EVALUATION OF EPIDIDYMAL SPERM BY THE CANNULATION TECHNIQUE AND THE EFFECTS OF *IN VIVO* STORAGE IN ANGUS BULLS

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CHAPTER I

INTRODUCTION

One of the important objectives of many researchers in the area of male reproduction is to improve the quality and quantity of semen produced. To do this they are continually trying to learn more about the changes the sperm cell undergoes while in the male reproductive tract. Extensive research has been conducted on the role of the mammalian testis but the contributions made by the epididymis are less well understood.

Recent research has shown that the epididymis is involved in the maturation and aging as well as the transport and storage of spermatozoa. This means that in the epididymis the sperm cells go through a process of changing from immature immotile cells to mature cells capable of fertilization. Then if not removed from the system, the sperm cells continue to age and eventually die and disintegrate.

Several methods have been used to obtain epididymal sperm for study, with the method of cannulation of the vas deferens showing the most promise. This cannulation method has been used to obtain sperm for biochemical and metabolism studies but no reports have been found on its use in studies concerned with the morphology of epididymal sperm.

This project was undertaken to (1) evaluate the morphology and output of epididymal sperm collected via a unilateral indwelling cannula in the vas deferens and to (2) determine the effect on the morphology and output of <u>in vivo</u> storage of epididymal sperm in Angus bulls. This study

involved a comparison of epididymal and ejaculated sperm characteristics as well as the effects of cannulation on epididymal sperm and on the histology of the testis and epididymides.

CHAPTER II

LITERATURE REVIEW

Little information is available on epididymal sperm in contrast to the vast amount of literature on ejaculate sperm. Although the epididymis is quite accessible in species where the testes are located outside the body in a scrotum, few researchers have conducted extensive investigations on epididymal sperm due to the lack of techniques for obtaining a continuous supply from the live animal. Only within the last ten years have such techniques been developed so that epididymal sperm can be routinely obtained and studied. The advent of the electron microscope and new staining techniques for sperm cells have aided the researchers in studying the progressive changes in the fine structure of the sperm cell. This review will cover only selected areas of epididymal sperm research which are pertinent to the research study undertaken.

Techniques for Collecting Epididymal Sperm

Three techniques have been used to obtain sperm from the epididymis and all have definite advantages and disadvantages. The oldest and most often used method of obtaining epididymal sperm is that of slaughtering or castration of the animal and removing the epididymal tissue. The second method is the fistulation of the vas deferens by a surgical technique to obtain epididymal sperm without contamination by the accessary gland secretions. The third technique is the cannulation of the vas

deferens.

Slaughter or Castration

Numerous papers reporting on epididymal function in the 1920's used the slaughter method. Young (1931) used guinea pigs to study the functions of the epididymis after killing the animals. Crabo and Gustafsson (1964) analyzed epididymal plasma and sperm concentration at six levels of the duct in bulls after the epididymides were removed at slaughter. Other studies using this method have been reported, including those by Crabo (1965) on bulls and boars, and Gustafsson (1966) and Lavon et al. (1971) on bulls. The big advantage of the slaughter method is that sperm can be obtained from several different areas of the epididymis at the same time. Also, it is a relatively easy and inexpensive method particularly if the animals are to be slaughtered anyway. The main disadvantages are that consecutive samples cannot be taken from the same animal and the sperm cells may be damaged during the interval between slaughter and cell recovery due to lack of blood circulation. Also, many slaughterhouse animals are sub-fertile so sperm obtained without the animal's previous history is always questionable. With this method no direct comparison between epididymal and ejaculated sperm can be made.

Fistulation of Vas Deferens

White, Larsen and Wales (1959) reported the first fistulation procedure for the vas deferens using rams. The fistula was about half an inch in diameter and located on the anterior neck of the scrotum. This fistula was established in only one vas deferens so sperm could be collected separately from each testis during ejaculation. Sixteen mature

rams were used with 13 of them producing semen out of the fistula 1 week after the operation. The fistulas remained patent for periods of from 1 month to 2 years with an average of 5 months.

Bennett and Rowson (1963) reported the establishment of a fistula in the vas deferens of a 2-year old bull. This fistula was located in the posterior dorsal region of the scrotum and a polyethylene tubing was left in the opening for 2 weeks to prevent closure. Complete patency was not obtained until 3 months after surgery but the fistula remained patent for 19 months and appeared to be permanent. Tadmor and Schindler (1966) used a combination of the previous techniques to establish fistulas in the vas deferens of rams. These fistulas were located in the posterior neck of the scrotum and a polyethylene tubing was left in them for as long as possible. Nineteen operations were completed on 12 rams (seven bilateral) with 12 operations successful. These fistulas stayed patent for a length of 10 to 145 days. Other fistulations in rams have been reported by Ewy and Bielanski (1962), Tischner (1967), and Zeltobrjuh, Loginova and Manuilov (1966). The most recent fistulation of the vas deferens was reported by Gilmore, Hooker and Chang (1971) in rats. They attached both vas deferens to an isolated end of the caecum to form a pouch which could be exteriorized. This fistula was established in 38 rats with limited success and stayed patent for various lengths up to 63 days.

The main advantage of unilateral fistulation is that repeated collections of epididymal sperm can be obtained from one individual and compared to ejaculated cells of the same individual. However, this technique has several drawbacks such as: the numbers of unsuccessful operations and the variability in length of patency of successful fistula-

tions; the need for a post-operative recovery period; the surgery may cause adverse effects on the sperm cells; the sperm leaving the fistula during collection is in contact with the skin and may become contaminated; and the fistula also has the tendency of being dirty because of the oozing of semen from it and thus, is an ideal site for bacteria growth and infection.

Cannulation of Vas Deferens

Cannulation is similar to fistulation with the exception that a tubing or cannula is surgically inserted and sutured in the vas with only the end of the cannula exteriorized. This provides a clean cannulation site and uncontaminated sperm can be collected into an attached vial for continuous quantitation. Amann, Hokanson and Almquist (1963) described a cannulation technique for the vas deferens in bulls. The cannula system involved two concentric polyethylene tubings with the inner one inserted in the vas and both exteriorized into the collection vial. The supporting apparatus and vial was attached to the posterior neck of the scrotum for continuous collection. This technique was used on two bulls and patency was achieved at first ejaculation. One bull stayed patent for 21 days while the other was still patent when slaughtered at 69 days. Amann (personal communications) indicated that a modification of this technique was being used regularly and successfully in his research. Barker and Amann (1971) reported bilateral cannulation of the vas deferens of two bulls with the cannulae staying patent from 3 to 48 days. The cannulation technique was used unilaterally by Wierzbowski and Wierzchos (1969) in three boars and the collection apparatus was located anterior to the scrotum. These cannulae remained patent for 24

to 93 days. Johnson, Pursel, and Kraeling (1971) cannulated the vas deferens of two boars with the cannula staying patent for 13 and 20 days.

Voglmayr et al. (1967) reported on a procedure for the cannulation of the rete testis of rams to collect sperm before entering the epididymis. Nine rams were used and patency length varied from 10 to 120 days. Voglmayr, Larsen and White (1970) reported on a similar procedure for cannulation of the rete testis in bulls. Three dairy bulls were cannulated and remained patent for up to 10 days.

The big advantage of the cannulation technique for the vas deferens is that epididymal sperm can be collected continuously from a living animal over an extended period. This method can be used for sperm and fluid quantitation, evaluation, and comparison to ejaculated semen. Its advantage over fistulation is that the vial allows for continuous collection which is not contaminated by contact with skin. The cannulation of the rete testis is of great importance because testicular sperm can be collected continuously without going through the epididymis. This permits actual sperm production to be determined since there is the chance that some sperm may be resorbed while passing through the epididymis. Also this technique should lend itself to investigations of the functions of the epididymis. The disadvantages of both cannulation techniques are the effects of surgery and post-surgical swelling on the testis, the required recovery period, and the variability of surgical success and duration of patency.

Effects of Surgery and Heat on Testis and Sperm

Researchers are often reluctant to report the negative side effects

of new techniques or procedures. Many of the research reports do not indicate that there were any adverse effects of surgery or post-surgical swelling or inflammation. The experience in the present study with the cannulation technique indicates that post-operative swelling and inflammation does occur, which causes an increase in temperature of the surgical site, even though all precautions were taken to conduct sterile surgery. Thus, one is led to assume that the surgery in earlier reports likewise caused post-operative swelling but was not always reported. Therefore, it appears important that the aspect of surgical effects and heat on the testis be briefly reviewed.

Cannulation and Fistulation Surgery

Two reports on the fistulation technique and three reports on the cannulations indicated some swelling and inflammation post-surgery. White et al. (1959) reported the application of antibiotic and cortical steroid ointment locally to reduce inflammation around the fistula in rams. They also indicated that inflammation was sufficient to prevent secretion from the fistula during the first 4 to 6 days. Bennett and Rowson (1963) noted that initially the fistula in the bull apparently closed and complete patency was not obtained until 3 months later which would suggest post-surgical swelling while the wound was healing. In the cannulations, Tadmor and Schindler (1966) indicated that antibiotics were applied locally and an initial swelling developed and remained for 10 to 14 days post-surgery in rams. Voglmayr et al. (1967) mentioned an unaccountable fluctuation in output from cannulae in the rete testis of rams during the first 1 to 3 days after operation. Barker and Amann (1971) reported that inflammation from surgery was not excessive. How-

ever, one bull had inflammation of the scrotum and some swelling persisted. Amann (personal communication, 1971) indicated that some post-operative swelling had been experienced but most had been overcome by using a better sterilization method for the cannulae. Several workers mentioned a post-operative recovery period of from 2 to 3 weeks before data was compiled. Therefore, from these reports it would appear that local swelling and inflammation is a problem associated with these surgical techniques.

Since these surgical techniques were performed in the neck of the scrotum and on the vas deferens in the spermatic cord, the area of swelling and inflammation did not extend directly into the testis. However, in the present study when swelling occurred it would usually involve the spermatic cord and most of the cannulated side of the scrotum. These conditions would hinder the functioning of the thermoregulatory mechanism of the scrotum and the pampiniform plexus so that the testis would not be kept at the proper temperature (about $7^{o}C$ below that of rectal temperature). Thus, the heat associated with these post-surgical conditions which could be compared to local heating or insulating of the scrotum, would still have a direct effect on the testis.

Scrotal Insulation and Local Heating

Much research had been conducted on the effects of heat on the testes of rams and has shown that spermatogenesis is impaired. Van Demark and Free (1969), in a review, summarized the effects of heat on the ram testis. Scrotal insulation caused an increase in the percentage of abnormal sperm, mostly tailless sperm, and a decrease in sperm output. In the testis the spermatocytes and spermatids stages were the most sen-

sitive to increased temperatures. The spermatozoa were less sensitive and appeared to be damaged during late development or in the caput epididymis. The causes of cell damage may be due to lack of adequate oxygen and substrate deficiency.

Some of the most recent research supports the previous findings. Sand and Dutt (1971) found that heat stress caused the blood flow in the pampiniform plexus artery to double after 24 hours and then decline to one half of normal by 5 to 7 days after stress. This decreased blood flow may cause a deficiency of oxygen and nutrients to the testes. Setchell, Voglmayr and Hinks (1971) used the cannulation of the rete testis technique to monitor testicular sperm fluid and concentration after heating the testis to 40.5°C for 3 hours. They allowed a surgical recovery period to minimize the chance that post-operative effects would be confounded with heat effects. Their data showed no change in fluid volume after heating but a profound fall in sperm concentration from 100 x 10^6 to as few as 1 x 10^3 sperm per ml about 22 days after heating. They suggested that heat had affected the pachytene primary spermatocyte stage. Voglmayr, Setchell, and White (1971) reported that the metabolic activity of testicular sperm was depressed and remained low for several weeks after heat stress. A histological study was reported by Samisoni and Blackshaw (1971) on ram testes heated to 40.5°C for 120 to 150 minutes. They found that the pachytene cells were heat sensitive and the cell membranes allowed for an increase in uptake of dye which would indicate the beginning of cell death. These cells also showed formations of autophagic vacuoles and an increase in lysosomal enzyme activity.

Less research on the effect of heat stress on bulls has been reported but the general consensus is that the reduced semen quality and

poorer conception rates during the summer is due to the effects of heat. Early studies on scrotal insulation reported effects similar to those on rams, indicating effects from increased percentages of abnormal sperm to the extreme of complete sterility following insulations of up to 16 days. Austin, Hupp, and Murphree (1961) conducted a scrotal insulation study on 12 Hereford bulls, 2 to 6 years old. The bulls were divided into 3 groups--controls (no insulation), 24-hour insulation, and 72-hour insulation. The mean scrotal skin temperature of the insulated groups increased approximately $3^{o}F$. In the insulation groups the number of live sperm and normal sperm decreased to 65 percent and 60 percent of the controls, respectively, in the second and third weeks post treatment. Both returned to control levels by the sixth week after treatment. Sperm concentration decreased significantly during the fourth through seventh weeks in the 72-hour group with the 24-hour group showing the same trend. The main abnormality noted was tailless heads with a small increase in coiled tails. When the scrotums of two Jersey bulls were heated to $38.4^{o}C$ for 13 days, the percentages of motile and live sperm decreased to zero by the second week and did not recover completely until 18 weeks post treatment (Gerona and Sikes, 1970). The principal abnormality noted was free heads during the first week.

To study the internal scrotal temperature of four dairy bulls, Graves, Ferguson and Miller (1970) implanted a thermistor into the stratum subdarticum of the scrotum and used radio telemetry to measure temperature changes under normal physiological conditions. A small incision was used to implant the thermistors and in every case the scrotal temperature increased during the next 24 hours presumably due to the surgery. In one bull cited, the scrotal temperature increased to the rectal

temperature level in 5 hours post-surgery. From these results it would appear that even the smallest degree of surgery on the scrotum will cause a rise in temperature of the local area. One can only speculate on how much increase in temperature would be caused by a major surgical technique performed in the area of the spermatic cord.

In summary, it would appear that most surgical techniques used to obtain epididymal sperm cause some initial swelling and inflammation of the local area and probably an increase in scrotal temperature, thereby causing a heat effect on the testis. These heat effects can be observed by an increase in abnormal sperm, a degeneration of certain germinal cells, and a decrease in sperm output.

Comparison of Epididymal and Ejaculated Sperm

Numerous studies have been reported on the characteristics of both epididymal sperm and ejaculated sperm; however, comparisons are difficult because the studies were done separately on each type (Salisbury and Van Demark, 1961). With the development of new techniques for obtaining repeated collections of epididymal sperm from the live animal, a direct comparison of the characteristics and output of epididymal and ejaculated sperm can now be made. One must remember that these results may be confounded with the effects of surgery; however, they may be still more accurate than results from the use of the slaughter technique. For purposes of this discussion, epididymal sperm will refer to those collected from the cauda or tail of the epididymis and ejaculated sperm as those which pass out through the penis.

Sperm Characteristics

In a review paper on epididymal sperm obtained by the slaughter of bulls and boars, Crabo (1965) indicated that sperm in the cauda were quiescent but that motility could be stimulated by dilution or oxygenation. Igboeli and Foote (1968) reported 41 percent motility of diluted cauda sperm from four bulls after slaughter. In contrast, White et al. (1959), using the fistulation technique in rams, reported good motility of epididymal sperm even when precautions were taken to exclude all air. They suggested that in the ram oxygen tension was sufficient in the epididymis to support motility. They also showed that the percentage of live sperm was approximately the same for the epididymal and ejaculated cells. Bennett and Rowson (1963) found the epididymal sperm collected via a fistula in a bull to be slow in progressive motility but they became much more active with an increase in temperature and nutrients. No comparison was made with ejaculated sperm, but the percentage of live cells was very high in the epididymal sperm. Zeltobrjuh et al. (1966) found the motility and survival lower for epididymal than for ejaculated sperm of rams. When the epididymal sperm obtained via a fistula was mixed with seminal plasma, as it is during ejaculation, the motility increased. Contrary to this, Bennett and Dott (1966) reported a decrease in motility in epididymal sperm obtained via a fistula in a bull when mixed with seminal plasma. This may have been due to the flow dialysis apparatus used to measure motility and the handling procedure of the sperm. They also indicated that seminal plasma did not change the percentage of cells responding to a differential live-dead stain.

Recently, interest has been generated on the effects of the vesicular gland secretions on the sperm cell and has stimulated the use of the

vesiculectomy technique. Faulkner, Hopwood and Wiltbank (1968) compared semen characteristics before and after vesiculectomies on bulls and found that motility and percent of live sperm decreased. Alexander et al. (1971) reported similar results on four Holstein bulls after vesiculectomies. Their values showed a decrease in motility from 57.3 to 41.4 percent ($P < .05$) and the percent live sperm decreased from 69.3 to 44.8 percent. The percent of abnormal sperm changed only slightly from 18.8 to 17.6 percent.

Sperm Morphology

The morphology of sperm from the cauda epididymis should be similar to ejaculated sperm because the cauda is the main site of sperm storage and is the source of the sperm included in an ejaculate (Bialy and Smith 1958). However, this is true only if the seminal plasma has no influence on the morphology of the cells. Faulkner et al. (1968) noted an increase in tail abnormalities in the sperm of bulls that had vesiculectomies, but they still had acceptable fertility. Bennett and Dott (1966) indicated a trend for the percentage of deformed heads, tailless heads, and coiled tails to increase when seminal plasma was added to epididymal sperm of bulls. These results are contrary to common belief.

Epididymal sperm collected from a vas deferens fistula in a bull showed many distal cytoplasmic droplets on their midpieces (Bennett and Rowson, 1963). White et al. (1959) also reported many droplets (51%) on epididymal sperm from a fistula in rams in comparison to only 4 percent on the ejaculated sperm. In support of these studies, an extensive study was conducted by Amann and Almquist (1962b) on the morphology of sperm from different areas of the epididymis of 47 normal dairy bulls

after slaughter. The authors reported the average cauda epididymal sperm to have the following morphology: 2.4% abnormal heads, 0.8% abnormal midpieces, 2.1% tailless sperm, 8.4% bent or broken tails, 0.4% coiled tails, 2.1% proximal droplets, and 67.9% distal droplets. There were an average of 72.5 percent live cells in 40 cauda epididymides and the sperm motility was 32.1 percent after dilution in normal saline. Another part of this study, involving 15 bulls given an exhaustion trial and then slaughtered, showed that the percent abnormal sperm in the cauda epididymides at slaughter was similar to that of the ejaculates collected during the depletions trials. Less than 3 percent of the ejaculated sperm had cytoplasmic droplets.

The morphology of the sperm cell has been studied for many years with the light microscope, but not until the development of the electron microscope have the fine ultrastructures of the sperm cell been defined and investigated (Salisbury and Van Demark, 1961; Saacke and Almquist, 1963). One of these fine structures that has had intense study in recent years is the acrosome cap. This structure consists of a double membrane system covering the anterior 60 percent of the sperm head. It has been shown that the acrosome is involved in the fertilization process and certain alterations result in either impaired fertility or sterility (Hancock, 1952; Saacke, Amann, and Marshall, 1968). Marshall and Saacke (1967) reported that a normal motile sperm has a uniform, tightly-fitting acrosomal cap. Alterations of this normal condition (roughened, raised, fragmenting, complete loss, etc.) may be caused by nutrition, environmental temperature, ejaculation frequency, and the normal aging process (Awa, 1970 and Saacke, 1970). Because of the acrosomes labile nature, Awa (1970) and Wells and Awa (1970) indicated that the examina-

tion of the acrosonal cap should be included as a part of the routine semen evaluation.

Sperm Production

Estimates of sperm production of animals has always been used to evaluate the spermatogenic function of the testis. It is of particular interest to A. I. organizations which can turn production into an economic advantage. Amann (1969), in a review, defined daily sperm production as the total number of sperm produced per day by the two testes. This production has been impossible to measure directly in living animals because of the necessity of quantitative collection of sperm out of the testes. Therefore, many techniques including the cannulation technique, have been developed to obtain an estimate of sperm production.

