Blockage of Sperm Transport Using Intraepididymal Calcium Chloride Injections in Rams

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J Anim Sci 1978. 46:1063-1065.

BLOCKAGE OF SPERM TRANSPORT USING INTRAEPIDIDYMAL CALCIUM CHLORIDE INJECTIONS IN RAMS[1]

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SUMMARY

Ten mature rams were paired on the basis of pre-treatment semen production and assigned randomly to one of two treatments. Treatments were bilateral intraepididymal injections of 3 ml of 50% (w/v) $CaCl_2$ in .9% NaCl (w/v) or 3 ml .9% NaCl. Semen was collected twice weekly for 5 weeks prior to treatment and for 7 weeks following treatment. Mounting times, ejaculate volume and sperm concentration were measured in all rams during the pre- and post-treatment periods. No differences between the groups were present for any characteristic during the pre-treatment period. However, reductions ($P<.01$) in sperm concentration and ejaculate volume were present by 12 days following $CaCl_2$ injection. Mounting time was not affected by $CaCl_2$ treatment. Mean values for the post-treatment period for the $CaCl_2$ *vs* the saline treatments were, respectively: mounting time, 69 and 77 sec; ejaculate volume, .43 and .80 ml and sperm concentration, .15 and 3.96×10^9 sperm/milliliter. While the number of spermatozoa was never less than 10^5 sperm/ejaculate in the $CaCl_2$-treated rams, all ejaculates were characterized by absence of motility and a predominance of fragmented spermatozoa.

(Key Words: Sperm Transport, Chemosurgery, Estrous Detection.)

INTRODUCTION

The impetus for the present study was the report by Koger (1976) who showed that a single injection of $CaCl_2$ solution under the horn buds of dairy calves prevented their development. Preliminary experiments suggested that percutaneous injections of $CaCl_2$ solutions into soft body tissues resulted in their destruction. Such tissue destruction with $CaCl_2$ was explored as a possible non-surgical method of selectively destroying components of the male reproductive system. This report describes a non-surgical method for blocking sperm transport in rams. The objective of this study was to determine the influence of intraepididymal injections of $CaCl_2$ on ejaculate volume, sperm concentration and libido in mature rams.

EXPERIMENTAL PROCEDURE

Semen was collected twice weekly from 10 mature rams of mixed breeding for a period of 5 weeks prior to treatment. Ejaculate volume (ml), sperm concentration and mounting time were determined for each ram at each collection. Sperm concentration was determined by direct hemocytometer counts. Mounting time was the time elapsed from exposure of the ram to a teaser ewe until mounting. All rams were given three-10 min opportunities to mount. At the end of the 5-week pre-treatment period, rams with comparable semen production were paired and assigned randomly to treatments. Five rams received bilateral percutaneous injections directly into the cauda epididymides with 3 ml of 50% (w/v) anhydrous $CaCl_2$ dissolved in sterile saline. This level was selected due to the results of pilot studies in which rams received intraepididymal injections of 1, 2, 3 or 5 ml of 50% $CaCl_2$. A dose of 3 ml was selected because necrosis was extensive and was limited to the epididymides. The 5 ml dose resulted in some necrosis of the ventral scrotum while lower doses did not cause widespread destruction of the epididymis. Injections were accomplished by inserting a 20 gauge 5.1 cm needle directly through the skin of the scrotum into the ventral cauda epididymides (figure 1) and injecting a total of 3 ml of $CaCl_2$. Careful

[1] Scientific Paper No. 4843. College of Agriculture Research Center, Washington State University, Pullman. Project No. 0237.

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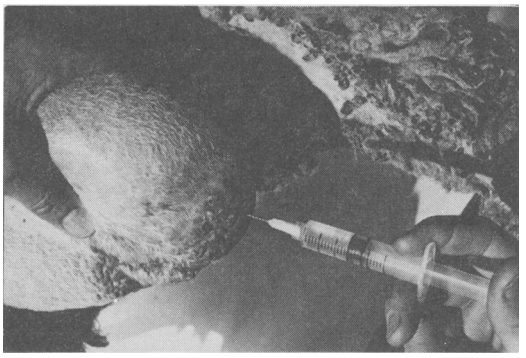

Figure 1. Injection of $CaCl_2$ into the cauda epididymis of a ram. Identification of the cauda epididymis by palpation should be done prior to insertion of the needle. The needle tip was repositioned without removal during the injection to facilitate wider distribution of $CaCl_2$ within the epididymis.

