm 3.3 \times 10^{8a}$	10	$1.4 \times 10^8 \pm 0.88 \times 10^{8a}$	-67
20	$10.8 \times 10^8 \pm 5.2 \times 10^{8a}$	186	$3.7 \times 10^8 \pm 2.1 \times 10^{8a}$	-11
200	$6.1 \times 10^8 \pm 2.2 \times 10^{8a}$	62	$5.5 \times 10^8 \pm 3.6 \times 10^{8a}$	29

Sperm cell numbers are Mean ± SEM (N = 5).

* Δ % represents the percent increase (+) or decrease (-) as related to the control (0-dose) values.

[a]Similar superscripts indicate no statistically significant ($P > 0.05$) differences as compared to the control(0-dose).

groups were not significant ($P > 0.05$). The effect of different doses of oxytocin on sperm output per collection during the first collection of individual bulls is shown in Table 2. The overall total output of sperm was increased by all doses of oxytocin. The maximal effect was found at 20 IU (417 percent). There was no dose-response relationship.

The effect of different doses of oxytocin on sperm output per collection during the second collection of individual bulls is shown in Table 3. Overall total output of sperm cells was increased at 20 and 200 IU oxytocin. At 2 IU oxytocin total output of sperm was below controls. Maximal effect was found at 200 IU.

In general, oxytocin treatment increased overall total output of sperm cells of both 15 and 75 minutes post injection. However, the larger effect was found at 15 minutes post injection. Values of the individual bulls showed large variability.

Discussion

This experiment shows an increase in overall total output of sperm cells when oxytocin was given regardless of first collection (Table 2) or second collection (Table 3). In the present experiment, the increase in output of sperm cells of the oxytocin treated bulls fluctuated between 99 and 417 percent over the controls, within 15 minutes after the oxytocin was given and between 80 and 167 percent within 75 minutes after oxytocin was given. No information was available to us on the effect of oxytocin on the electroejaculate in bulls. Similiar to our findings in the bull, in the ram, oxytocin treatment increased the volume of semen and the number of spermatozoa per ejaculate (Knight and Lindsay, 1970). No dose-response relationship in the present work was found when oxytocin was injected at 2, 20, and 200 IU in the first collection. Four-

TABLE 2

EFFECT OF DIFFERENT DOSES OF OXYTOCIN ON SPERM OUTPUT PER COLLECTION DURING THE FIRST COLLECTION OF INDIVIDUAL BULLS.*

Dose of Oxytocin (IU)	TOTAL SPERM COUNT PER INDIVIDUAL BULL (In millions)					Overall total output of sperm per dose size (in millions)	Δ %**
	Bull A	Bull B	Bull C	Bull D	Bull E		
0	0	1.8	327	686	28	1042	0
2	.77	.009	246	1726	100	2073	99
20	.11	3.7	779	2476	2126	5385	417
200	1275	.042	252	824	689	3040	192

*The first collection was taken 15 minutes post oxytocin injection (IM).

** Δ % is the percent change as compared to the control group (received no oxytocin).

TABLE 3

EFFECT OF DIFFERENT DOSES OF OXYTOCIN ON SPERM OUTPUT PER COLLECTION DURING THE SECOND COLLECTION OF INDIVIDUAL BULLS*

Dose of Oxytocin (IU)	TOTAL SPERM COUNT PER INDIVIDUAL BULL (In Millions)					Overall Total output of sperm per dose size (in millions)	Δ%**
	Bull A	Bull B	Bull C	Bull D	Bull E		
0	399	.38	.36	512	116	1028	0
2	438	.012	250	1.6	18	707	-31
20	64	0.0	1177	235	373	1849	80
200	1959	.013	412	230	143	2743	167

*The second collection was taken 60 minutes after the first collection and 75 minutes after the initial oxytocin injection.

** Δ% is the percent change as compared to the control group (zero oxytocin).

hundred seventeen percent increment in the sperm output was found at 20 IU dose size. At 200 IU, the increment was only 192 percent above the controls (Table 2). It has been proposed, that the inhibitory effect of a relatively large dose of oxytocin on the sperm output of the seminiferous tubules may have been due to tonic contraction of the seminiferous tubule wall (Niemi et al., 1965). This may partially explain the lack of response between dose of oxytocin and sperm output in our experiment.