The oldest method used extensively to estimate sperm production has been the daily sperm output which is the total number of ejaculated sperm collected over a period of time expressed on a per day basis. Amann and Almquist (1961) indicated that only if an animal is ejaculated at a sufficiently high frequency can sperm output be used to estimate total sperm production. They suggested that daily collections be made for 7 to 10 days, to allow for a preliminary stabilization, before recording daily sperm output data by ejaculation. Two other methods used to estimate sperm production are quantitative testicular histology and testicular homogenates. These methods are based on the sampling of testis tissue and the enumerating of a certain germinal cell stage to use in a formula for the calculation of daily sperm production. For large investigations the homogenates are preferred due to less time required and the use of larger samples. The cells most often evaluated are either

spermatids-stage I, round spermatids, or primary spermatocytes.

The newest methods used for estimating sperm production are the cannulation or fistulation of the vas deferens and the cannulation of the rete testis. These methods appear to be the most accurate, provided that cannulation and surgery do not alter the rate of sperm production or affect the physiology of the testis. Estimates obtained from the cannulation of the rete testis would be better than those from the vas deferens because the passage through the epididymis would be bypassed. Estimates of daily sperm production vary among the techniques used, the breed and age of the animals, the environmental conditions involved and others. Table I gives a summary of some estimates of daily sperm production in cattle. It should be noted that the estimates of daily sperm production made from ejaculated daily outputs are considerably lower (50% or more) than the estimates from testicular homogenates or histology. This difference is thought to be due to the phenomenon of sperm resorption in the epididymis (Amann and Almquist, 1961; Lambiase and Amann, 1968). Table II gives a summary of the daily sperm output from the cannulation and fistulation studies of the vas deferens and rete testis. There is much variation in daily sperm output among the studies. One may question the validity of the results of Ewy and Bielanski (1962) in rams and wonder if an error of 10^3 was made in reporting the data. It appears that the daily sperm output via one vas deferens in rams is about 1-2 x 10^9 and in bulls is about 2-3 x 10^9. These values for bulls are approximately half of the output obtained during ejaculation which would be expected since sperm comes from both vas deferens during ejaculation. The amount of testicular sperm obtained from one rete testis in bulls should be approximately half of the estimates of daily sperm pro-

TABLE I

ESTIMATES OF DAILY SPERM PRODUCTION IN CATTLE

Specie	No. of Animals	Age (yrs)	Method of Estimation[b]	Daily Sperm Production (x 10^9)	References
Bos indicus	4	3.5	Eja-1X/MWF	2.0	Sayed and Oloufa (1957)
Beef					
Angus and Hereford	11	5	Eja-2X/MWF	3.9	Almquist (1969)[a]
Ang. and Here.	7	1.5	Eja-1X/wk	0.614	Almquist and Cunningham (1967)
Ang. and Here.	5	1.5	Eja-3X/wk	1.57	Almquist and Cunningham (1967)
Ang. and Here.	5	1.5	Eja-6X/wk	1.71	Almquist and Cunningham (1967)
Hereford	8	2,5	Eja-9X/wk	3.53	Hupp et al. (1962)
Hereford	4	4.0	Eja-4X/wk	1.04	Austin et al. (1960)
Shorthorn	7	1.5	Eja-3.5X/wk	1.35	Swierstra (1966)
Hereford	5	1.0	Test. Hom.	7.71	Hafs et al. (1968)[a]
Shorthorn	7	1.5	Test. Hist.	5.3	Swierstra (1966)
Dairy					
Holstein	8	3.0	Eja-2X/MWF	5.15	Amann and Almquist (1962a)
Holstein	30	3-6	Eja-2X/MTh	5.82	Hahn et al. (1969)[a]
Holstein	8	3.0	Test. Hom.	9.31	Amann and Almquist (1962a)

TABLE I (Continued)

Specie	No. of Animals	Age (yrs)	Method of Estimation[b]	Daily Sperm Production (x 10^9)	References
Holstein	5	1.0	Test. Hom.	6.54	Macmillan and Hafs (1968)
Holstein	8	3.0	Test. Hist.	11.88	Amann and Almquist (1962)
Hol. and Ayr.	3	1-3	Cannula in rete testis	7.32	Voglmayr et al. (1970)

[a]References in Amann (1969).

[b]Eja - Ejaculation, Test. Hom. - Testicular Homogenate, Hist. - Histology.

TABLE II

DAILY SPERM OUTPUT FROM ONE VAS DEFERENS OF CANNULATION AND FISTULATION STUDIES

Specie	Method of Collection	No. of Animals	Vol. (ml)	Conc./ml (x 10^9)	Daily Sperm Output (x 10^9)	References
Rams	fistula	13	.17	6.696	1.138[a]	White et al. (1959)
Rams	fistula	2	-----	------	2.0	Tischner (1967)
Rams	fistula	5	-----	------	.0016-.0026	Ewy and Bielanski (1962)
Rams	fistula	12	.1-.3	5-10	.5-3.0[a]	Tadmor and Schindler (1966)
Rams	cannula in rete testis	9	40	0.137[a]	5.5[a]	Voglmayr et al. (1967)
Bulls	fistula	1	.3-.6	7.632	2.29-4.59[a]	Bennett and Rowson (1963)
Bulls	cannula	2	-----	------	2.8	Amann et al. (1963)
Bulls	cannula	--	.4-.8	------	1.5-3.0	Amann (Personal Communication)
Bulls	cannula in rete testis	3	40	0.0916	3.66[a]	Voglmayr et al. (1970)
Boars	cannula	3	-----	------	3.18-6.06	Wierzbowski and Wierzchos (1969)
Boars	cannula	2	-----	------	12.5	Johnson et al. (1971)

[a]Values calculated from data.

duction from testicular homogenates and histology, but the estimate of Voglmayr *et al.* (1970) is lower. This may be due to the effects of surgery or cannulation on the rate of sperm production. Also note the small volume of fluid that flows out of a vas deferens (.1-.8 ml) compared to that from a rete testis (40 ml). This difference is thought to be due to the resorption of fluid in the caput epididymis (Crabo, 1965).

Sperm Fertility

The fertilizing capacity of cauda epididymal sperm of the bull has not been extensively studied, but Igboeli and Foote (1968) reported that the non return rates for cauda and ejaculated sperm were very similar (69% *vs.* 75%). Zeltobrjuh *et al.* (1966) indicated that three out of four ewes inseminated with epididymal sperm became pregnant. Reports on laboratory animals indicate that cauda sperm are much more fertile than sperm obtained from higher areas of the epididymis. This supports the theory of maturation changes in the sperm during their passage through the epididymis.

Effects of *In Vivo* Storage of Epididymal Sperm

The epididymis should be thought of as an organ for sperm development in which these cells are constantly changing; ripening until an optimal functional capacity is attained and then aging if their residence is prolonged (Young, 1931). This concept is still valid today and research has proven that sperm cells do go through a process of maturation and will age if retained in the epididymis too long.

Sperm Maturation and Transport

Before one can study the effects of storage or the aging of sperm in the epididymis, the normal maturation changes and transport times must be understood. The sperm cells undergo many changes in the epididymis such as: the cytoplasmic droplet shifts from the neck to the distal end of midpiece, the acrosome becomes tightly bound to the sperm head, the capacity of motility and fertility is acquired, plus many biochemical changes not well understood (Crabo, 1965; Noyes, 1968, Barker and Amann, 1971). The isotope labeling technique has permitted the determination of the transport time through the epididymis. The sperm enter the epididymis the next day after they are shed in the tubules and traverse the epididymis of the bull in 8 to 11 days (Noyes, 1968). Koefoed-Johnson (1960) reported that this transport time can be shortened by increasing the frequency of ejaculation.

Since, under normal conditions, only half of the sperm that enter the top of the epididymis emerge at the tail, there must be resorption, disintegration or liquefaction of sperm by the epididymis (Noyes, 1968; Amann and Almquist, 1961; Amann, 1969). During the normal process, sperm may also be lost from the epididymis by masturbation, urination, and phagocytosis. Another difficulty in studying the aging of sperm is the theory that waves of sperm mix during their passage through the epididymis thereby causing a mixture of different ages in the different areas of the duct. Since about 70 percent of the sperm in the male tract is stored in the cauda epididymis, researchers have zeroed in on this site to study sperm aging. Two methods used to study sperm aging are frequency of ejaculation and the ligation and isolation of certain areas of the epididymis.

Storage by Frequency of Ejaculation

By varying the frequency of ejaculation, one can indirectly simulate different storage periods of sperm in the cauda. However, this method of storage is hindered by all the mechanisms that remove sperm from the epididymis (resorption, masturbation, and urination) so actual storage is not accomplished. Many studies have been reported on the effects of ejaculation frequency on sperm characteristics and output. Hafs, Hoyt and Bratton (1959) used 10 dairy bulls to compare the semen characteristics of bulls ejaculated once a week to those ejaculated daily. They found that daily collection caused a significant increase in average percent motility (63 vs. 69%), but no significant effects on percentage of live or abnormal sperm or on conception rates. Of course, the sperm output per week was considerably more for the bulls collected daily than weekly. Several other reports indicated little difference in sperm characteristics but significant increases in weekly output of bulls collected at high vs. low frequencies (Dukelow, Frederick and Graham, 1960; Amann and Almquist, 1961; Almquist and Cunningham, 1967). Table II showed this increase in output with the increase in collection frequency.

Awa (1970) compared weekly collections to 4 times per week collections on six bulls and found no significant differences in average percent of motility, or of live or abnormal sperm. However, he did report significant decreases in percent capless and abnormal acrosomes of sperm from bulls collected four times per week. He indicated that sexual rest caused a deterioration of the acrosomal state and frequent collections improved the acrosome quality considerably. Amann and Almquist (1962b) compared the sperm characteristics in the cauda epididymides of bulls at

sexual rest (SR), ejaculated 6-7 times weekly and depleted (SR-D) before slaughter. They found a significant increase in tailless heads from the SR-D over the 6-7 times. The SR had a non-significant increase in bent and broken tails and total abnormals over the other two groups. They suggested that if sperm degeneration can be detected morphologically, it would be characterized by bent or broken tails and then tailless heads and eosinophilic sperm.

Storage by Ligation or Vasectomy

The second method of studying sperm aging *in vivo* is by the ligation or isolation of certain areas of the epididymis. This method has the advantages of preventing the loss of sperm by masturbation or urination and may prevent the entrance of sperm from the testis. But the mechanism of resorption is still involved and the effects of surgery are again a problem. Amann (1962) concluded from a detailed study on 14 dairy bulls that the testis histology and sperm production are not affected by ejaculation frequency, rate of sperm removal by collection, or closure of the excurrent duct by vasectomy. This means that even though the sperm is blocked from passing out of the epididymis the testis is continually producing and sending sperm into the epididymis. Almquist and Amann (1961) concluded that sperm production and the storage of sperm in the duct system are independent. Amann and Almquist (1962a) reported on a study of extra-gonadal sperm reserves in nine bulls 23 weeks after unilateral vasectomy. They found that the rate of sperm resorption in the epididymis on the intact sides from bulls collected 8 times weekly was 57 percent compared to 96 percent on the vasectomized sides. This indicated that the rate of resorption was dependent upon the number of

sperm present in the cauda. They also concluded that the cauda was the major site of sperm resorption.

The effects of vasectomies or ligations of the epididymis on the morphology of the stored sperm are limited in the bull. Amann and Almquist (1962a) conducted unilateral vasectomies on nine bulls and found no significant effects on sperm morphology in the cauda after 23 weeks. This suggested to them that destruction of sperm is very rapid in the epididymis. Most bulls had a spermatocoele in the vas deferens with a high percent of abnormal (mostly tailless heads), low percent motility and low percent live. They also isolated the cauda unilaterally in one bull and found an increase (23 to 62%) in bent and broken tails over the intact side at 26 days storage and a decrease (77 to 57%) in live cells. In another bull they found an increase (2 to 76%) in tailless heads over intact side at 70 days storage and a decrease (74 to 0%) in live cells. This indicated to them that tail abnormalities were the first indications of sperm degeneration.

Barker and Amann (1971) reported an immunofluorescent analysis on epididymal sperm and tissues after vasectomies and epididymal ligations and found that the stereocilia and apical cytoplasm in the caput epididymis absorbed sperm-specific proteins. They also noted the effects of storage in spermatocoeles formed by ligations in the numerous tailless heads and capless acrosomes. These data supported the theory of Saacke and Marshall (1968) and Saacke (1970) on the progressive changes in the acrosome during aging. This aging process has been outlined as follows: first the cell membrane increases in permeability, followed by the swelling of the anterior part of the acrosome and the loss of the cell membrane; finally the outer membrane of the acrosome roughens and frag-

ments with the loss of the internal stainable material, thus, leaving the inner acrosome membrane attached to the sperm head with the formation of the equatorial segment.

Studies on the effects of epididymal ligation and storage of sperm in vivo have also been reported on rams and rabbits. Salamon (1967) bilaterally ligated the corpus-cauda epididymal junctions in eight rams and ejaculated them every third day for 34 days. He found a decrease in percent motility from about 80 percent on the second day to zero at 19 to 28 days post-ligation. During the period of 2 to 34 days post-ligation, the percent live decreased (75.4 to 48.9%) and the percent total abnormals increased (8.5 to 35.5%) with the tailless heads being the most prevalent abnormality. These results indicate a degenerative process. He also noted a very rapid disappearance of sperm from the areas anterior to the ligations suggesting resorption.

Most studies on sperm aging and its effects on fertility have been conducted on rabbits (Glover, 1962; Gaddum and Glover, 1965; Tesh and Glover, 1966; Bedford, 1967; Paufler and Foote, 1968; Tesh and Glover, 1969). In general, they reported an increase in coiled tails and tailless heads due to aging of sperm in the epididymis. The effects of aged sperm on fertility were an increase in pre-implantation losses, then an increased post-implantation mortality and finally fertility was lost entirely with still older sperm.

Storage by Cannulation and Clamping

Another possible approach to study the in vivo storage of epididymal sperm would be by cannulating the vas deferens and then clamping the cannula shut to cause storage in the epididymis. With this technique

the sperm can be evaluated daily for a base period before storage, then following a storage period, daily collections can be again evaluated and compared to pre-storage values to determine the effects of storage. This technique has the advantages of preventing sperm loss by masturbation or urination, allowing a comparison of daily samples before and after storage, and the results are not confounded with the effects of ligation of the epididymis just prior to storage. The disadvantages are that sperm can still enter the epididymis and mix with the aging cells and resorption can still remove aged sperm (which is a phenomenon that has not been prevented). This method has not been reported in the literature but shows promise as a new procedure. Therefore, it was used in the following study to determine the effects of *in vivo* storage of epididymal sperm in Angus bulls.

CHAPTER III

MATERIALS AND METHODS

Animals and Management

Six pairs of half-sib Angus bulls 2 to 3-years of age were used as the experimental animals in this study. These 12 bulls were born in the spring of 1969 in lines 7, 8, 9 and 10 of the Beef Cattle Improvement Study (Project 1256) at the Fort Reno Livestock Research Station. They ran with their dams under normal range conditions until being weaned at about 7 months of age and placed immediately on a 140 day feedlot test. At the completion of this test they were placed on pasture for about 8 months, and in late 1970 were brought to Stillwater and held in drylot until used. The bulls were fed a daily feed of approximately 10 pounds of native prairie hay and 10 pounds of a ground mixed ration, the composition of which is given in Table III, during both the holding and the experimental periods. The bulls were never exposed to cows either prior to or during the experimental period and were maintained as nearly as possible at sexual rest during the holding period.

A description of the 12 bulls used in this study is given in Table IV. They were used in half-sib pairs with each pair composing a trial. The six trials, each of about 60 days duration, ran consecutively. The first trial began in January 1971 and the last trial terminated in March 1972. The bulls in the first trial were 2-year-olds weighing 980 pounds and the bulls in the last trial were 3-year-olds weighing 1230 pounds.

TABLE III

COMPOSITION OF RATION FED BULLS

Ingredient	% of Ration
Steam rolled barley	68.5
Cottonseed hulls	15.0
Soybean meal (44% protein)	5.0
Molasses	5.0
Dehydrated alfalfa (17% protein)	5.0
Salt (trace mineralized)	0.5
Limestone	1.0
(Vitamin A -- 750 IU/pound of ration)	

TABLE IV

DESCRIPTION OF BULLS AND EXPERIMENTAL DESIGN

Experimental Bull No.	Calf No. Project 1256	Trial No.	Cannulation Date	Age at Cannulation (mos)	Weight at Cannulation (lbs)	Termination Date
1	7940	1	1-11-71	22	980	4-3 -71
2	7948	1	2- 5-71	21	985	4-22-71
3	8910	2	5-25-71	27	1000	8-11-71
4	8915	2	5-25-71	27	1000	8-11-71
5	0901	3	8-17-71	30	1070	10-25-71
6	0943	3	8-17-71	29	1100	10-25-71
7	8925	4	10-27-71	30	1139	12-29-71
8	8949	4	10-26-71	30	1139	11-15-71
9	9909	5	12- 2-71	33	1175	1-26-72
10	9924	5	12- 2-71	33	1032	1-28-72
11	7912	6	2- 4-72	36	1230	3-10-72
12	7949	6	2- 4-72	34	1230	3-11-72

Each trial began with a 2 to 3 week acclimatization period of confinement. This involved putting the pair of bulls in a box stall in the basement of the Animal Nutrition Laboratory where the temperature was controlled at about 70°F. This period was designed to allow spermatogenesis to begin functioning under a controlled environment. During the last week of acclimatization, each bull was electroejaculated two times on each of 2 different days to determine whether motile sperm were present in the ejaculate and to remove aged sperm from the cauda epididymis. The two collections on the second day were pooled, evaluated and used as the pre-cannulation data.

Following surgery and a 4 to 5 hour recovery period, the two bulls were moved to the Physiology environmental chamber where they remained for the entire experimental period. This climatic controlled chamber was kept at a constant temperature of 70°F and at about 50% relative humidity with slight fluctuations of 5% being experienced periodically. Each bull was haltered and tied in a pipe stall which allowed no lateral movement but did allow the bull to move back and forth for about 4 feet and to lay down. A pipe head gate was located at the front of the stall and used during electroejaculation and when changing the cannulation vial. The concrete floor was covered with saw dust which was changed twice daily to insure cleanliness. The bulls were fed and watered twice daily and the cannulation device checked for possible difficulties. At the termination of each trial the bulls were castrated and tissue samples collected for histological study.

Cannulation Materials and Procedure

A preliminary trial was conducted on Bulls 1 and 2 during early

1971 to determine the success of the surgical procedure, the duration of cannula patency and to establish techniques for the collection and evaluation of the sperm. On the basis of this preliminary work, the procedure was standardized for the remaining 10 bulls.

To obtain a continuous quantitative collection of epididymal sperm, the unilateral vas deferens cannulation technique of Amann et al. (1963) and Amann (personal communication, 1970) was used with modifications. The cannula consisted of a polyethylene tubing[1] (.011 in. ID. x .024 in. OD.) surrounded by a Silastic tubing[2] (.040 in. ID. x .085 in. OD.). About 3 inches of the polyethylene tubing projected from the Silastic casing which had a collar constructed from Silastic Adhesive[3] at its end. The collection device consisted of a polyethylene disk (1 in. wide x 1½ in. long) which was constructed from a 1000 ml polyethylene laboratory squeeze bottle. A polyethylene elbow (¼ in. OD. x 1½ in. long), which was made from a squeeze bottle spout, ran through the center of the disk. A polyethylene snap cap from a 5 ml glass vial was fitted on to the elbow so this glass vial could be used as the collection container and removed easily. The cannulae and supporting devices were sterilized in Nolvasan for 24 hours and then rinsed with normal saline solution before surgery.

Surgery was performed in the Veterinary Research large animal surgery room by two faculty members of the College of Veterinary Medicine.

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[1] Intramedic PE-10, Clay-Adams Inc., New York.

[2] Silastic Medical Grade, Dow Corning Corp., Midland, Michigan.

[3] Silastic Medical Adhesive, Dow Corning Corp., Midland, Michigan.