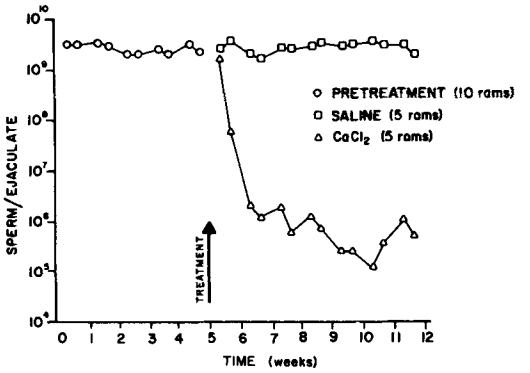

Figure 2. The influence of intraepididymal injections of $CaCl_2$ on ejaculated spermatozoa. Individual points are the means of all collections for each treatment.

palpation of the cauda epididymides was conducted prior to each injection to insure that the solution was deposited into epididymis rather than adjacent tissues. The needle tip was repositioned during the injection to facilitate distribution of the $CaCl_2$ within a large portion of the cauda epididymis. Rams (5) receiving percutaneous injections of 3 ml of sterile saline served as controls. Semen was collected 2 days after treatment and ejaculate volume, sperm concentration and mounting time were determined twice weekly for each ram. Semen was collected twice weekly for a period of 7 weeks following treatment. Since the pre-treatment was used only to assign rams to treatments based on semen production the data for the pre-treatment and post-treatment were analyzed separately by split-plot analysis of variance. The following model was used:

$$Y = T_i + R_{ij} + D_k + TD_{ik} + E_{ijk}$$

where T_i = the i^{th} treatment group
R_{ij} = the j^{th} ram in the i^{th} treatment group
$D = k^{th}$ day.

RESULTS AND DISCUSSION

Ejaculate volume and concentration of spermatozoa were reduced ($P<.01$) for the post-treatment period following injections of $CaCl_2$, when compared to the saline-treated rams (table 1). The reduction in number of sperm per ejaculate was not linear across the post-treatment collection period (figure 2). The number of sperm per ejaculate was reduced ($P<.01$) by 12 days following injection of $CaCl_2$ (figure 2). Saline injections into the

TABLE 1. INFLUENCE OF INTRAEPIDIDYMAL $CaCl_2$ INJECTIONS ON EJACULATE VOLUME, CONCENTRATION OF SPERMATOZOA AND LIBIDO IN RAMS (MEANS FROM 5 RAMS PER TREATMENT)

Item	Ejaculate volume (ml)	Sperm/ml of semen ($\times 10^9$)	Mounting time (sec)[a]
Pre-treatment			
NaCl	.94 ± .05[b]	4.63 ± .25[b]	110.7 ± 20.9[b]
$CaCl_2$	1.06 ± .04[b]	4.30 ± .29[b]	66.6 ± 10.9[b]
Post-treatment			
NaCl	.80 ± .05[b]	3.96 ± .24[b]	76.8 ± 11.6[b]
$CaCl_2$	.42 ± .03[c]	.15 ± .07[c]	69.3 ± 7.8[b]

[a]Time elapsed from exposure to ewe until mounting.

[b,c]Means (± standard error) within the pre- and post-treatment periods with different superscripts were significantly different ($P<.01$) as determined by the ANOVA.

epididymides did not affect ejaculate volume or sperm concentration. Mounting time was not influenced by either treatment. Some rams did not mount at each opportunity. However, this was not a consistent occurrence and did not differ among the two treatment groups.

While the number of spermatozoa was never less than 10^5 sperm per ejaculate in the $CaCl_2$-treated rams, all ejaculates were characterized by total absence of motility and head-tail fragmentation. Rams displayed no sign of pain during or after the injection of $CaCl_2$ or saline. Rams injected with $CaCl_2$ displayed slightly swollen epididymides, however, by approximately 1 week after injection the swelling was not evident.

The use of sclerosing or necrotizing agents for fertility alterations has been previously attempted (Freeman and Coffey, 1973; Freeman, 1975). Freeman and Coffey (1973) tested the influence of intra-vasal injections of either 90% ethanol, 10% silver nitrate, 36% acetic acid, 3.6% formaldehyde, 3% sodium tetradecylsulfate, 5% potassium permanganate or 3.6% formaldehyde in 90% ethanol on fertility of male rats. They found that all compounds except potassium permanganate resulted in complete loss of fertility in treated males as measured by litters produced by females bred to the treated male rats.

The present study suggests that chemosurgical blockage of sperm transport with $CaCl_2$ injections is effective in causing reduced sperm output without depressing libido in rams. This finding indicates that injections of $CaCl_2$ into the epididymides may be an effective nonsurgical procedure for producing infertile males for detection of estrus. Such a technique would significantly reduce expense and inconvenience of surgery. This technique does not, however, eliminate the possibility of intromission and thus does not eliminate the possibility of disease transmission. While post-treatment fertility was not directly evaluated in this experiment, the lack of motility and severe head-tail fragmentation in ejaculates from $CaCl_2$-treated rams suggests that these animals were sterile. Further data are needed in this regard. Rams treated with $CaCl_2$ also had reduced ejaculate volume. Presumably this was due to reduced sperm numbers and reduced contribution to seminal fluid volume by epididymal secretions. The reduced number of spermatozoa was undoubtedly due to blockage of sperm transport through the cauda epididymis.

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