In general, a larger overall total output of sperm cells at the 15 minute collection was found than at the 75 minute collection. This was probably due to depletion of sperm cells during the first collection and also due to depletion or utilization of the injected oxytocin. It has been reported that long term administration of oxytocin induced refractoriness to oxytocin in the ram (Knight and Lindsay, 1970).

During natural mating, no release of endogenous oxytocin has been found. Conversely, significant increase of plasma concentrations of oxytocin were reported during electroejaculation in the bull (Schams et. al., 1982). This is suggestive that in our experiment the effect of exogenous oxytocin could have been enhanced by the release of endogenous oxytocin.

When average number of sperm cells per collection at 15 and 75 minutes post oxytocin injection were calculated, no significant difference was found between group means (Table 1). This was probably due to both individual variation and to the small number of degrees of freedom in this experiment.

CHAPTER III

EXPERIMENT II—UTEROTONIC EFFECT OF SEMEN OBTAINED FROM OXYTOCIN INJECTED BULLS; TIME-RESPONSE RELATIONSHIP

Introduction

The passage of glycoproteins (Sairam et al., 1980; Razdan et al., 1977), steroids, (Eiler and Graves, 1977; Graves and Eiler, 1979; Eiler and Graves, 1981), amino acids (Armstrong et al., 1982a), antibiotics (Eliason et al., 1980; Schramm et al., 1980) and alkaloids (Swanson et al., 1978; Gerber and Lynn 1976; Taylor et al., 1980) into the ejaculate from blood has been reported. Because of the chemical configuration of oxytocin (octapeptide), it was expected that this hormone could pass into the ejaculate after being injected systemically. If oxytocin injected prior to ejaculation is transferred in significant amounts in the semen, from blood, the uterotonic effect of semen should be increased. It has been suggested that release of oxytocin may be part of the physiology of ejaculation (Schams et. al., 1982). The objective of this research was to determine the uterotonic effect of semen of bulls injected with oxytocin.

Materials and Methods

Four eighteen-month old Angus bulls were used in this study. Blood was taken with the use of indwelling catheters in the jugular vein. Synthetic oxytocin (W.A. Butler Co., Columbus, Ohio) was injected both intravenously (400 IU) and intramuscularly (200 IU) at the same time. Bulls were electroejaculated at 5, 15, 30, and 60-minutes post oxytocin injection. Six to eight ml semen was

collected each time. Blood samples were drawn during electroejaculation at 0-time and 15 and 60-minutes post oxytocin injection. Control bulls were subjected to the same treatment as treated bulls, but saline was injected in place of oxytocin. Zero-time blood was used as a baseline value. Semen and plasma were frozen until the uterotonic effect was tested.

The uterotonic effect of semen and plasma samples was tested by using the rat uterus preparation bioassay (Tallarida and Jacob, 1979; Munsick, 1960; Schild, 1942). Briefly, this assay consisted of priming mature rats with estrogen 24 hrs prior to testing. The animals were then killed by cervical dislocation and the uterine horns were excised and suspended in a tissue bath (Phipps & Bird, Inc.) (60 ml capacity) at a constant temperature (39°C). The bath contained Van Dykes solution without magnesium (Munsick, 1960; vanDyke and Hasting, 1978) which was aerated with 95% 0_2 and 5% CO_2. Motility was recorded by means of tension transducer (Gould-Statham) connected to a polygraph (Gilson). Tension-time index was calculated by integrating the area under the tracing. Area was measured by using a digitizer (Hewelett Packard). Semen and plasma threshold volumes were found. In addition, the uterotonic effect of two times threshold and four times threshold volume was measured.

The threshold volume was the volume of semen or plasma added to the tissue bath to elicit an increment of about 25 percent baseline motility. Two times threshold and four times threshold values were determined by adding two and four times respectively the threshold volume as seen in Figure 2. At the end of the experiment the dry weight of the uterine horn was measured.