Immediately before surgery the bull was given an injection of a tranquilizer to immobilize him so he could be strapped in lateral recumbency on a hydraulically controlled tilt surgical table. A basical anesthesia (Halothane) was utilized with sterile surgical procedures. An incision was made in the left posterior neck of the scrotum and the left spermatic cord and vas deferens isolated. The vas deferens was ligated about 3 to 4 inches above the caput epididymis and severed proximal to the ligation. The inner polyethylene cannula was inserted into the lumen of the proximal end of the vas deferens up to the Silastic collar. This collar was then sutured (3 times) to the wall of the vas deferens. The exteriorized end of the cannula was pushed through a hole in the scrotum made by a 12 gauge needle about 2 inches to the right and above the incision so the cannula would not interfere with the healing of the incision. Penicillin-streptomycin and dexamethasone[4] were mixed and used locally in the incision to reduce infection and inflammation. After closure of the incision, the polyethylene disk and elbow were threaded on the cannula and the disk was attached to the scrotum by four double suture loops. The loops were passed through small pieces of rubber tubing on the scrotal side to prevent their cutting through the skin. Immediate patency of cannula was usually confirmed by sperm dripping out of the end. This end of the cannula was placed in the collection vial which as snapped on to the elbow and taped to prevent accidental loss. After the surgery, which lasted about 2 hours, the bull was moved to the padded recovery room, which was adjacent to the surgery room, for a 4 to 5 hour period. Surgery was usually performed on the second bull immediately after com-

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[4]Azium, Schering Corp., Dallas, Texas.

pletion of the first using the same procedure. Daily intramuscular injections of penicillin-streptomycin were given for the first several days post-surgery and at later periods when swelling of the scrotum was observed. It was assumed that the antibiotics had no deleterious effects on the sperm cells.

No major problems were experienced during the surgery except for occasional difficulty in inserting the cannula in the vas deferens and keeping it in place while the collar was being sutured to the vas. The greatest difficulty with this technique was the post-surgical swelling and inflammation which will be discussed with the results.

General Collection Procedures

The standard procedure was to move the two bulls from the recovery room to the environmental chamber where they remained for the entire experimental period. During this period, the cannulation collection vial on each bull was changed at 24 hour intervals and the volume and concentration of the epididymal sperm in the vial was measured. Samples were also examined immediately under the light microscope to detect gross increases in the number of tailless sperm and the presence of foreign cells and bacteria. Beginning on about the fourth day after cannulation, each bull was electroejaculated, using a Nicholson electroejaculator[5], three times per week (Monday, Wednesday, and Friday afternoons). At the time of collection care was taken to prevent cold shock of samples at all stages of collection, processing and evaluation. All glassware was placed on a slide warmer at 37°C and a polyethylene bottle

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[5]Nicholson Manufacturing Co., Denver, Colorado.

of warm water surrounded the ejaculate collection tube. Two clean 5 ml collection vials were prepared with 4 drops (0.12 ml) of warm 2.9% sodium citrate buffer for collection of the sperm from the cannula. The procedure used during each ejaculation was as follows. The 24-hour collection vial for the cannula was replaced with one of the clean vials and ejaculation of the bull began. During the period of pre-ejaculation drippings from the penis, a few drops of sperm would flow out of the cannula. At least three drops were allowed to flow out since this was estimated to be the volume of semen present in the cannula. When it was felt that a fresh sperm sample could be obtained directly from the cauda epididymis, the vial was replaced by the second warmed vial. Immediately after the bull had ejaculated, the collection from the penis (ejaculate) and the sperm in the cannula vial (epididymal) were placed on the slide warmer. The volumes of the ejaculate and the two epididymal samples were determined and the samples examined immediately for percent and rate of motility. Twice weekly (Mondays and Fridays) stained slides were prepared of the ejaculate and the second epididymal sample with a live-dead differential stain and the Wells-Awa acrosome stain. Sperm concentration was determined in all ejaculates and in all 24-hour cannulation vials and output was calculated.

Storage Treatment Procedures

When the surgical area of a bull showed no swelling and the percentages of abnormal and live epididymal sperm had returned to approximately the pre-cannulation values (usually after about 35 days), the storage treatment procedure was imposed on that bull. Only 4 bulls (3, 6, 7 and 9) recovered sufficiently from the swelling with the cannula still patent

so that the storage treatment could be implemented. This treatment procedure involved the ejaculation of the bull daily for 10 days and the evaluation of all criteria of the ejaculate and the second epididymal sample as described above. These observations constituted the pre-storage data. The cannula was then completely closed for 6 days. This was accomplished by clamping two small animal metal sultures with the prongs flattened over the end of the cannula with a pliers. After the sixth day, the cannula was cut just above the sutures so the cannula could flow again. Post-storage data was obtained on these bulls by the same procedure practiced pre-storage of ejaculating and evaluating samples daily for 10 days. However, only the cannulae in Bulls 3 and 6 flowed post-storage, and the cannulae of Bulls 7 and 9 did not flow even after many different methods were tried in an effort to reestablish the flow. These methods included ejaculating, manually massaging the epididymis, pushing a stylus into the cannula, and flushing the cannula with saline. Following the 10 day post-storage period, Bulls 3 and 6 were returned to the post-cannulation collection and evaluation regime and remained on it for the duration of cannula patency.

Evaluation of Sperm Characteristics and Morphology

The following procedures were used to evaluate the ejaculate and epididymal sperm.

(a) Sample Volume: The ejaculate volume was obtained directly from the 15 ml graduated tubes used for collection. The volume of epididymal secretions collected in the 24-hour cannulation vial was obtained to the nearest 0.01 ml by the use of a graduated 0.2 ml pipette. The volume of epididymal sperm

obtained during ejaculation was estimated by the number of drops flowing from the cannula, with each drop considered to be 0.01 ml.

(b) Sperm Concentration: After mixing each sample to insure homogeneity, the sperm concentrations in both the ejaculate and 24-hour vial was determined by a hemocytometer count (Salisbury and Van Demark, 1961). The procedure involved drawing a small quantity of semen into a red blood cell dilution pipette and diluting it to a ratio of 1:200 with a 3 percent sodium chloride solution. After mixing thoroughly, a small drop of this mixture was placed in the counting chamber and the number of sperm counted in a given area using the light microscope. The concentration of cells per ml was then calculated from the count obtained and the dilution factors involved. The concentration of the epididymal samples during ejaculation was assumed to be the same as that of the previous 24-hour vial because the concentration of these samples were similar when checked periodically.

(c) Sperm Motility: A drop of semen was gently mixed with a drop of warm 2.9 percent sodium citrate dihydrate solution on a prewarmed slide. A cover slip was placed over the suspension and the slide examined on the prewarmed stage of a light microscope (X 430). The percentage of motile cells was expressed in units of 5 from 0 to 100. The rate of progressive motility was scored on a scale from 0 to 4 with the following descriptions: 0 = no movement, 1 = rocking, 2 = slow, 3 = intermediate, and 4 = very rapid and vigorous.

(d) Percent Live Sperm: The percentage of live sperm was determined using the nigrosin-eosin Y live-dead differential stain (Hancock, 1952). The stain was prepared by dissolving 6 grams of water-soluble nigrosin and 1 gram of eosin Y in 60 ml of glass distilled water. Smears of sperm from the ejaculate were prepared by mixing two drops (one drop = 0.03 ml) of semen with four to eight drops of stain (depending on the concentration of the sample) in a warm shell glass vial and allowing it to stand for 3 minutes. A small drop of the mixture was then placed on a clean microscopic slide, smeared and dried on the slide warmer at 37°C. The same procedure was used for the epididymal samples with the exception that one drop of the sperm-buffer mixture in the collection vial was mixed with five to ten drops of stain. All stained slides were prepared in duplicate. Percentages of live sperm cells were determined by counting the number of unstained cells included in the first two hundred sperm cells counted using the light microscope (X 430). All stained and partially stained cells were considered dead. The average of two counts per sample was used as the mean percent live sperm of the sample.

(e) Percent Abnormal Sperm: This percentage was determined from the live-dead slides (disregarding the live-dead differentiation) by counting the number of abnormal cells per two hundred sperm and taking the average of two such counts as the mean percent abnormal. The classification of abnormal sperm as described by Salisbury and Van Demark (1961) was used and expanded. The total abnormal sperm in both the pre- and post-

storage slides was categorized into percentages of freeheads, abnormal heads and mid-pieces, gross abnormal tails and abnormal tail ends. The gross abnormal tails included broken, bent, coiled and hooked. A smooth bend of the tail back on itself more than 135° was classified as hooked. The abnormal tail ends included all tails that had a small coil at their tip or were hooked more than 135° backward.

(f) Percent Aged Acrosomes: This percentage was determined by using the Wells-Awa stain which was prepared according to the following procedures (Awa, 1970).

Solution (A): consisted of a one percent solution of water-soluble eosin B[6] (total dye content 88 percent) in glass-distilled water.

Solution (B): consisted of a one percent solution of water-soluble fast green FCF[6] (total dye content 90 percent) in glass-distilled water.

On the day of semen collection one volume of solution (A) was mixed with two volumes of solution (B) and 1.7 volumes of 95% ethyl alcohol to provide the acrosome stain. All stains, solutions and glassware were prewarmed on the slidewarmer. Smears from the ejaculate were prepared by mixing two drops of semen with six to ten drops of stain (depending on the concentration) in a shell glass vial and allowing it to stand for 2 to 3 minutes. Then a small drop was placed on a clean microscopic slide, smeared and air-dried at 37°C. The procedure for the epididymal

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[6]Allied Chemical and Dye Corporation, Pharmoceutical Laboratories, National Amiline Division, New York.

sample was to mix one drop of the sperm-buffer mixture in the collection vial with 15 to 25 drops of acrosome stain in a shell glass vial and allow it to stand for 4 to 5 minutes, then smearing as described for the ejaculate.

When the sperm-buffer mixture in the cannulation collection vial was very concentrated (with a higher ratio than 1 to 1), the procedure included a dilution step which diluted the sperm to a ratio of 1 volume sperm cells to 2 volumes buffer. One drop of this diluted sperm was mixed with three drops of buffer in a glass vial in another dilution step. Two drops of this second diluted sperm was mixed with four to six drops of acrosome stain and allowed to stand 3 minutes before smearing slides.

All stained slides were prepared in duplicate. The percentage of aged acrosomes was determined by counting the number of sperm with aged acrosomes per two hundred cells using the light microscope under oil immersion at 970X with the blue filter. The alterations of the acrosome that were classified as aged were roughened, ruffled, elevated, incomplete, disintegrating, and capless as described by Awa (1970). The small roughened or frosty appearance was not classified as aged.

Histological Studies

After the cannula stopped flowing, the experimental period was terminated by castration of the bull. The testes, epididymides and the portion of the spermatic cord containing the cannulation site were removed and immediately placed in 35^{o}C normal saline solution. The cannulation site was dissected to determine the gross effects of surgery on the tissue and to attempt to determine the cause of cannula stoppage.

Gross observations and comparisons were made on the cannulated and non-cannulated systems. Samples of sperm were obtained from the vas deferens, cauda and caput epididymides of both systems by dissecting a small area of each region and massaging the sperm out into a vial and diluting it with buffer. These samples were evaluated for motility, percent live, percent abnormal and percent aged acrosomes. The epididymides were dissected from the testes and each testis was weighed to the nearest 0.1 gram. The vas deferens, cauda epididymis and a cross-section through the testis of each system was placed in Bouins solution for later histological evaluation. Tissue from the upper and lower regions of the vas deferens, bottom of cauda epididymis and an area of testis was sectioned, stained and examined with a light microscope by the Veterinary Pathology Department for histological changes due to surgery and cannulation. A comparison between the cannulated and non-cannulated systems was made.

Statistical Analysis

The experimental data were divided into three periods (recovery, pre-storage, and post-storage) and summarized within bull within period by calculating means and standard errors for the different sperm criteria. Data from bulls that reacted similarly during each period were pooled. For the cannulation recovery period data from Bulls 3, 6, 7, and 9 (storage group) were pooled as were the data from Bulls 4, 5, 10 and 11 (non-storage group). Data from Bulls 3, 6, 7 and 9 were pooled for the pre-storage period and from Bulls 3 and 6 for the post-storage period. Epididymal and ejaculated sperm criteria were compared within bull, within groups, and between groups for all above periods. Also

recovery, pre-storage and post-storage criteria were compared within sperm type. Testis weights were compared between cannulated and non-cannulated systems and the association between testis weight and sperm output was determined by simple correlations. All comparisons were tested for significant differences using the Students "t" test and all analyses were made according to Steel and Torrie (1960).

CHAPTER IV

RESULTS AND DISCUSSION

Preliminary Study

It should be obvious that before good quantitative results can be obtained from a cannulation study, the cannulae must flow for a sufficient period of time and the effects of surgery and cannulation must be overcome. It seemed important to grant first priority to a preliminary study to permit an estimate of the time intervals involved in cannula patency and surgical recovery. Also, since the vas deferens cannulation techniques of Amann et al. (1963) had not been used extensively by other workers or used to study the morphology of epididymal sperm, another question to answer was the feasibility of using this technique to monitor cauda epididymal sperm.

From the preliminary study conducted on Bulls 1 and 2, it was found that the cannula would flow during ejaculation and a fresh sample of epididymal sperm could be collected routinely. Also, the cannulae would stay patent for long periods (81 and 57 days for Bulls 1 and 2, respectively). However, it was also found that swelling and inflammation occurred post-surgery and lasted about 6 days. Figures 1 to 4 show the epididymal and ejaculate sperm characteristics of Bulls 1 and 2 during the cannulation period. The cannula of Bull 1 was clamped shut for 4 days to determine the initial effects of sperm storage which will be discussed in a later chapter. There was a marked increase in percent