Sodium thioglycolate (thioglycolic acid, sodium salt; Sigma Chemical Company, St. Louis, Missouri) was used to break the disulfide bonds in oxytocin

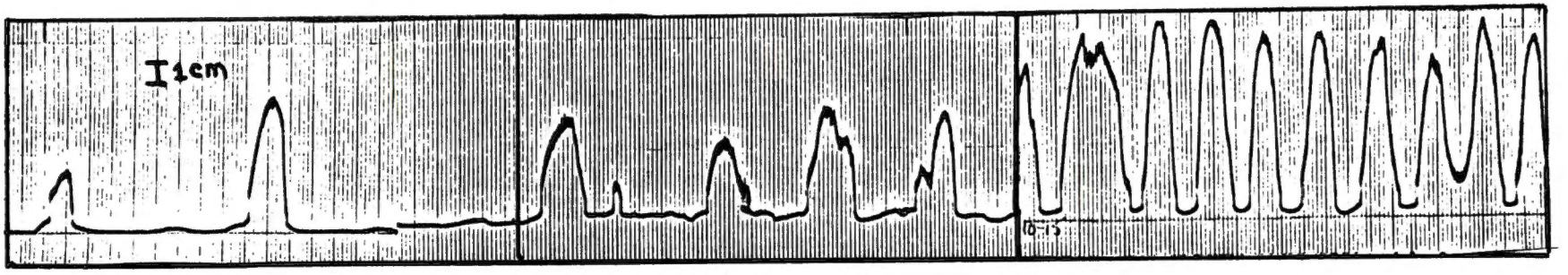


Figure 2. Polygraph Tracings of Rat Uterus Contractions in Response to Oxytocin.

Scale: 1 cm equals 50 mm Hg.

in order to test if oxytocin was responsible for the uterotonic effect of the blood and semen samples. Samples, along with a standard oxytocin solution were incubated 2 hours with thioglycolate (1 mg/ml). The uterotonic activity of each solution was then tested using the rat uterus preparation.

Design and Statistical Analysis

The experimental design was time-response relationship. Separation of the means was accomplished by using Students t-test. Significance is reported at 0.05 level.

Results

The volume of semen and plasma added to tissue bath in order to find the threshold dose is shown in Table 4. No significant difference was found in the volume of semen needed to reach threshold at 5, 15 and 60 minutes as compared to control. Significant difference was found between 30 minutes and the control group.

No significant difference was found in the volume of plasma needed to reach threshold at 15 and 60 minutes as compared to the control group. The uterotonic effect of semen was significantly larger than that of plasma at 60 minutes. However, no other significant differences were found between semen and plasma.

The uterotonic effect of semen and plasma from control and oxytocin treated bulls, when two times threshold and four times threshold volumes were added into the rat uterus preparation is summarized in Table 5. At two times threshold, the uterotonic effect of semen was increased between 47 and 61 percent over the control. However, at the 5 minute sample the uterotonic

TABLE 4

VOLUME (ul) OF SEMEN AND PLASMA ADDED TO THE TISSUE BATH (60 ml) TO FIND THRESHOLD* DOSE IN CONTROL BULLS AND OXYTOCIN INJECTED BULLS

Sample	Minutes After Oxytocin was Injected into Bull				
	Control	5	15	30	60
Semen (µl)	$39 \pm 10.7^{a,x}$	50 ± 14.4^{a}	$31 \pm 6.3^{a,x}$	100 ± 15.8^{b}	$58 \pm 8.3^{a,x}$
	(0)	(27)	(-20)	(155)	(48)
Plasma (µl)	$24 \pm 0.5^{a,x}$	NM	$21 \pm 2.5^{a,x}$	NM	$25 \pm 0.1^{a,y}$
	(0)		(-14)		(3)

*Sample volumes are Mean ± SEM µl.

NM Not Measured.

a,b Values with similar superscripts are not statistically significant ($P > 0.05$) and those with different superscripts are significantly different from controls within each row.

( ) In parenthesis is percent increase (+) or decrease (-) as related to the controls.

x,y Values with similar superscripts are not statistically significant ($P > 0.05$) and those with different superscripts are significantly different when compared with each other in the same column.

TABLE 5

UTEROTONIC* EFFECT OF SEMEN AND PLASMA FROM CONTROL AND OXYTOCIN TREATED BULLS WHEN 2 TIMES THRESHOLD AND 4 TIMES THRESHOLD VOLUMES WERE ADDED INTO THE RAT UTERUS PREPARATION.