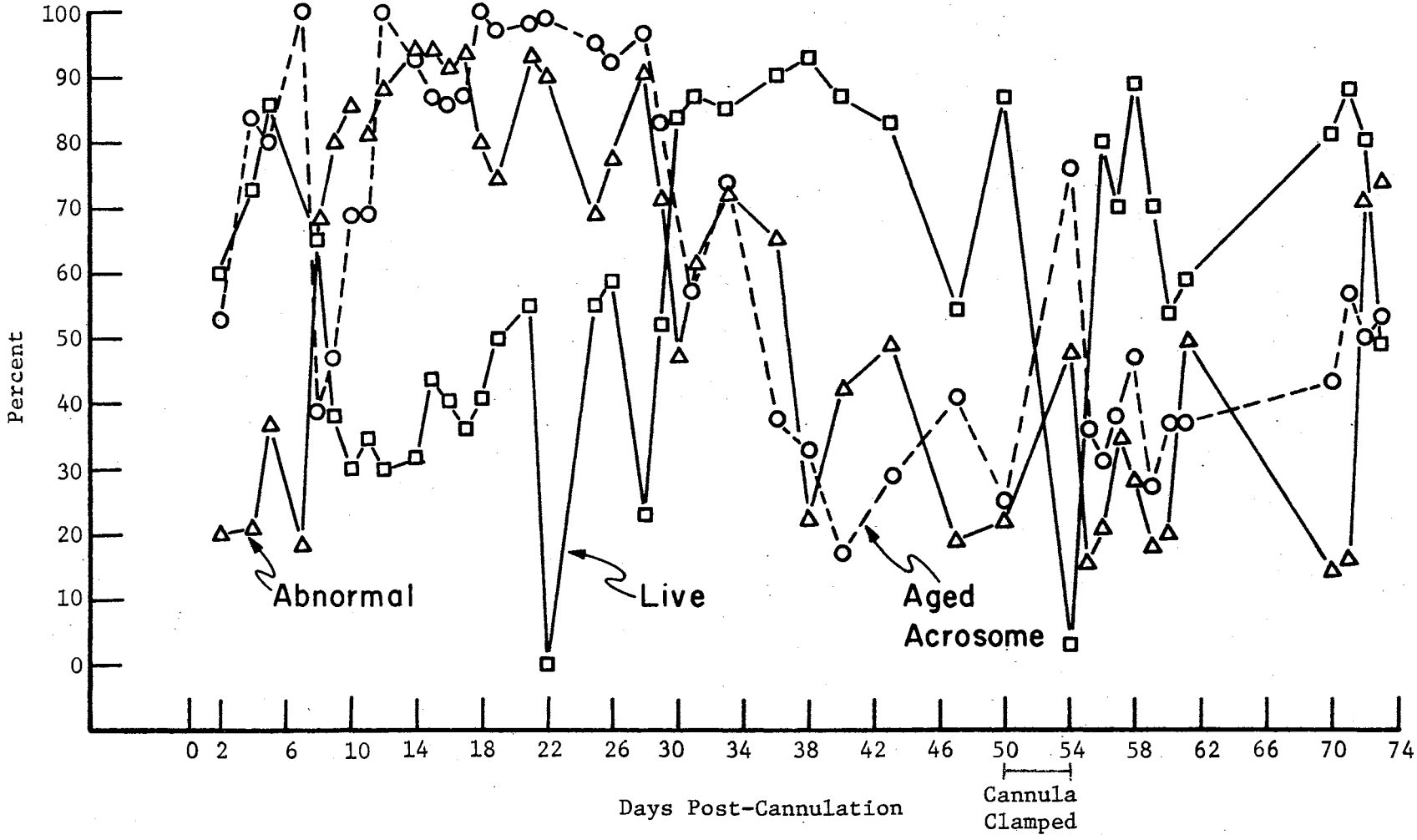

Figure 1. Bull 1 - Epididymal Sperm Characteristics Post-Cannulation

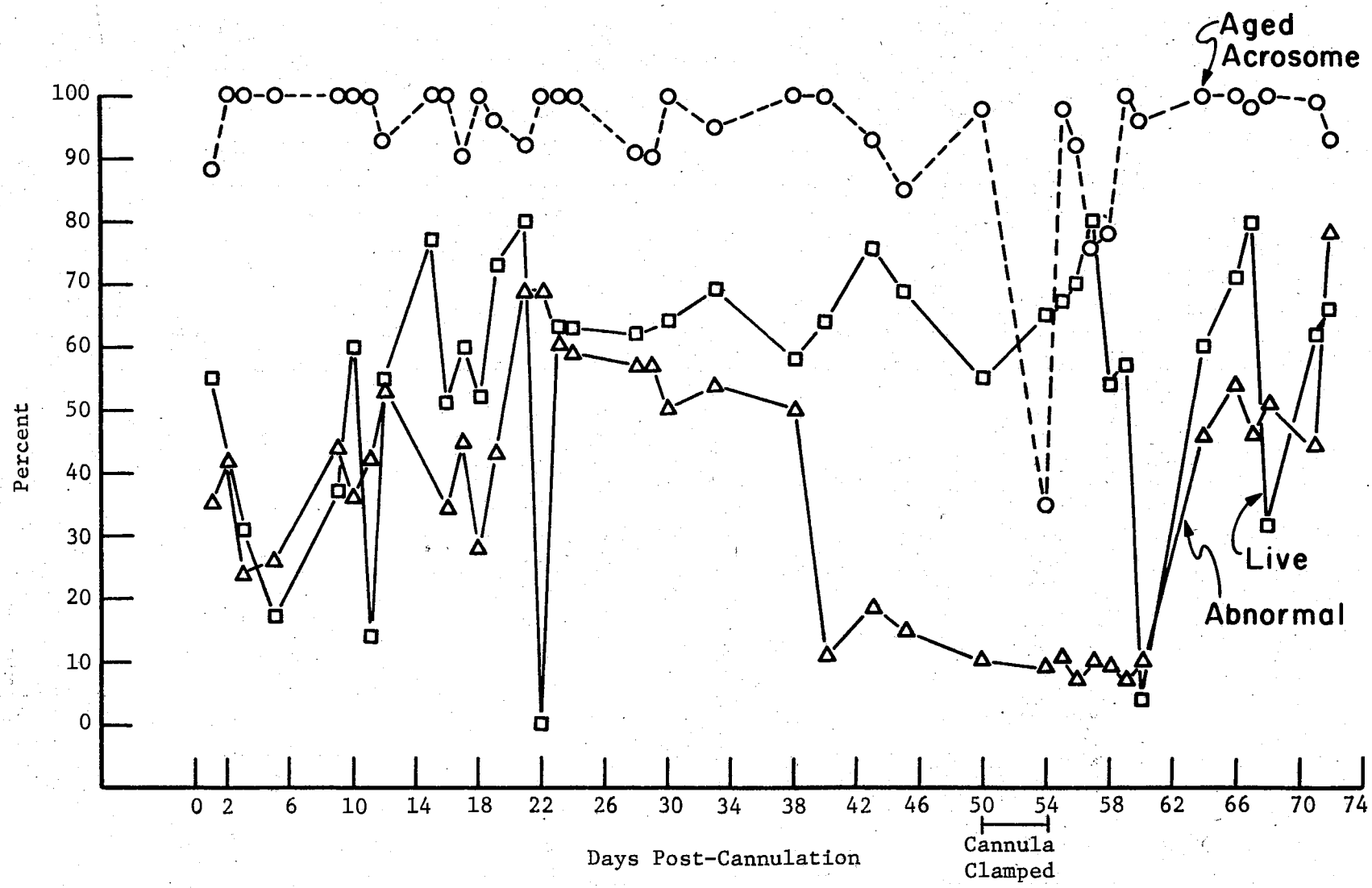

Figure 2. Bull 1 - Ejaculated Sperm Characteristics Post-Cannulation

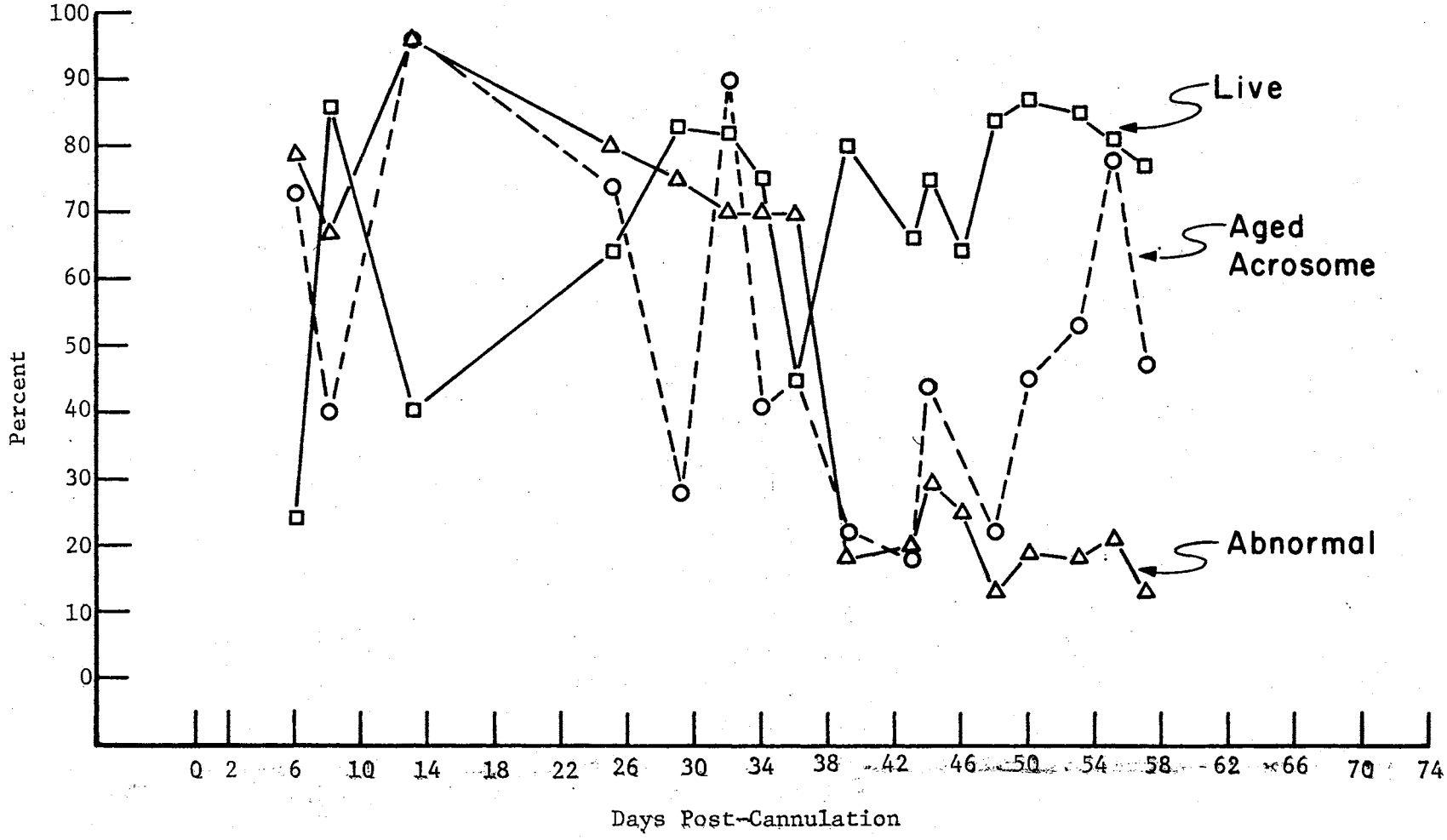

Figure 3. Bull 2 - Epididymal Sperm Characteristics Post Cannulation

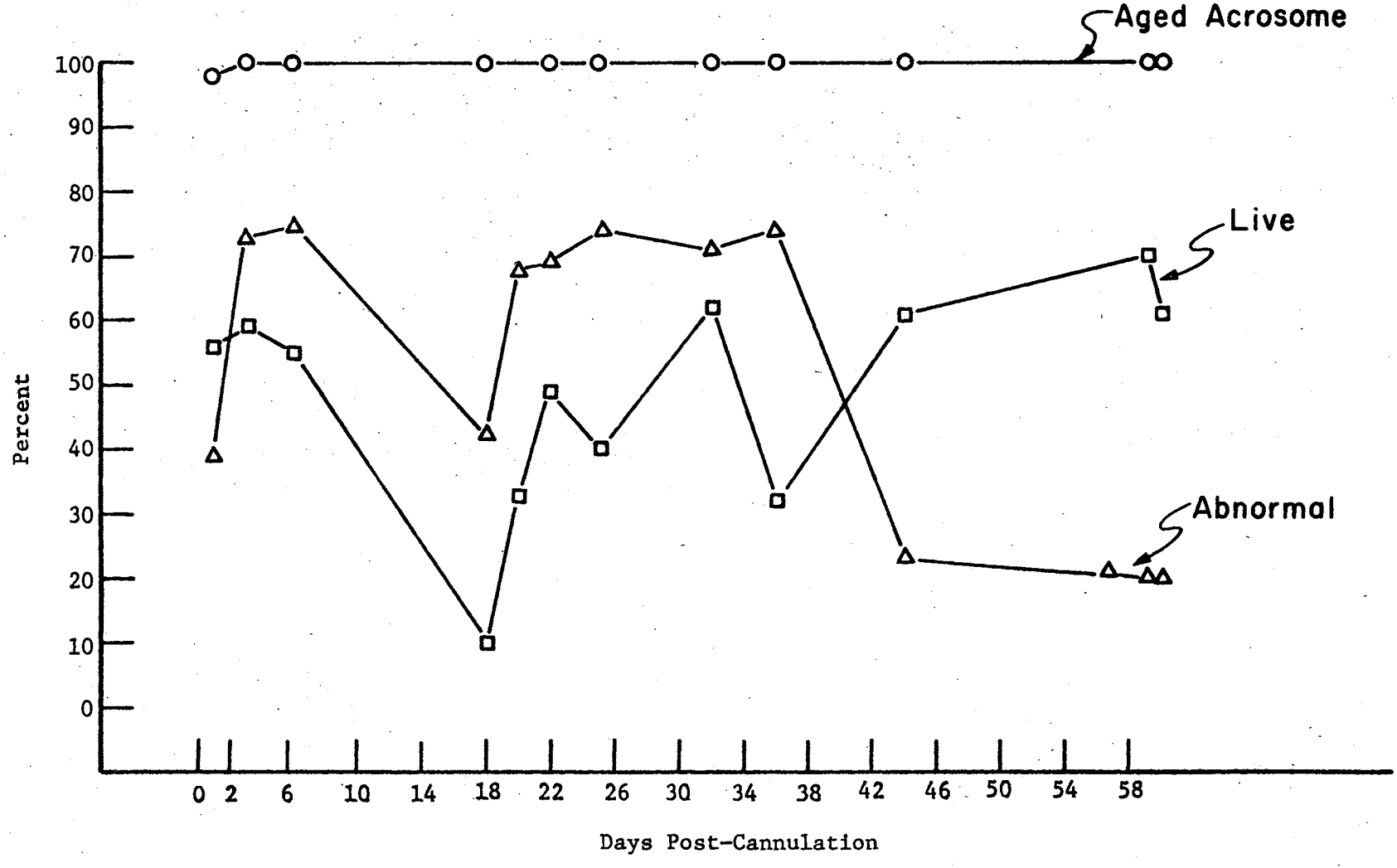

Figure 4. Bull 2 - Ejaculated Sperm Characteristics Post Cannulation

abnormal sperm from post-cannulation day 8 to 38 (Bull 1) and day 13 to 36 (Bull 2). The percentage of live sperm decreased during these same periods. From these results, it was concluded that the technique could be used to study epididymal sperm but a post-cannulation recovery period of from 30 to 40 days was needed to allow the sperm cells to recover. When the percentages of live and abnormal sperm had recovered to the pre-cannulation levels, the sperm samples could be evaluated with the minimum confounding of the effects of surgery.

Success and Difficulties of Cannulations

The remaining five trials were conducted according to the schedule outlined in Table V which gives a summary of the cannula patency lengths and causes of cannula stoppage. Ten of the 12 cannulations were successful when considering the patency lengths. The cannulae of the two unsuccessful cannulations flowed only a few days and were thought to be due to the cannula collar coming loose from the cannula in Bull 8 and the cannula becoming kinked in Bull 12. Of the 10 successful cannulations, two (Bulls 5 and 11) were terminated because of swelling before the cannulae lost patency. In another two bulls (7 and 9) the cannulae were clamped shut for the storage treatment and did not resume patency after removal of the clamp. Therefore, excluding Bulls 7, 8, 9, 11, and 12, the average cannula patency length was 66 days with a range from 50 to 81 days. These patency lengths are similar to those reported by Amann *et al.* (1963) and longer than those of Barker and Amann (1971) for bulls.

The main causes of patency stoppage, as shown in Table V, were fibrosis in the surgical area and difficulties associated with keeping

TABLE V

SUMMARY OF CANNULATION PATENCY LENGTHS AND CAUSES OF STOPPAGE

Trial	Bull No.	Cannulation Date	Patency Length (Days)	Causes of Stoppage
1 - Preliminary	1	1-11-71	81	Fibrosis around vas
	2	2- 5-71	57	inner cannula pushed out
2	3	5-25-71	71	Fibrosis and closure of vas
	4	5-25-71	69	Fibrosis and leakage of vas
3	5	8-17-71	69 - T	----------
	6	8-17-71	66	Inner cannula pushed out
4	7	10-26-71	50 - C	----------
	8	10-26-71	3	Cannula collar came off
5	9	12- 2-71	46 - C	----------
	10	12- 2-71	50	Collar loose from vas
6	11	2- 4-72	35 - T	----------
	12	2- 4-72	4	Kink in cannula

T - Terminated due to swelling.

C - Cannula clamped for storage experiment and failed to reestablish flow.

the inner cannula in the vas and the collar attached to the vas deferens. The fibrosis was a result of an inflammatory reaction in the tissues. In an effort to prevent the inner cannula from being pushed out, a smaller Silastic tubing (.020 in. ID x .037 in. OD) was used as the outer cannula in Bulls 11 and 12 and shrunk on to the polyethylene inner cannula by using toluene. This cannula modification could not be tested for its success because of the early termination of Bull 11 and the cannula kinking in Bull 12.

The major difficulty experienced during the early part of the cannulation period was the swelling and inflammation which occurred in the scrotum and at the cannulation site. Table VI gives a summary of the time intervals and degrees of initial swelling as well as the swelling exhibited during the latter portion of the cannulation period. With the exception of Bull 3 the initial swelling occurred in all bulls on about the third day post-cannulation and persisted for an average of 10 days. The degree of swelling exhibited was arbitrarily determined using a scale of slight, medium and large with the greatest degree of swelling attained given in Table VI. Much variation was observed between bulls for the degree of initial swelling. Bulls 4, 5, 10, and 11 had the longest interval and greatest degree of initial swelling while Bulls 7, 9, and 12 experienced the least degree of swelling.

The trauma of manipulation of the spermatic cord and the vas deferens during surgery was probably a major cause of this swelling. However, in the event other factors were also involved, several different procedures were tested to see whether they would prevent, or reduce, this initial swelling. In trial 2, dexamethazone[7] was injected intramuscularly

[7]Azium, Schering Corp., Dallas, Texas.

TABLE VI

SUMMARY OF POST-CANNULATION SWELLING OF THE SCROTAL AREA OF THE BULLS

Bull No.	Initial Swelling		Later Swelling	
	Interval (Post-Cannulation Days)	Greatest Degree[a]	Interval (Post-Cannulation Days)	Greatest Degree[a]
1	1-7	medium	-----	------
2	2-7	medium	-----	------
3	----	------	43-56 70-78	large medium
4	2-15	large	43-49	medium
5	2-15[b]	large	-----	------
6	3-12	medium	51-53 66-69	slight large
7	3-8	slight	56-62	large
8	1-15	medium	-----	------
9	6-11	slight	40-43	medium
10	2-16	large	25-29 42-51	slight large
11	4-20	large	30-35	large
12	4-8	slight	-----	------

[a]Scale of values range from slight to large.
[b]Swelling on spermatic cord remained for most of experimental period.

at cannulation and 3 days later; in trial 4, a Silastic tubing was used instead of the polyethylene tubing for the inner cannula to reduce tissue reaction; and in trial 6, a new sterilization procedure for the cannula and device was used. However, none of these modifications prevented the initial swelling. It was assumed that the dexamethazone did not effect the sperm production of the bulls. Not only did the use of the Silastic tubing fail to reduce post-surgical swelling, it resulted in further difficulties. The Silastic cannula was difficult to insert into the vas deferens at cannulation because of a stylus which had to be placed inside the tubing due to its great flexibility. Also it was difficult to obtain semen out of the cannula during ejaculation which may have been due to the pressure of the contracting vas deferens collapsing it.

The new sterilization procedure tried was washing the cannula and device in Ivory soap, rinsing twice in distilled water, soaking in Nolvason 24 hours, using formalin vapors 24 hours, and rinsing in saline before use. Forceps were used to handle materials after washing in soap. This procedure probably gave the best protection but the cannula still may not have been completely sterile and a superior sterilization procedure may need to be developed. Amann (personal communication 1971) indicated similar difficulties and was using a gas sterilization procedure to overcome them.

Swelling reoccurred during the latter portion of the experimental period in some bulls (Table VI). This swelling was one of the reasons that the storage treatment could not be imposed on Bulls 4, 5, 10 and 11.

Recovery Period

Sperm Characteristics and Effects of Cannulation

Since the initial post-surgical swelling could not be prevented, a recovery period was allowed during which the effects of cannulation on the epididymal and ejaculate sperm were evaluated. The length of this recovery period varied greatly between bulls. The sperm of some bulls did not recover before the experimental period was terminated and the sperm of other bulls recovered rapidly and the storage treatment procedures were imposed on them. Therefore, a standard length of the recovery period could not be established. To utilize the maximum data from all the bulls, the recovery period for each bull was classified as that time interval when the epididymal sperm cells were returning to the pre-cannulation levels after the effects of swelling and while the bull was collected 3 times per week under the recovery period regime. The four bulls which recovered sufficiently for the storage treatment to be imposed were classified as the storage group and included Bulls 3, 6, 7, and 9. The four bulls whose sperm samples did not recover sufficiently from the surgery or who exhibited scrotal swelling later in the cannulation period which prevented the implementation of the storage treatment were classified as the non-storage group (Bulls 4, 5, 10 and 11).

Figures 5 through 8 show the percentages of motile, live and abnormal sperm and of aged acrosomes for the epididymal and ejaculate samples of Bulls 6 and 4 collected during this recovery period.

To facilitate the discussion of the sperm characteristics, only the characteristics of Bull 6 representing the storage group bulls and the characteristics of Bull 4 representing the non-storage group bulls will be discussed in detail.

The epididymal sperm of Bull 6 (Figure 5) showed the effects of surgery and swelling by an increase in percent abnormals (7 vs. 29%) and a

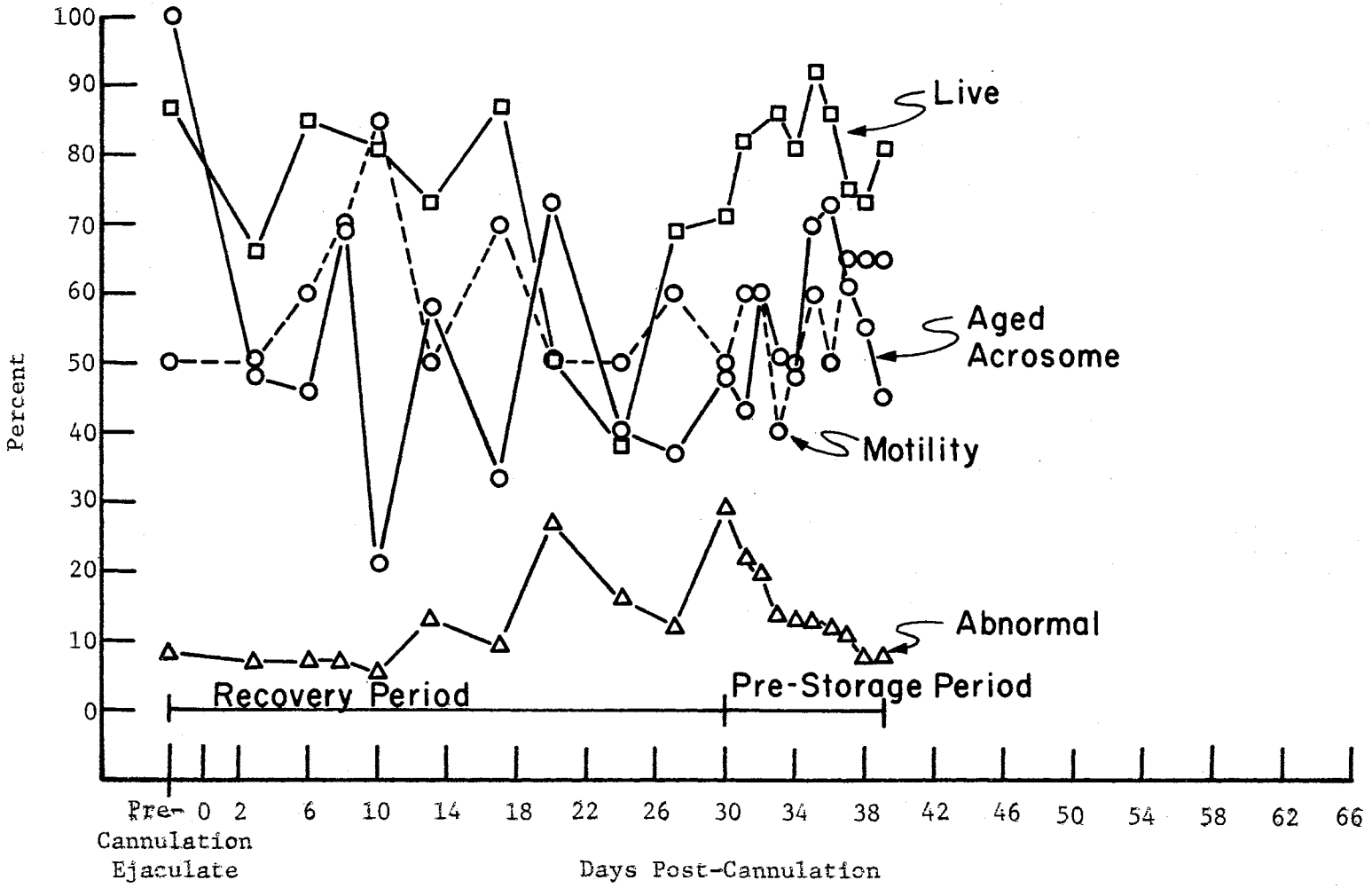

Figure 5. Bull 6 - Epididymal Sperm Characteristics Post Cannulation

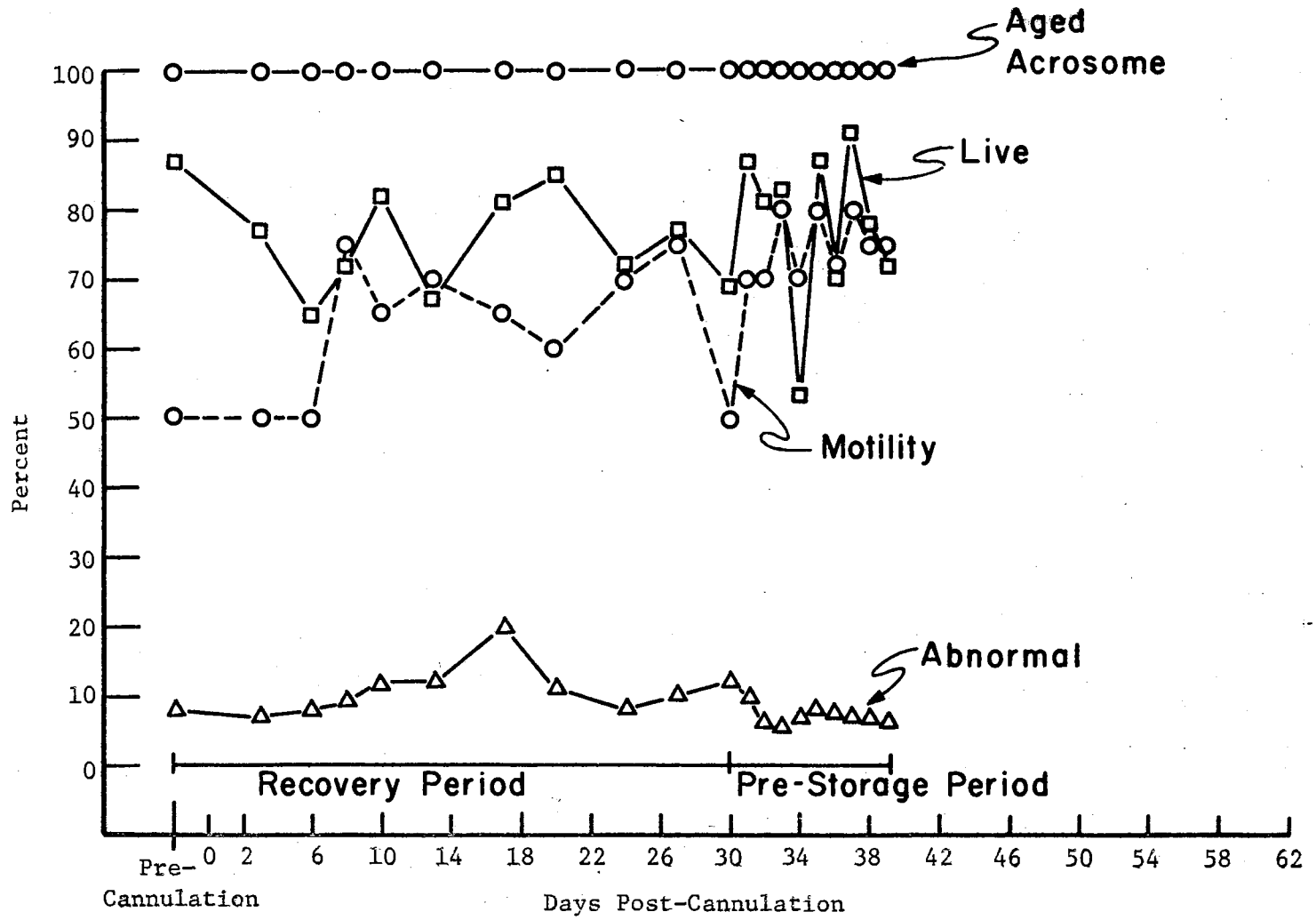

Figure 6. Bull 6 - Ejaculated Sperm Characteristics Post Cannulation

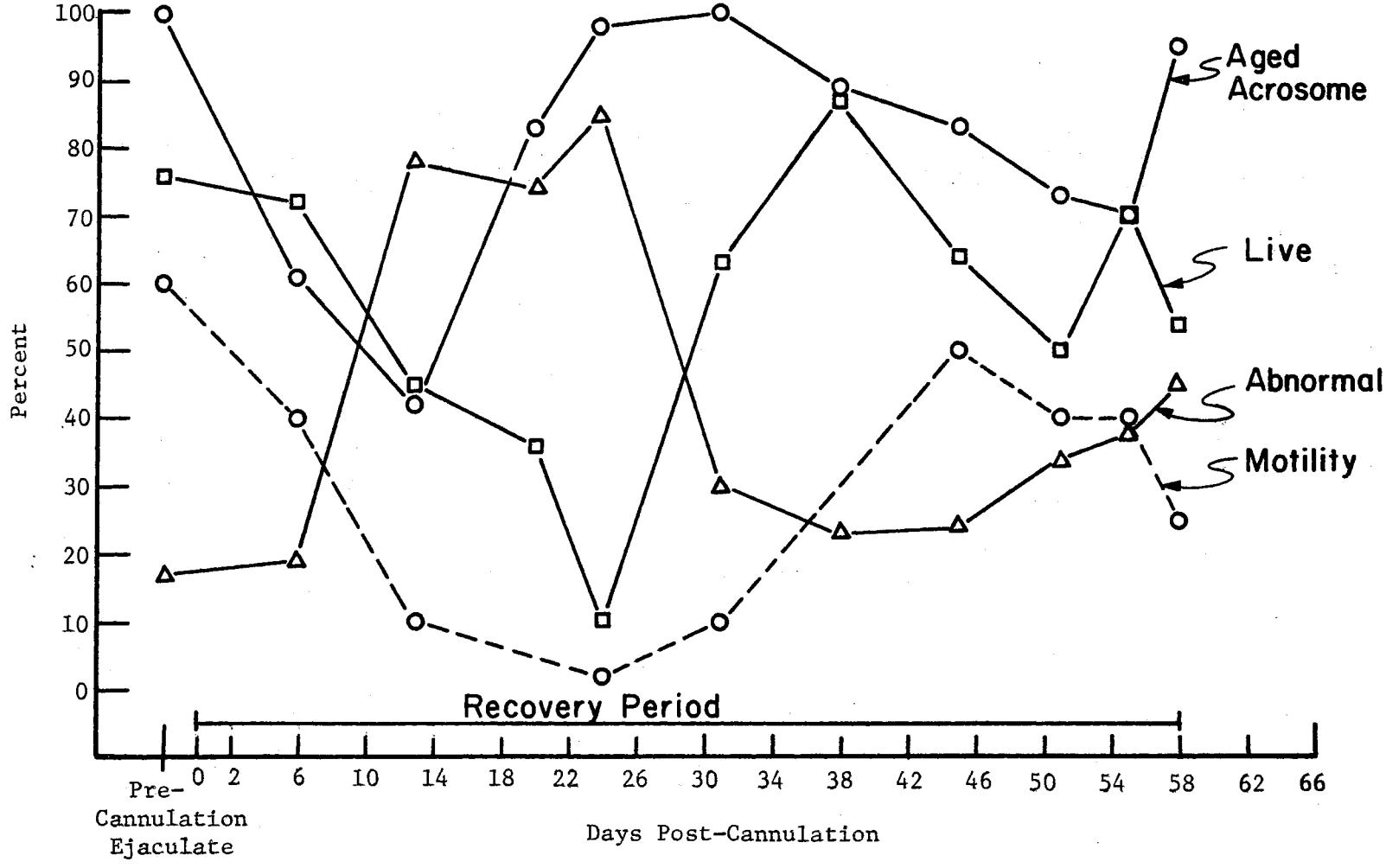

Figure 7. Bull 4 - Epididymal Sperm Characteristics Post Cannulation

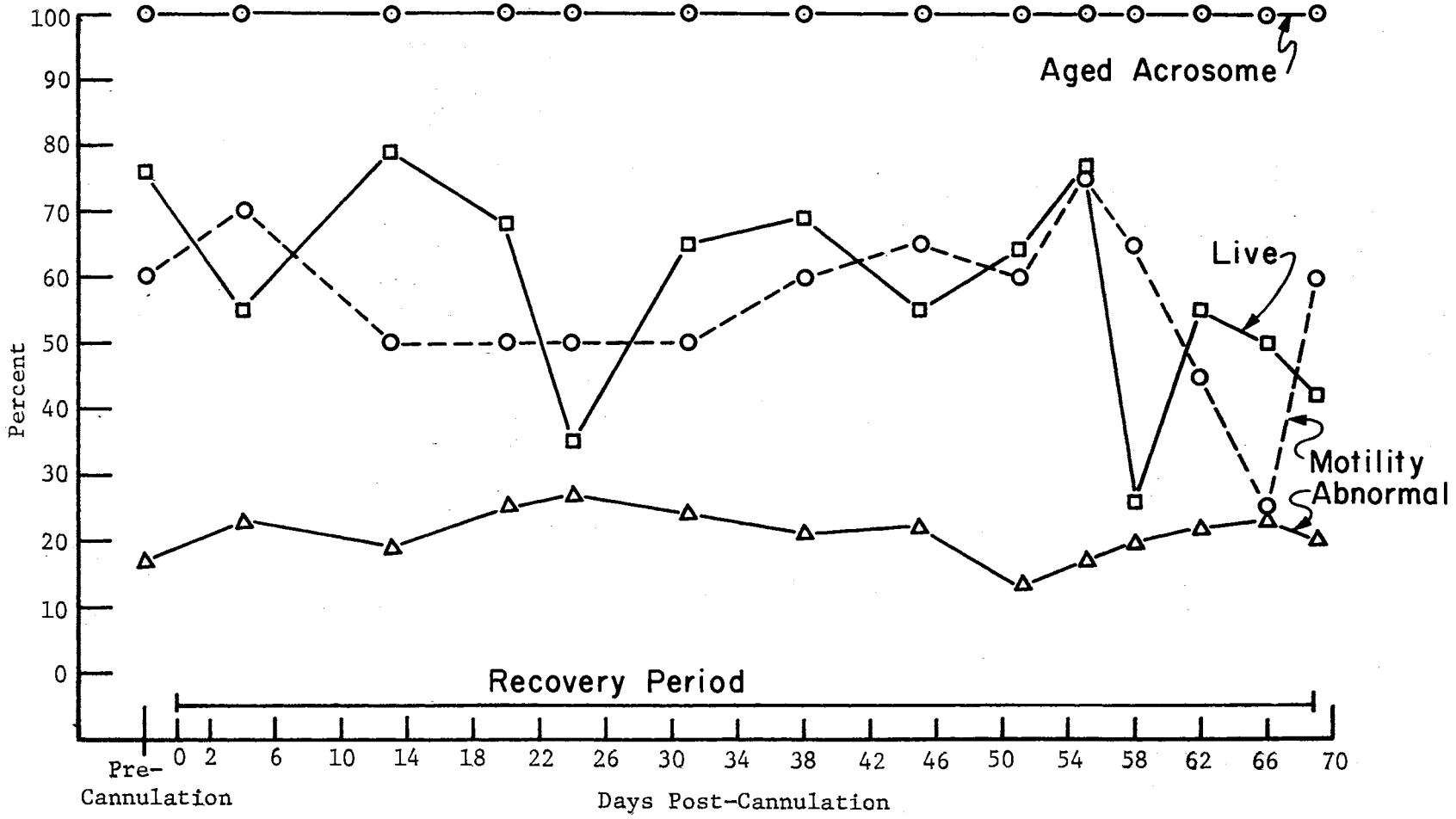

Figure 8. Bull 4 - Ejaculate Sperm Characteristics Post Cannulation

decrease in percent live sperm (87 vs. 38%) during the period of 18 to 33 days post-cannulation compared to the pre-cannulation values. These results are similar to the effects of scrotal insulation and local heating on the testis reported by Van Demark and Free (1969), Samisoni and Blackshaw (1971), and Austin et al. (1961). This time interval after surgery would indicate a heat effect on the spermatids and spermatocyte stage. This statement is based on the estimates of the time intervals between various stages of the spermatogenic cycle. The interval between the spermatid stage and the passage of sperm out of the cauda epididymis was estimated at 15 to 25 days and the interval between the spermatocyte stage and the cauda epididymal sperm was estimated at 25 to 45 days (White, 1968). The effect of heat on these stages was also reported by Van Demark and Free (1969), Setchell et al. (1971), and Samisoni and Blackshaw (1971).

The epididymal sperm of Bull 4 (Figure 7) showed a drastic increase in percent abnormal sperm (19 to 85%) and a big decrease in percent live sperm (72 to 10%) after Day 6 and did not recover until Day 38 post-cannulation. The main sperm abnormalities noted were tailless heads, degenerating heads, and loops at the bottom of the midpieces which indicates the heat effect on the testis as reported by Austin et al. (1961). The percent aged acrosomes increased (42 to 100%) and the percent motility decreased (40 to 2%) during this interval.

The ejaculate sperm from Bull 6 (Figure 6) and Bull 4 (Figure 8) showed only slight effects of surgery during the recovery period although the percent abnormals in Bull 6 increased from 8 to 20% and the percent live of Bull 4 decreased from 76 to 35% during short intervals. These results would suggest that the effect of cannulation and swelling was

confined mainly to the cannulated system and had little influence on the non-cannulated system.

The percentages of aged acrosomes in the ejaculates were 100 percent for both bulls during the entire cannulation period and this result was also observed in all of the other bulls. Several valid theories can be given for the cause of the 100% aged acrosomes in the ejaculate samples. These theories are: an error in the acrosome staining procedure, the effect of cannulation and scrotal swelling, the stress effects on the bulls under this experimental procedure, the effect of seminal plasma on the sperm cells, and the long lasting effects of sexual rest on the sperm. However, the only theory that could be tested was the error in the dilution and staining procedure.

In this study, the ejaculate samples had a much lower concentration than samples normally collected by the AV, due to the use of an electroejaculator and due to the fact that sperm from only one testis was mixed with all the seminal plasma during the process of ejaculation. Since the acrosome stain was developed and tested using concentrate sperm samples, the staining procedure contained a dilution step using 2.9% sodium citrate buffer. With the dilute ejaculate samples in the present study, the dilution step was deemed unnecessary and omitted. However, subsequent testing of the necessity for this dilution step in dairy bull ejaculates indicates that this buffer plays a critical role in the acrosome staining procedure. When the ejaculate sperm in these bulls were stained without using the buffer dilution step, all the acrosomes showed exactly the same elevated and roughened condition as was observed in the present study. Therefore, it appears that the omission of this dilution step in the present study was the cause of the 100% aged acrosomes in

the ejaculates. Also, this dilution step was the only difference in the staining procedure between the epididymal and ejaculate samples and the epididymal samples reacted normally so this would add support to the necessity of the dilution step. Because of this technique error on the ejaculate samples, the comparison of aged acrosomes between the epididymal and ejaculate samples is invalid and has been omitted from this study.

Figures 9 through 20 show the sperm characteristics for the epididymal and ejaculate samples for the remaining six bulls on which data was obtained during the recovery period. The graphs for the bulls in the storage group include the pre-storage period also which will be discussed in a later chapter. The epididymal sperm of the storage group bulls (3, 7, and 9) showed similar effects to those of Bull 6 but of mostly a smaller magnitude. The motility and aged acrosome percentages showed much variation during the recovery period. The epididymal sperm of the bulls in the non-storage group (Bulls 5, 10, and 11) reacted similarly to those of Bull 4 but showed variations in the amount of change of the characteristics and in the length of the recovery period. The ejaculate sperm characteristics of all bulls showed only minor changes during the recovery period.

The comparison of post- and pre-cannulation values for the ejaculate data indicated there was little effect from the cannulation procedure. Since, during normal ejaculation, the cauda epididymis provides most of the sperm in the ejaculate, the cauda epididymal sperm characteristics (excluding percent motility and percent cytoplasmic droplets) should be similar to those of the ejaculate. If the ejaculate sperm was not affected by cannulation, then a comparison of the epididymal and ejaculate