SAMPLE		PERCENT CHANGE** ON TENSION - TIME INDEX (AREA UNDER TRACING)							
		Dose: 2 times threshold volume				Dose: 4 times threshold volume			
		Minutes after oxytocin injection				Minutes after oxytocin injection			
	Controls	5	15	30	60	5	15	30	60
SEMEN	0	-42	47	61	58	66	153	-22	5
PLASMA	0	NM	87	NM	116	NM	23	NM	16

*Uterotonic effect expressed as tension-time index, which is a measure of area under the tracing.

**Percentage change is the increase (+) or decrease (-) as related to the controls.

NM Not measured.

effect of semen was below that of controls. With the exception of the 5 minute group, two times threshold values were above the control values in both semen and plasma. The tension-time index of plasma was larger than that of semen at 15 and 60 minute samples.

With the exception of the 30 minute group, four times threshold values were above the control values for both semen and plasma. Contrary to what happened at two times threshold, the tension-time index of semen was larger than that of plasma at 15 minutes at four times threshold. Notice at 30 and 60 minutes there was minimal increase in the tension-time index over the controls. At 30 minutes there was decreased value as compared to controls.

No significant difference was found between the dry weights of the uteri used to determine the uterotonic effect of semen, in the bioassay. This, plus the constant volume of Van Dykes Solution in the bath, standardized the concentration of semen between assays.

Addition of thioglycolate inactivated the uterotonic effect of the oxytocin solution, but did not inactivate the uterotonic activity of either semen or plasma.

Discussion

The uterotonic effect of semen on the rat uterus preparation was not affected by injecting oxytocin into the bull. This is suggestive that none or very little of the injected oxytocin passed into the ejaculate from blood. It is doubtful, then, that any hypothetical release of oxytocin during mating may have been ejaculated. If any oxytocin was released during electroejaculation (Schams et al., 1982) it may only serve the physiology of the male reproductive tract but not that of the female (Voglmayr, 1975). It has been proven that many

exogenous substances (Swanson et al., 1978: Gerber and Lynn 1976; Taylor et al., 1980) and exogenous hormones can be readily ejaculated and passed into the female reproductive tract after being injected or given orally to the male (Eiler and Graves 1977;1981; Graves and Eiler 1979; Sheth et al.,1975; DeAloysio et al., 1974; Shoenfeld et al., 1978; Armstrong et al., 1982; 1982a).

What was striking was the occurance that the uterotonic effect of plasma of bulls injected with oxytocin was not any larger than bulls receiving no oxytocin. The half life of oxytocin is from one to several minutes. Therefore, some uterotonic effect was expected to be present in the blood. In this work, the first blood sample was taken 15 minutes after oxytocin injection. The results indicate that within this period of time the injected oxytocin was totally cleared (Table 4). In general, the uterotonic effect of plasma and semen was very much alike (Table 4). The uterotonic effect of semen was probably not due to oxytocin because, thioglycolate treatment did not inhibit the uterotonic effect of semen or plasma on the rat uterus preparation. The disulfide bonds of the oxytocin molecule are chemically disrupted by thioglycolate which renders oxytocin biologically inactive. When oxytocin was added to control semen, thioglycolate inactivated, the the additive uterotonic effect of the oxytocin. This is further evidence that the uterotonic effect of semen from bulls injected with oxytocin was not due to the presence of oxytocin in the semen.

CHAPTER IV

GENERAL DISCUSSION

Summary

In this research, it was found that oxytocin treatment did not significantly ($P > 0.05$) affect the mean sperm output per group. The lack of significance was probably due in part, to the large individual variation and the small degreees of freedom used in this study. However, the total output of sperm (the summation of individual bulls) per treatment group was increased the most in the 20 IU oxytocin treated group (Table 2). It is speculated that if this positive affect to 20 IU of oxytocin is consistent, it may be of practical value for use in electroejaculation. Further research is needed in this area.

Secondly, the uterontic effect of the ejaculate was not affected by the injection of oxytocin prior to electrojaculation. This is suggestive that if there is release of oxytocin during mating in the male, the passage of oxytocin into the bull ejaculate is doubtful. The role of the released oxytocin, if any, would be to serve the ejaculatory physiology in the male without affecting the motility of the female reproductive tract.

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