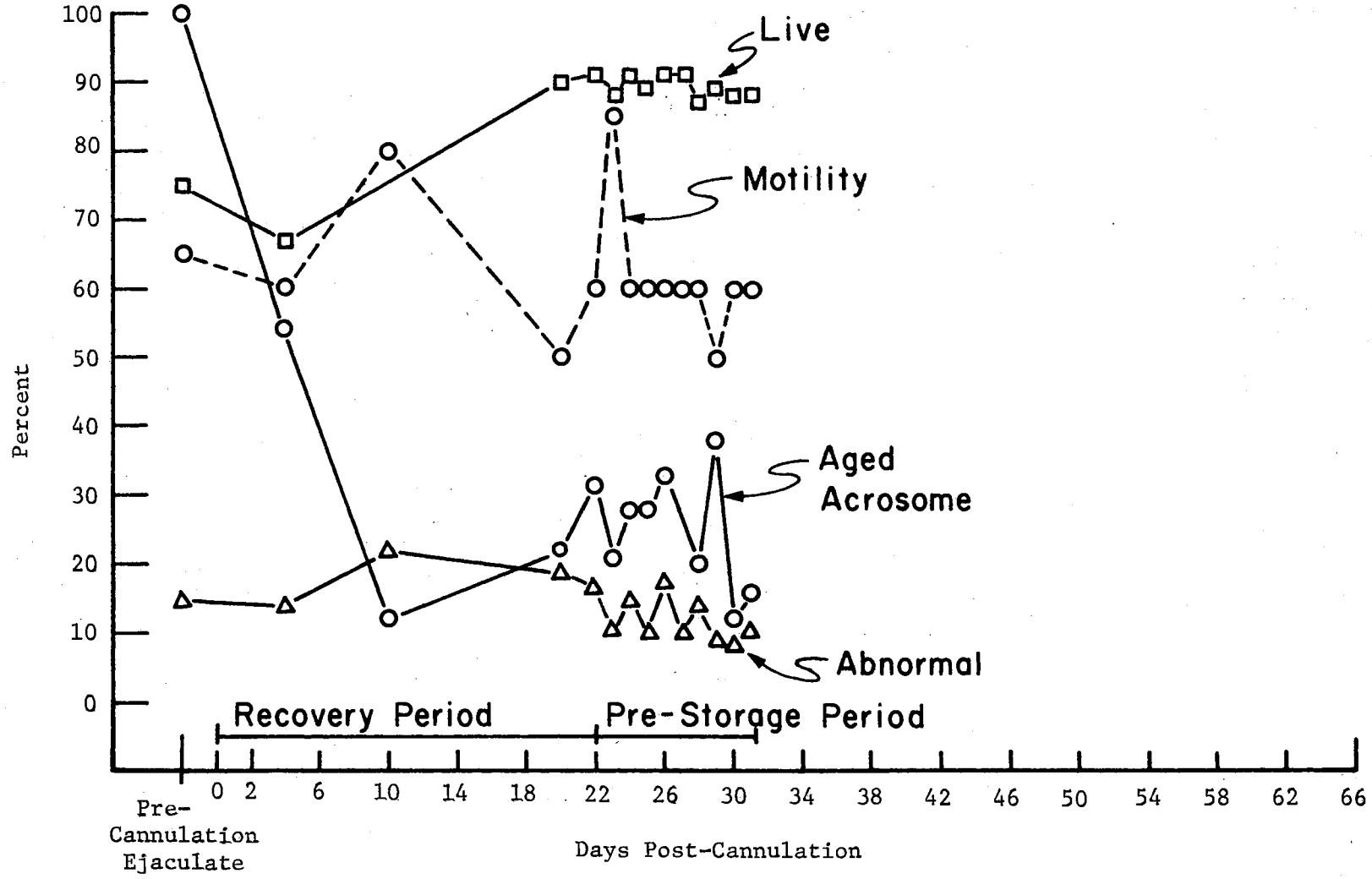

Figure 9. Bull 3 - Epididymal Sperm Characteristics Post Cannulation

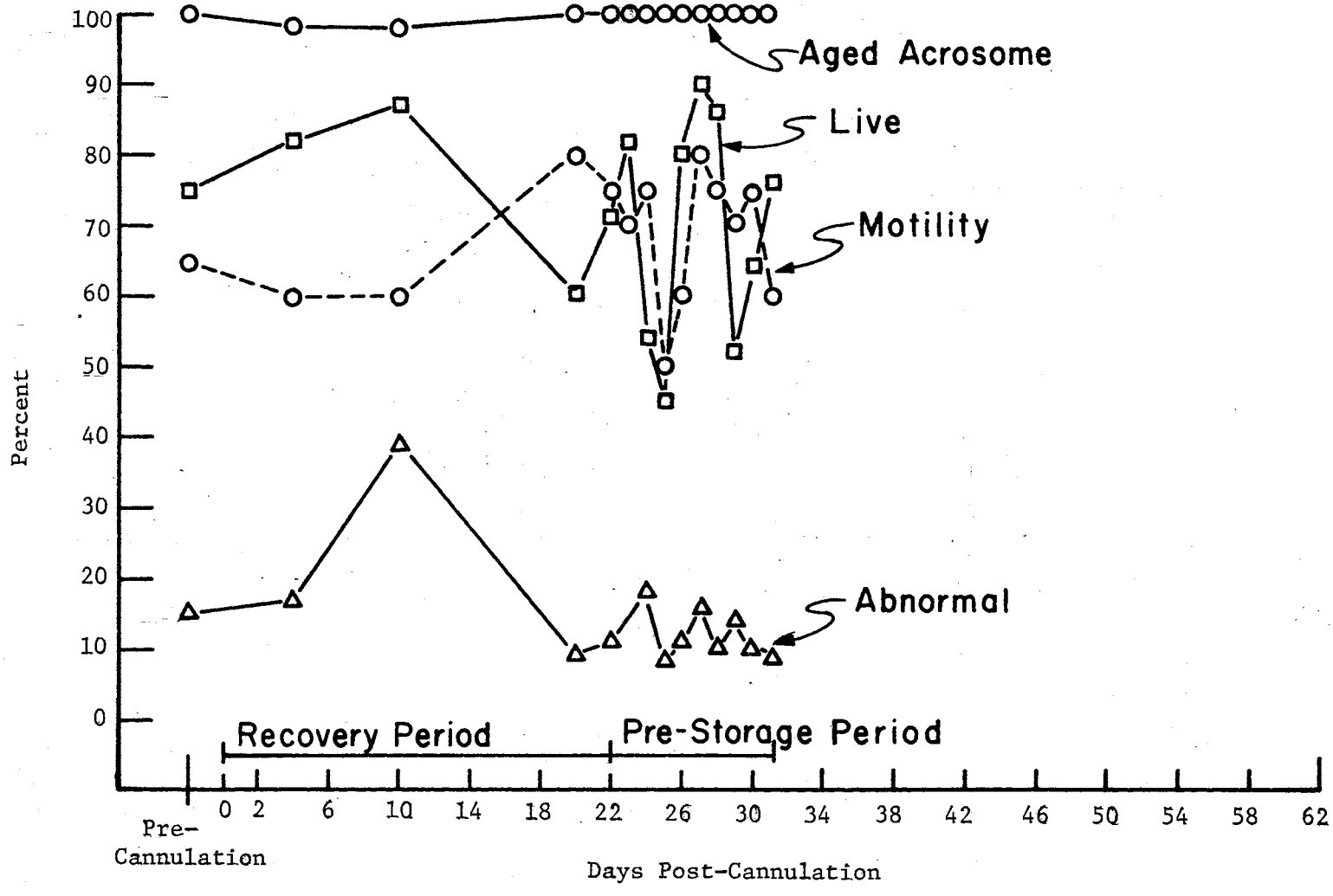

Figure 10. Bull 3 - Ejaculate Sperm Characteristics Post Cannulation

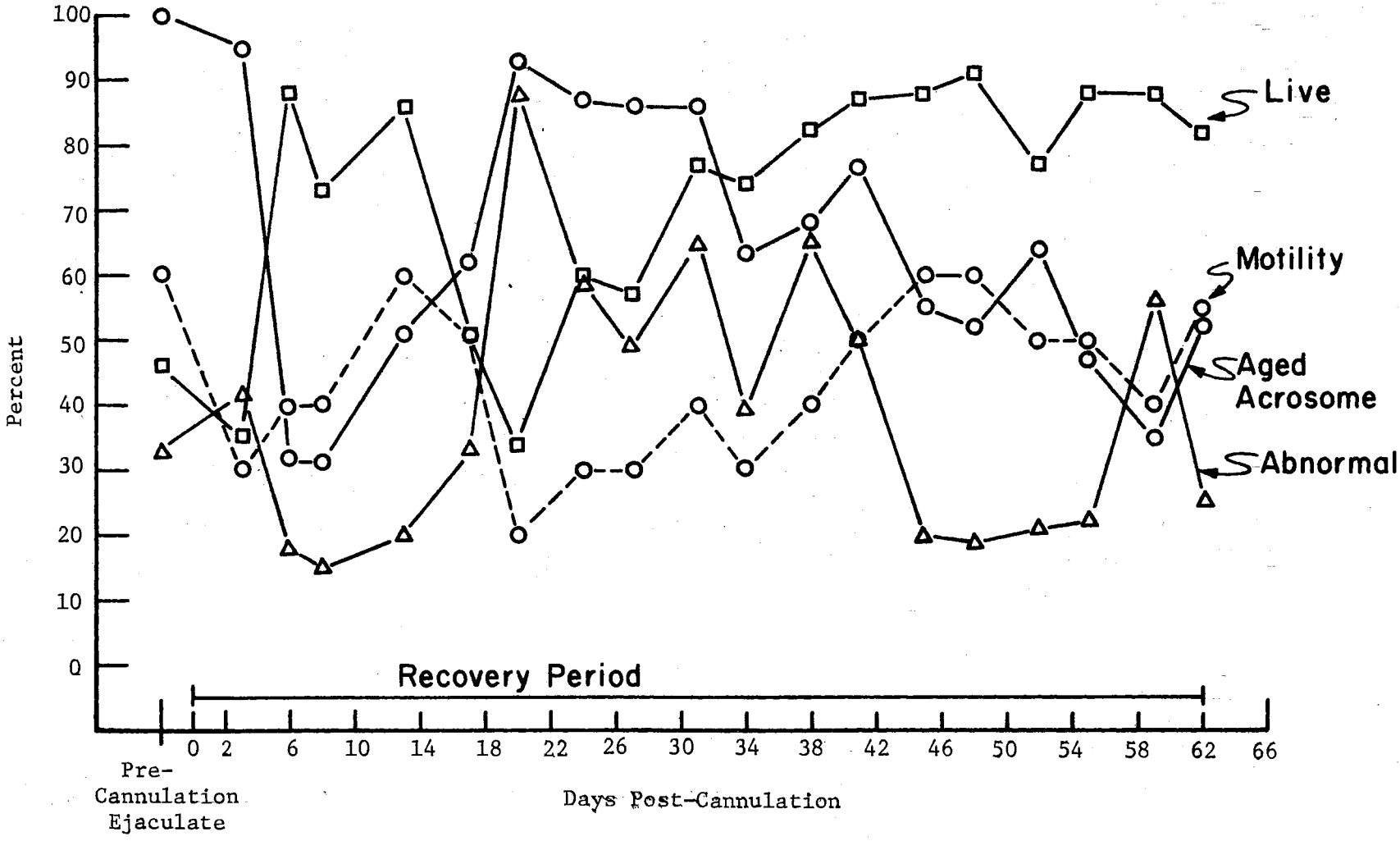

Figure 11. Bull 5 - Epididymal Sperm Characteristics Post Cannulation

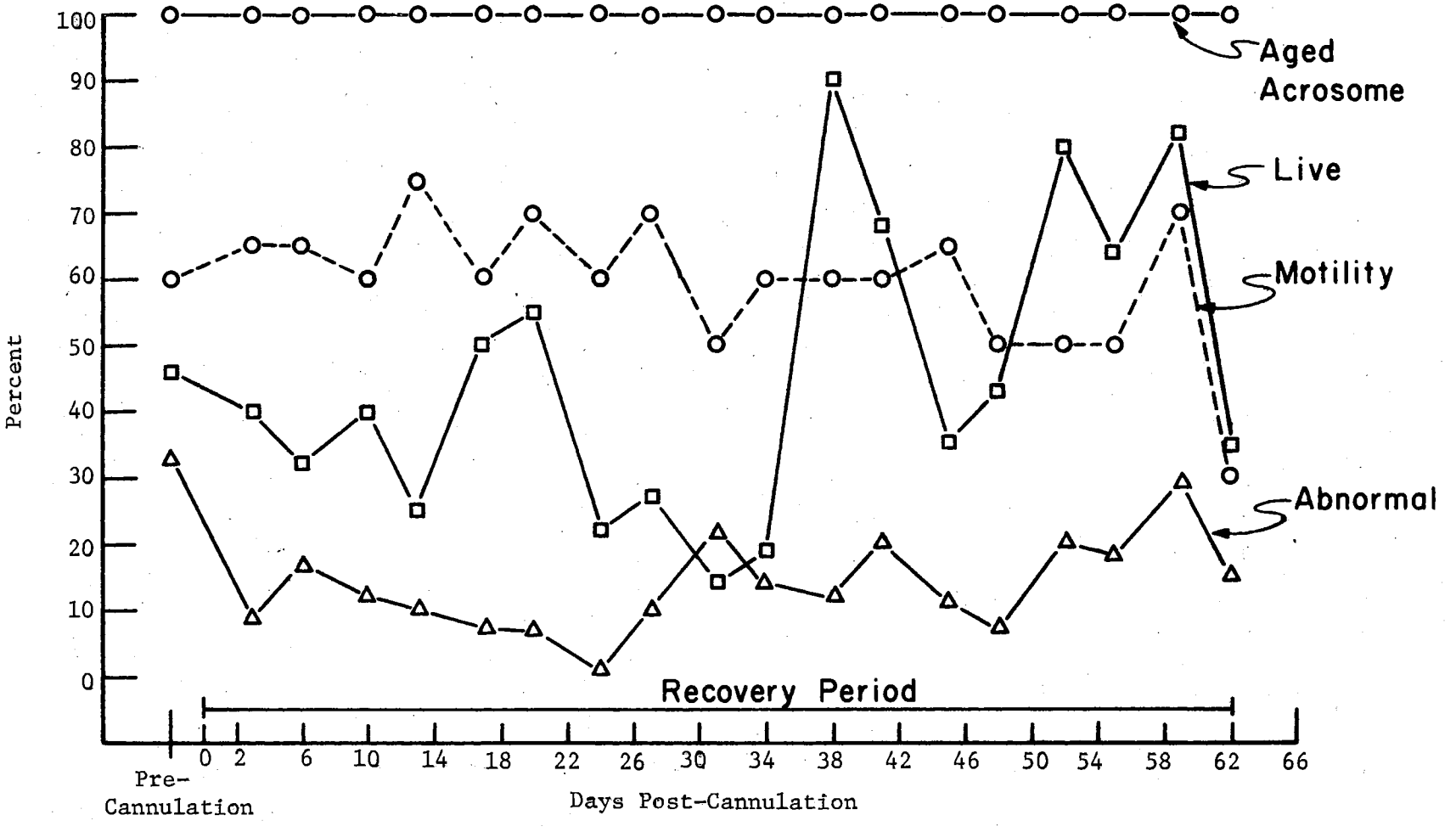

Figure 12. Bull 5 - Ejaculate Sperm Characteristics Post Cannulation

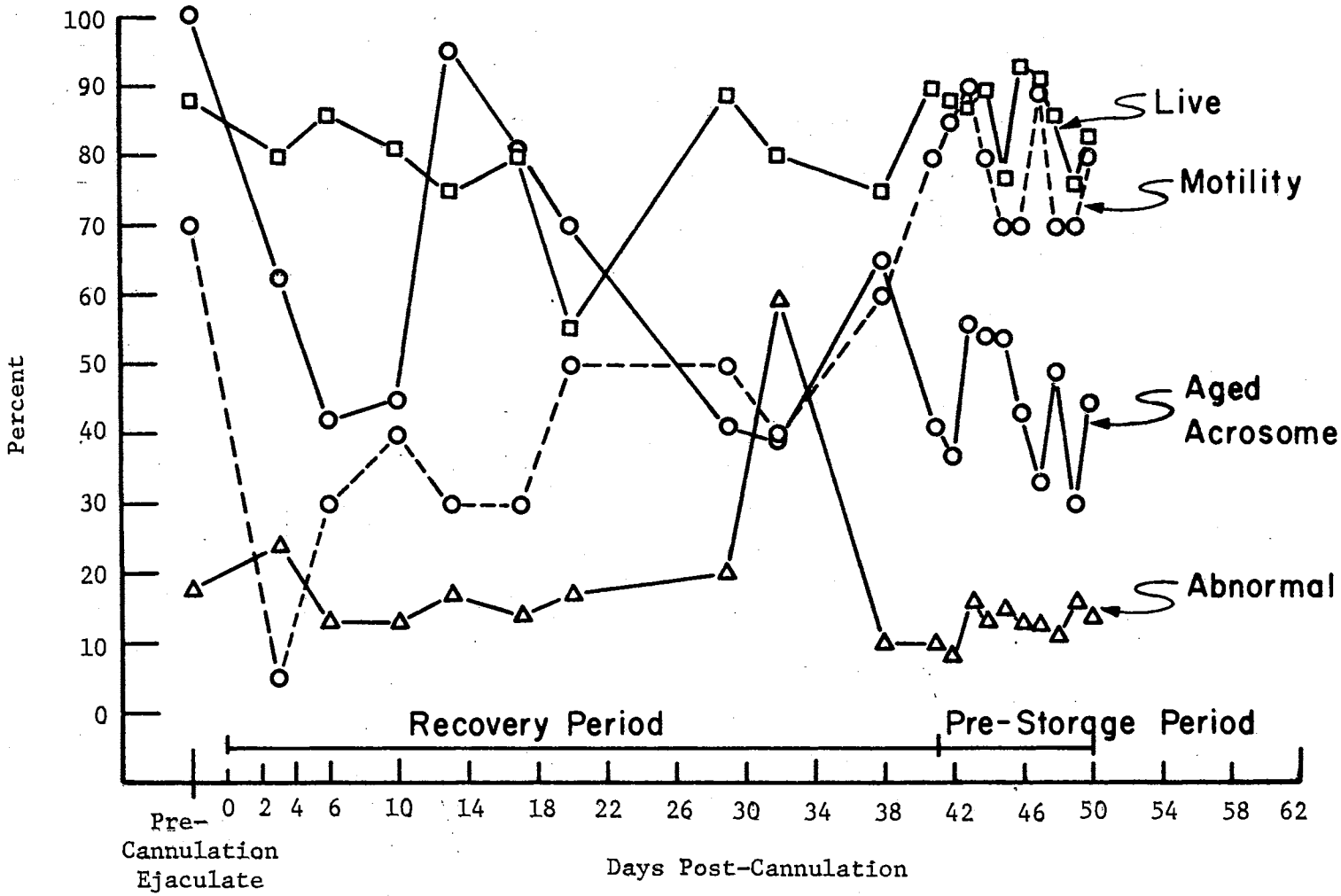

Figure 13. Bull 7 - Epididymal Sperm Characteristics Post Cannulation

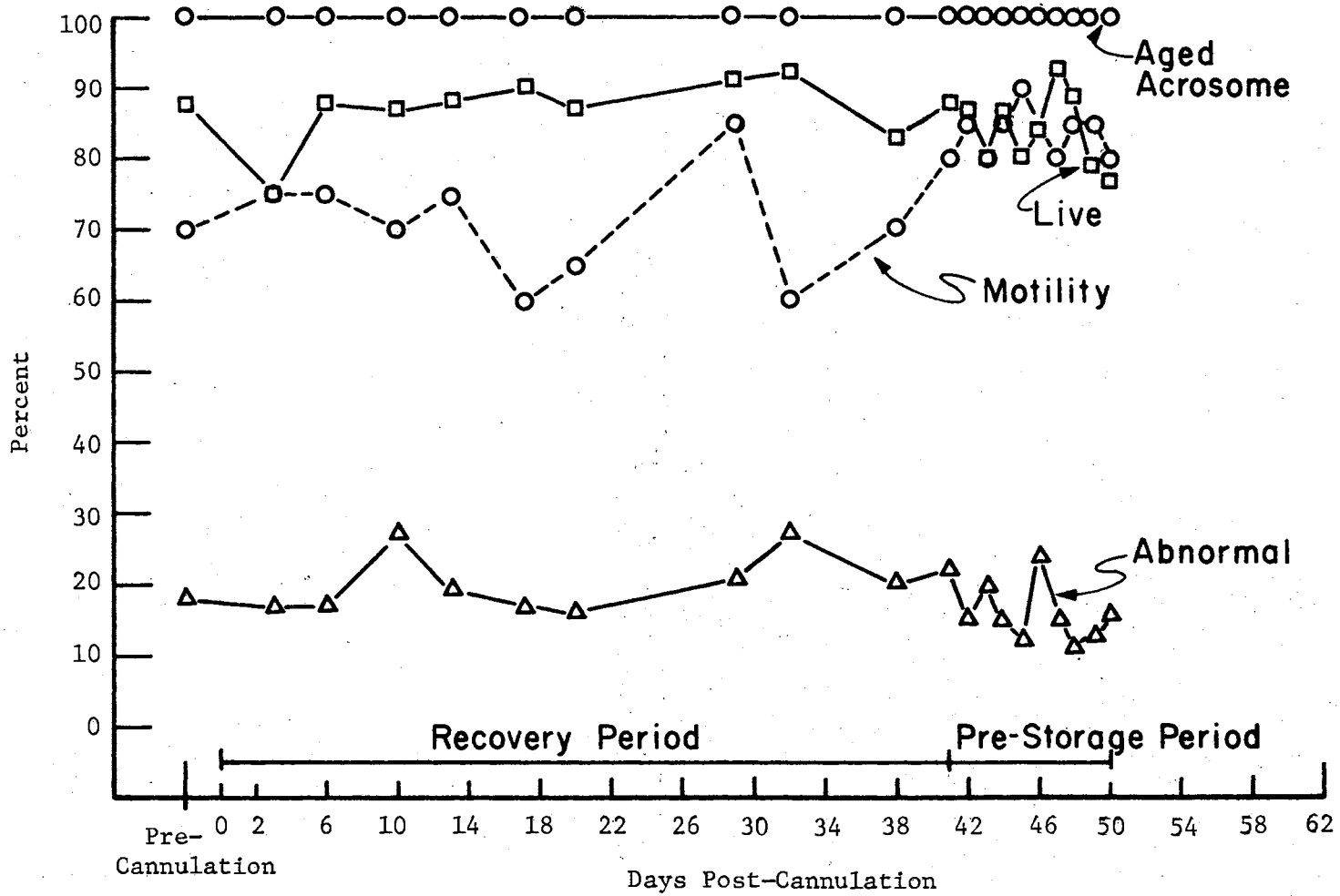

Figure 14. Bull 7 - Ejaculated Sperm Characteristics Post Cannulation

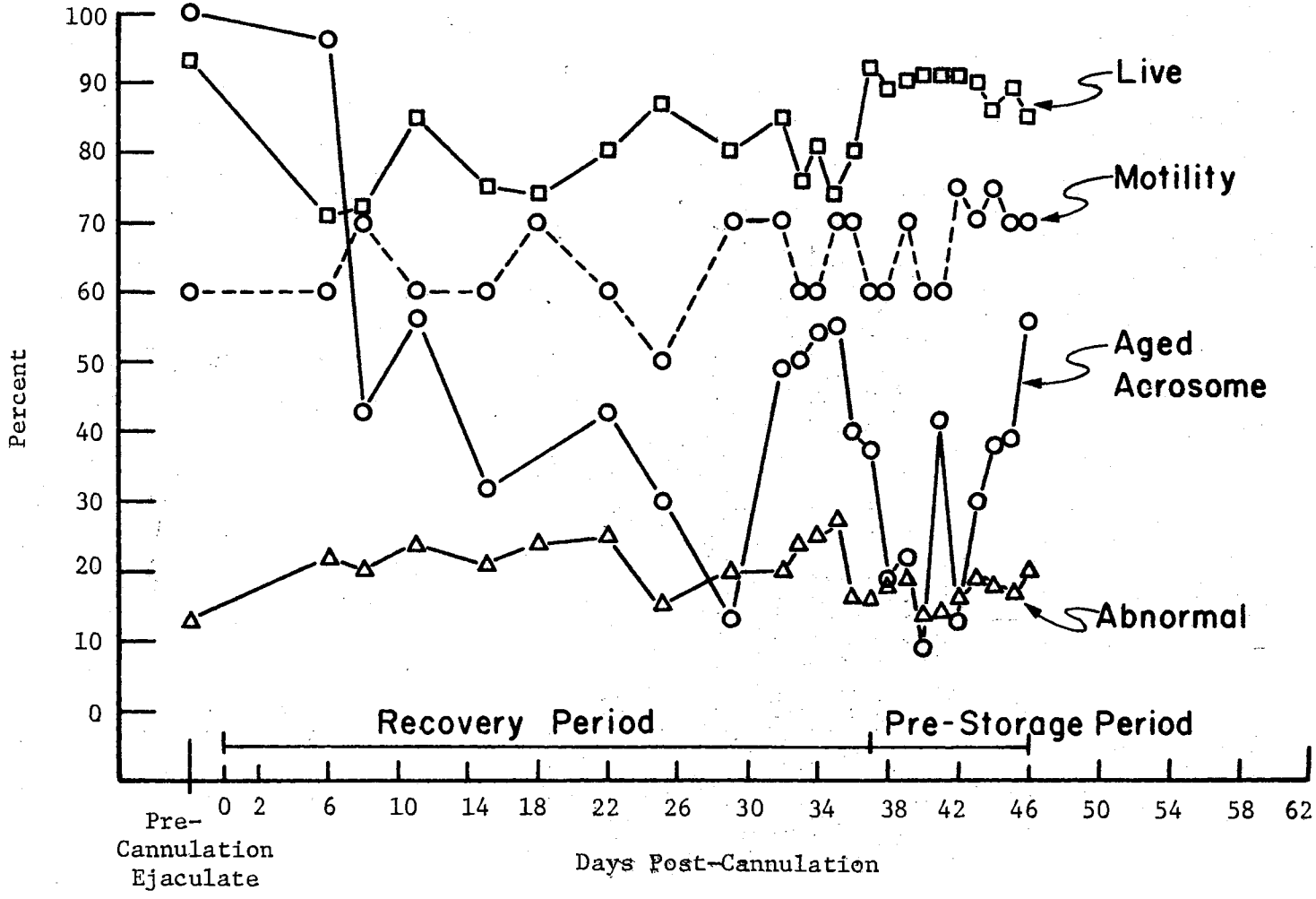

Figure 15. Bull 9 - Epididymal Sperm Characteristics Post Cannulation

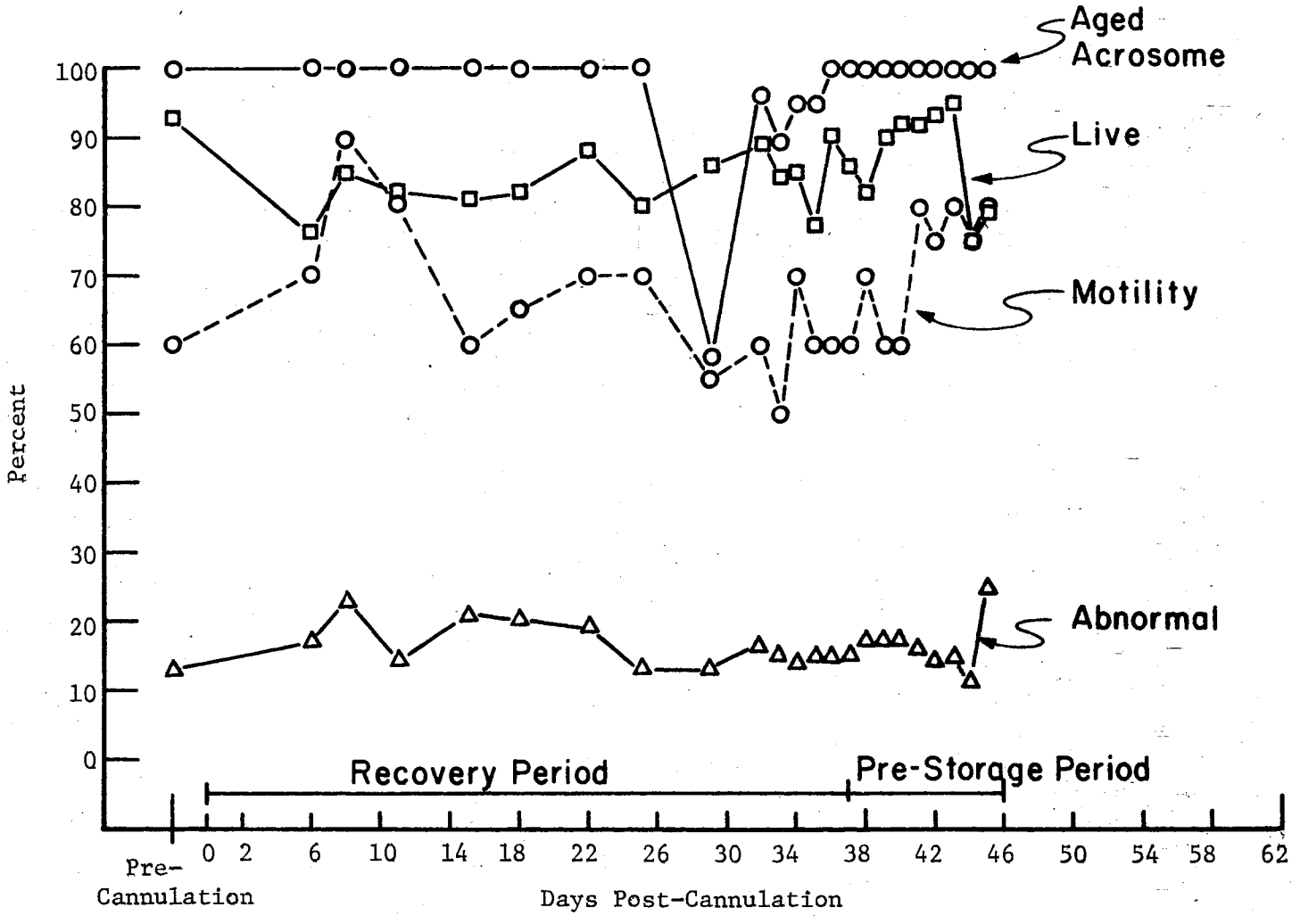

Figure 16. Bull 9 - Ejaculated Sperm Characteristics Post Cannulation

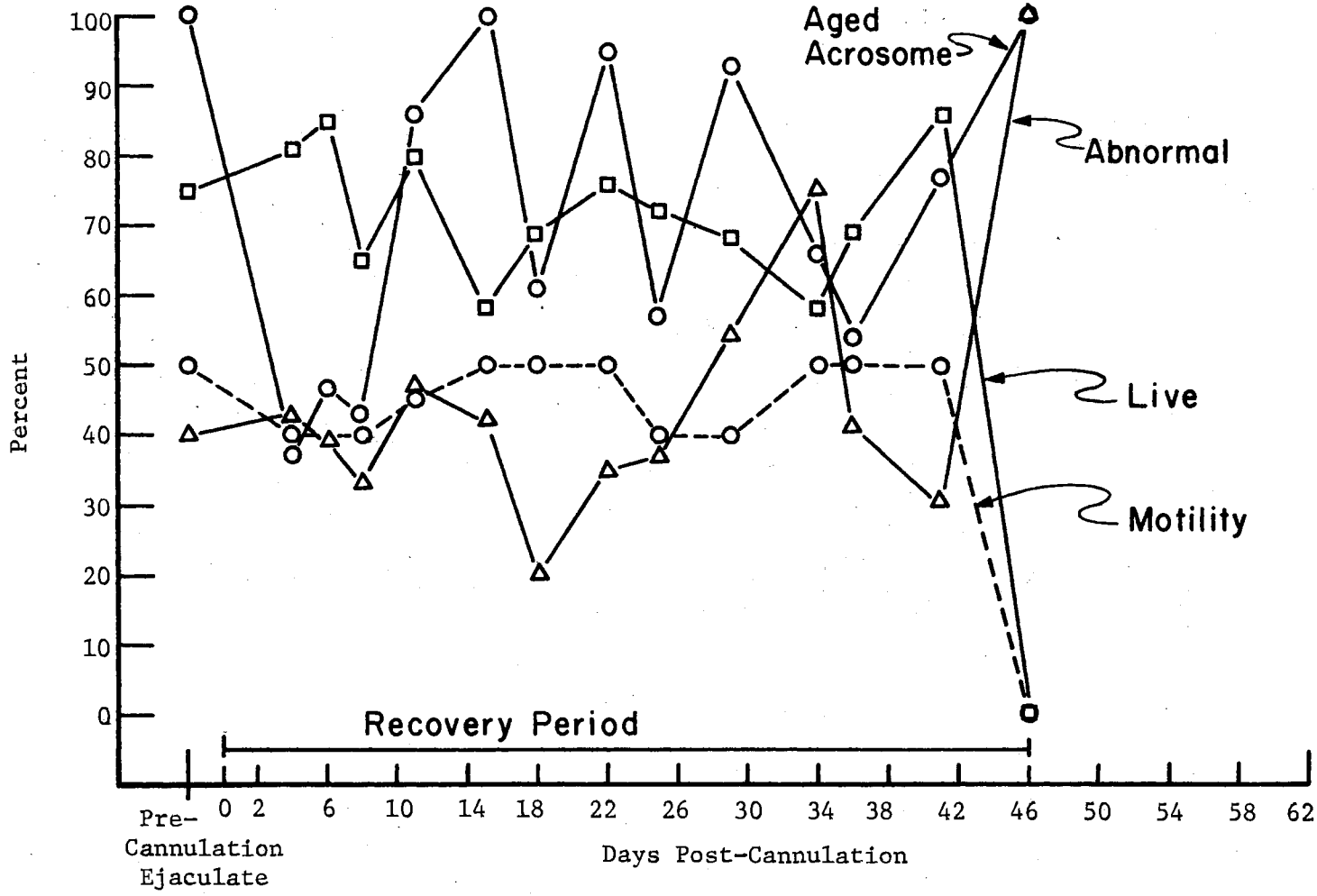

Figure 17. Bull 10 - Epididymal Sperm Characteristics Post Cannulation

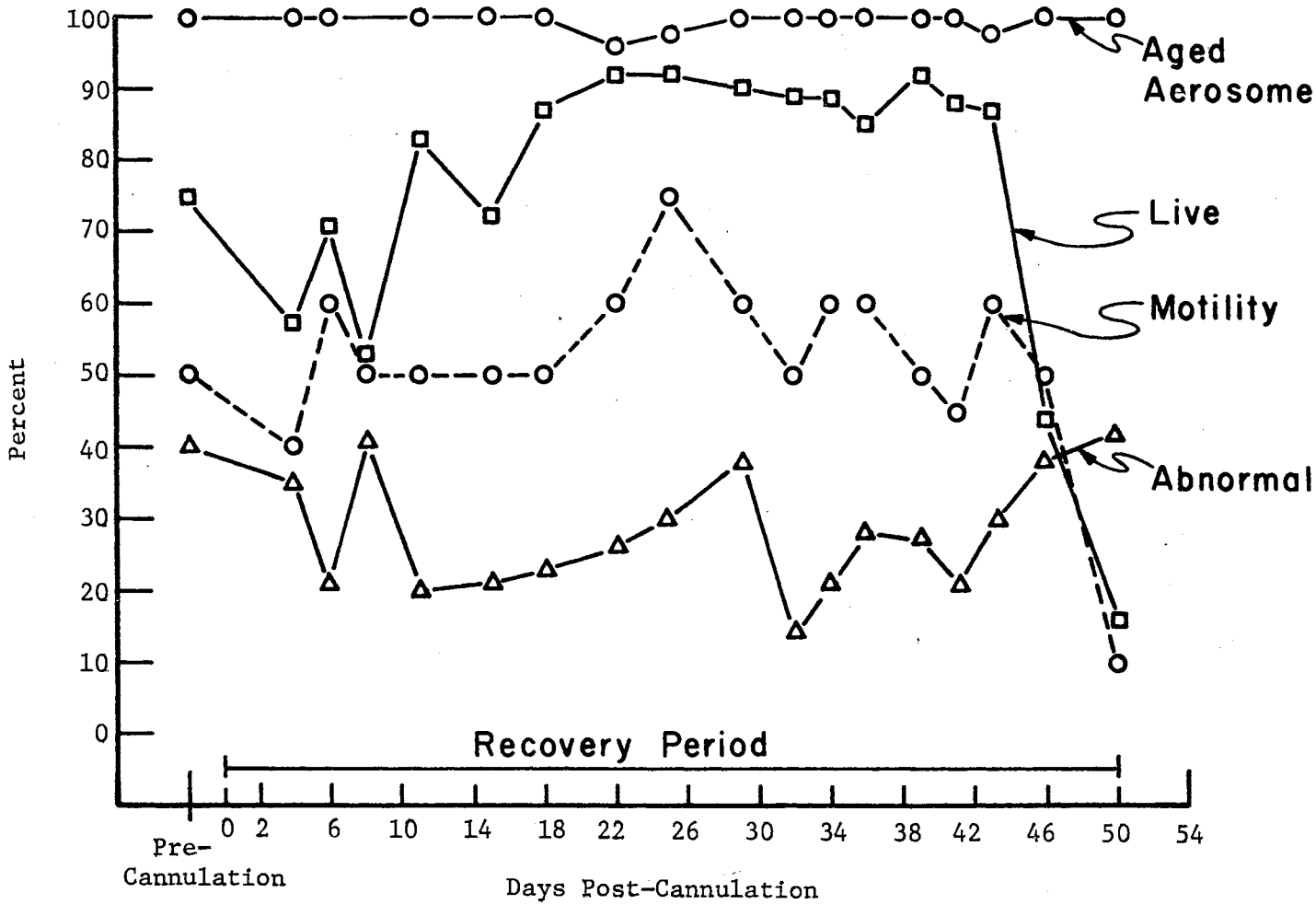

Figure 18. Bull 10 - Ejaculated Sperm Characteristics Post Cannulation

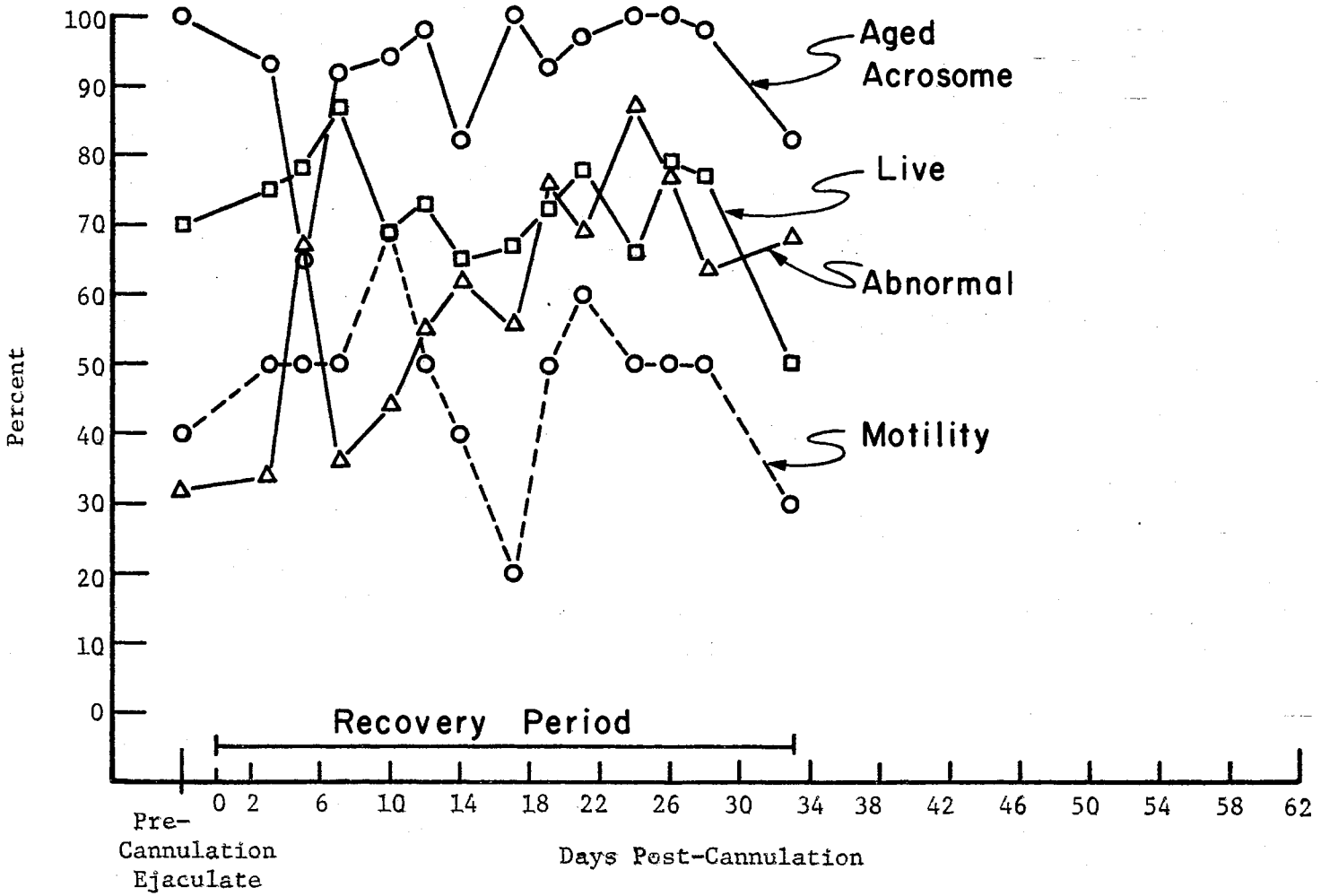

Figure 19. Bull 11 - Epididymal Sperm Characteristics Post Cannulation

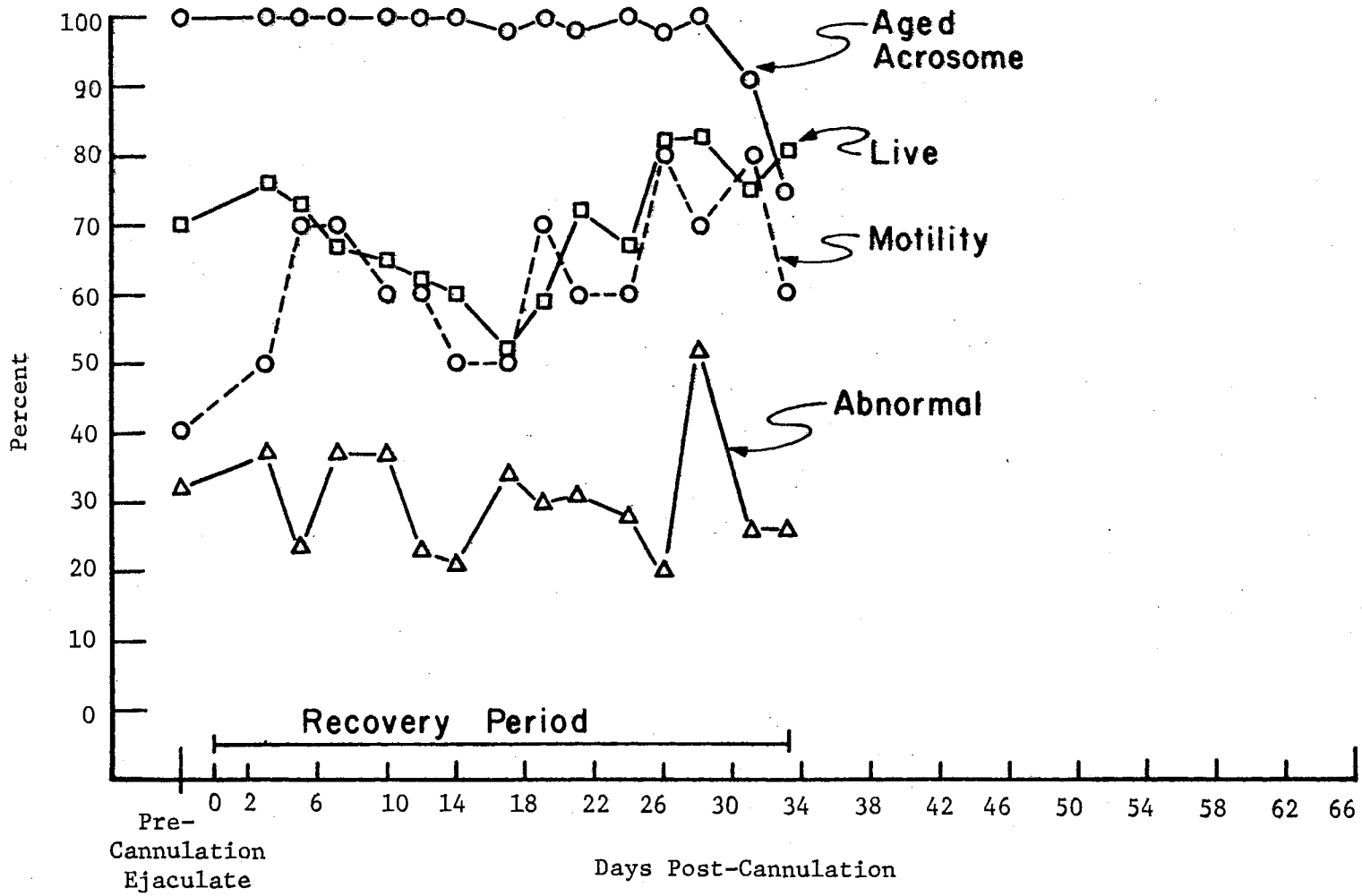

Figure 20. Bull 11 - Ejaculated Sperm Characteristics Post Cannulation

sperm characteristics may help determine the effects of cannulation. The within bull comparisons of the epididymal and ejaculate sperm characteristics are given in Table VII and show much variation between bulls. Because of the large variations between bulls, and to help summarize these results, the data were pooled within sperm type for the storage and non-storage groups and compared in Table VIII. In the pooled ejaculate characteristics, the percent motility and live sperm were significantly lower for the non-storage group than the storage group; and there was a trend for the percent abnormals to be higher. This would indicate that the storage group had better quality sperm than the non-storage group.

In the epididymal sperm characteristics, the non-storage group was significantly ($P < .01$) higher in percent abnormal (47.8 vs. 18.1%) and aged acrosomes (76.2 vs. 45.9%) with a trend for being lower in percent motility and live. This suggests that the greater degree and duration of inflammation in the non-storage group, as indicated in Table VI, may have caused this poorer quality semen.

Table IX gives a comparison of epididymal and ejaculated sperm characteristics within bull groups and overall groups. In the storage group (which was the least effected by surgery as shown in Table VI), there was a trend for the epididymal percent and rate of motility to be lower than the ejaculate. The percent live and abnormals were similar between the epididymal and ejaculate samples. In the non-storage group, the epididymal sperm was significantly lower in percent and rate of motility and higher ($P < .01$) in percent abnormals (47.8 vs. 23.3%). This again indicates the lower quality of the epididymal sperm of the non-storage bulls, probably due to their greater swelling after cannula-

TABLE VII

COMPARISONS OF EPIDIDYMAL AND EJACULATE SPERM CHARACTERISTICS WITHIN BULL DURING THE RECOVERY PERIOD[a]

Bull No.	Sperm Type[b]	Length of Period (Days)	No. of Samples	Motility (%)	Rate of Motility	Live (%)	Abnormal (%)	Aged Acrosome[c] (%)
Bull 3								
	Eja.	21	3	66.7 ± 6.67	3.17 ± 0.17	76.3 ± 8.29	21.7 ± 8.97	
	Epid.	21	3	63.3 ± 8.82	2.83 ± 0.33	77.3 ± 6.74	18.3 ± 2.33	29.3 ± 12.70
	Diff.			3.4	.34	1.0	3.4	
Bull 4								
	Eja.	69	13	55.8 ± 3.57	3.12 ± 0.08	56.9 ± 4.38	21.2 ± 1.00	
	Epid.	69	10	25.2 ± 5.48	1.95 ± 0.30	55.1 ± 6.83	44.9 ± 7.85	79.4 ± 5.77
	Diff.			30.6**	1.17**	1.8	23.7*	
Bull 5								
	Eja.	69	18	59.4 ± 2.49	3.17 ± 0.12	45.6 ± 5.41	13.4 ± 1.59	
	Epid.	69	18	43.1 ± 2.83	2.67 ± 0.19	73.2 ± 4.30	39.3 ± 2.71	63.1 ± 4.87
	Diff.			16.3**	0.5*	27.6**	25.9**	
Bull 6								
	Eja.	29	9	64.4 ± 3.17	3.22 ± 0.12	75.3 ± 2.28	10.8 ± 1.30	
	Epid.	29	9	60.6 ± 4.12	2.33 ± 0.22	70.2 ± 5.59	11.4 ± 2.27	48.3 ± 6.02
	Diff.			3.8	0.89**	5.1	0.6	
Bull 7								
	Eja.	40	9	70.6 ± 2.69	3.56 ± 0.06	86.8 ± 1.71	21.2 ± 2.27	
	Epid.	40	9	37.2 ± 5.34	1.72 ± 0.17	77.9 ± 3.23	20.8 ± 4.98	60.0 ± 6.6
	Diff.			33.4**	1.84**	8.9*	0.4	
Bull 9								
	Eja.	36	13	66.2 ± 2.95	3.35 ± 0.09	83.5 ± 1.20	16.6 ± 0.90	
	Epid.	36	13	63.8 ± 1.80	3.23 ± 0.12	78.5 ± 1.45	21.8 ± 1.00	45.9 ± 5.35
	Diff.			2.4	0.12	5.0*	5.2**	
Bull 10								
	Eja.	50	17	51.8 ± 3.26	3.29 ± 0.06	75.7 ± 5.21	28.0 ± 2.02	
	Epid.	50	13	41.9 ± 3.74	2.19 ± 0.22	66.7 ± 6.11	45.8 ± 5.80	70.5 ± 6.27
	Diff.			9.9	1.1**	9.0	17.8*	
Bull 11								
	Eja.	35	14	63.6 ± 2.69	3.46 ± 0.04	70.3 ± 2.46	30.4 ± 2.30	
	Epid.	35	13	47.7 ± 3.43	2.85 ± 0.12	72.0 ± 2.51	61.0 ± 4.40	91.8 ± 2.80
	Diff.			15.9**	0.61**	1.7	30.6**	

[a]Values are mean ± standard errors.

[b]Eja. - Ejaculate; Epid. - Epididymal; Diff. - Difference.

[c]Aged Acrosome values in ejaculate omitted due to technique error.

*($P < .05$).

**($P < .01$).

TABLE VIII

COMPARISON OF BULL GROUPS FOR EPIDIDYMAL AND EJACULATE SPERM CHARACTERISTICS DURING THE RECOVERY PERIOD[a]

	No. of Samples	Motility (%)	Rate of Motility	Live (%)	Abnormal (%)	Aged Acrosome[b] (%)
Ejaculate						
Storage Group	34	67.0 ± 1.31	3.33 ± 0.09	80.5 ± 2.79	17.6 ± 2.53	
Non-Storage Group	62	57.7 ± 2.52	3.26 ± 0.08	62.1 ± 6.78	23.3 ± 3.82	
Difference		9.3**	0.07	18.4*	5.7	
Epididymal						
Storage Group	34	56.2 ± 6.38	2.53 ± 0.33	76.0 ± 1.94	18.1 ± 2.34	45.9 ± 6.33
Non-Storage Group	54	39.5 ± 4.92	2.40 ± 0.21	66.8 ± 4.13	47.8 ± 4.64	76.2 ± 6.18
Difference		16.7	0.11	9.2	29.7***	30.3**

[a] Values are means ± standard errors.

[b] Ejaculate aged acrosome values omitted due to technique error.

* ($P < .05$).

** ($P < .02$).

*** ($P < .01$).

TABLE IX

COMPARISON OF EPIDIDYMAL AND EJACULATE SPERM CHARACTERISTIC DURING THE RECOVERY PERIOD WITHIN BULL GROUPS[a]

Sperm Type	No. of Samples	Motility (%)	Rate of Motility	Live (%)	Abnormal (%)	Aged Acrosome[b] (%)
Storage Group						
Ejaculate	34	67.0 ± 1.31	3.33 ± 0.09	80.5 ± 2.79	17.6 ± 2.53	
Epididymal	34	56.2 ± 6.38	2.53 ± 0.33	76.0 ± 1.94	18.1 ± 2.34	45.9 ± 6.33
Difference		10.8	0.8	4.5	0.5	
Non-Storage Group						
Ejaculate	62	57.7 ± 2.52	3.30 ± 0.08	62.1 ± 6.78	23.3 ± 3.82	
Epididymal	54	39.5 ± 4.92	2.40 ± 0.21	66.8 ± 4.13	47.8 ± 4.64	76.2 ± 6.18
Difference		18.2**	0.9***	4.7	24.5***	
Overall Means						
Ejaculate	96	62.4 ± 1.42	3.30 ± 0.06	71.3 ± 3.67	20.5 ± 2.29	
Epididymal	88	47.9 ± 4.03	2.48 ± 0.19	71.4 ± 2.28	33.0 ± 2.60	61.0 ± 4.42
Difference		14.5***	0.82***	0.1	12.5***	

[a]Values are means ± standard errors.

[b]Ejaculate aged acrosome values omitted due to technique error.

*($P < .05$).

**($P < .02$).

***($P < .01$).

tion.

The overall means for the epididymal sperm were significantly lower than the ejaculate for percent and rate of motility. The percent abnormals were significantly ($P < .01$) higher for the epididymal sperm because of the high value for the non-storage group. In conclusion, it appears that the effects of cannulation on epididymal sperm characteristics depend on the degree and duration of swelling and inflammation of the surgical area. Most bulls showed some effects of the initial swelling by increased percent abnormals and decreased percent live during the recovery period. The ejaculate sperm was not noticeably affected by cannulation, but no determination on the acrosome state could be made.

Sperm Output and Effects of Cannulation

The cannulation technique of the vas deferens is a good method to estimate daily sperm output because no sperm are lost due to masturbation or urination; however, the resorption phenomenon in the epididymis still influences the output. Since daily volumes and concentration of epididymal sperm were obtained during the entire experimental period, good estimates of daily sperm output could be calculated. Also, estimates of ejaculate output were obtained through the non-cannulated system so comparisons between the two methods could be made. However, one must be concerned about the effects of surgery and swelling on sperm output via cannulation.

The sperm output data in this study was divided into three periods: recovery, pre-storage, and post-storage periods. The recovery period used for the sperm output data was the same as that used for the sperm characteristics. Since the length of the recovery period varied con-

siderably between the bulls, the recovery period for each bull was classified as that time interval when the epididymal sperm cells were recovering from the effects of swelling and while the bull was collected three times per week under the recovery period regime. The pre-storage period applied only to the four storage group bulls and was the 10-day period immediately before enforced storage of epididymal sperm when the bulls were ejaculated daily. The post-storage period involved only two bulls and was the 10-day period immediately after enforced storage when daily collections were obtained. The pre- and post-storage periods will be discussed in later sections.

A summary of the average output of epididymal sperm for each bull and the overall means for the recovery period are given in Table X. The first criterian that was met in summarizing the epididymal sperm output was that the cannula had to flow before that day's output was included. The volume during ejaculation refers to the volume obtained from the cannula during the entire ejaculation process. The total volume of the epididymal sperm is the volume during ejaculation plus the volume obtained in the previous 24-hour collection vial. For 130 samples, the average volume of epididymal sperm flowing from the cannula during ejaculation of eight bulls was 0.127 ± .025 ml. This is a sizeable quantity and should provide enough sperm cells to conduct biochemical and metabolism studies on fresh samples routinely. The overall mean daily total volume of sperm flowing through the cannula of eight bulls during 318 days was 0.265 ± .031. This volume is within the range reported by Tadmor and Schindler (1966) working with fistulas in the vas deferens in rams, but is slightly lower than the 0.3 to 0.6 ml reported by Bennett and Rowson (1963) and 0.4 to 0.8 ml reported by Amann (personal communi-

TABLE X

SUMMARY OF EPIDIDYMAL SPERM OUTPUT FROM ONE VAS DEFERENS DURING THE RECOVERY PERIOD[a]

Bull No.	No. of Samples	Volume During Eja.[b] (ml)	No. of Samples	Daily Total Volume[c] (ml)	Sperm Conc. per ml ($X10^7$)	Daily Sperm Output ($X10^7$)
3	8	0.120 ± 0.018	20	0.22 ± 0.032	445.1 ± 29.8	106.5 ± 17.30
4	20	0.128 ± 0.023	51	0.17 ± 0.019	90.0 ± 15.7	15.8 ± 3.13
5	28	0.160 ± 0.011	64	0.19 ± 0.016	372.2 ± 12.0	75.6 ± 8.12
6	11	0.082 ± 0.009	27	0.40 ± 0.037	460.8 ± 18.0	196.1 ± 22.36
7	16	0.038 ± 0.006	40	0.24 ± 0.017	506.9 ± 22.3	132.6 ± 11.55
9	17	0.268 ± 0.027	36	0.38 ± 0.044	434.9 ± 27.5	183.2 ± 27.20
10	17	0.066 ± 0.010	49	0.31 ± 0.033	365.4 ± 26.6	139.8 ± 19.00
11	13	0.151 ± 0.020	31	0.22 ± 0.038	354.2 ± 30.3	73.6 ± 10.90
Overall Mean	130	0.127 ± 0.025	318	0.265 ± 0.031	378.7 ± 45.3	115.4 ± 21.3

[a]Values are means ± standard errors.

[b]Volume obtained during each entire ejaculation process.

[c]Total daily volume obtained including volume during ejaculation and volume in previous 24-hour collection vial.

cation 1970) in bulls. The smaller quantity in the present study may be due to using young beef bulls instead of the mature dairy bulls that were used in other studies. The average concentration of the epididymal samples was 378.7 ± 45.3 x 10^7 per ml and the total daily sperm output was 115.4 ± 21.3 x 10^7. The large standard errors indicate the large amount of variation between bulls which was partially caused by the greater effects of cannulation on the non-storage group. These values are again lower than previous reports (Bennett and Rowson, 1963; Amann et al., 1963; Amann, personal communication 1970).

Table XI gives a summary of the average ejaculate output from one testis of each bull collected three times per week during the recovery period and the overall means. From the eight bulls and 134 collections, the average volume, sperm concentration and sperm output per ejaculate was 7.0 ± .37 ml, 29.5 ± 5.91 x 10^7 and 197.6 ± 37.2 x 10^7, respectively. Much variation was also observed between bulls for the ejaculate which may have been partially due to the difference in the response of the bulls to electroejaculation. Electroejaculation was used instead of the AV because the bulls had no previous collection experience. Since the concentration and output values represent only the sperm from one testis, they were doubled to compare with other ejaculated sperm studies. Converting this doubled output to a daily basis for comparisons, the daily output was 1.6 x 10^9. This is very comparable to the 1.57 x 10^9 output reported by Almquist and Cunningham (1967) for five 1½-year-old Angus and Hereford bulls collected three times per week. It is higher than the reports of Austin et al. (1960) who collected four 4-year-old Hereford bulls four times per week and Swierstra (1966) who collected seven 1½-year-old Shorthorn bulls three and one-half times per week. It was

TABLE XI

SUMMARY OF EJACULATE SPERM OUTPUT (3X/WK) FROM ONE TESTIS DURING THE RECOVERY PERIOD[a]

Bull	No. of Samples	Volume Per Eja. (ml)	Sperm Conc. per ml ($X10^7$)	Sperm Output Per Eja. ($X10^7$)
3	8	6.0 ± 0.62	13.2 ± 1.24	76.5 ± 7.6
4	24	7.4 ± 0.59	20.2 ± 3.29	150.8 ± 27.4
5	24	6.1 ± 0.46	20.6 ± 3.61	114.3 ± 21.3
6	11	8.6 ± 0.75	25.1 ± 2.60	200.6 ± 12.2
7	16	6.3 ± 0.51	27.4 ± 3.44	181.6 ± 32.8
9	17	6.1 ± 0.45	56.2 ± 8.54	334.2 ± 62.5
10	20	7.0 ± 0.62	55.2 ± 5.56	376.4 ± 51.5
11	14	8.4 ± 0.69	18.1 ± 2.4	146.2 ± 20.1
Overall Mean	134	7.0 ± 0.37	29.5 ± 5.91	197.6 ± 37.2

[a]Values are means and standard errors.

considerably lower than the 5.15×10^9 daily output of eight 3-year-old Holstein bulls collected six times per week (Amann and Almquist, 1962c).

The sperm output data during the recovery period was divided into bull groups within type and compared to determine if the degree of swelling and inflammation had an effect on sperm output (Table XII). For the epididymal sperm, there was a trend in the non-storage bull group for the volume and concentration to be about one third lower and the sperm output to be one-half that of the storage group. Since no pre-cannulation output data was obtained to determine differences between groups, one can only assume from these data that the lower output of the non-storage group was probably due to the greater swelling experienced by this group (Table VI). This assumption is substantiated to some extent by the similarity in the ejaculate sperm output of the two groups. These data would also suggest that unilateral cannulation of the vas deferens has little effect on the contralateral testis sperm output.

Comparisons were also made between the epididymal and ejaculate sperm output within bull groups and for the overall means (Table XIII). This table shows the large differences in volume and concentration between the epididymal and ejaculate samples. These differences are due to the mixing of seminal plasma with the epididymal sperm during ejaculation which markedly increases the volume and decreases the concentration. The sperm output per ejaculate collected three times per week was higher than the epididymal daily output in both groups and overall. However, when the output was compared on a daily basis in the storage group, the epididymal output was greater, 154.6×10^7 vs. 84×10^7, although the difference was not significant. This indicates that the ejaculate output was only 54% as great as the epididymal. This greater output of

TABLE XII

COMPARISON OF BULL GROUPS FOR EPIDIDYMAL AND EJACULATE OUTPUT DURING THE RECOVERY PERIOD[a]

Groups	Volume During Eja.[b] (ml)	Total Volume[c] (ml)	Sperm Conc. per ml ($X10^7$)	Sperm Output ($X10^7$)
Epididymal				
Storage	0.127 ± 0.05	0.31 ± 0.046	461.9 ± 15.9	154.6 ± 21.1
Non-Storage	0.126 ± 0.02	0.22 ± 0.032	295.5 ± 68.6	76.2 ± 25.3
Difference[d]	0.001	0.09	166.4	78.4
Ejaculate				
Storage	6.74 ± 0.61		30.5 ± 9.1	198.2 ± 52.9
Non-Storage	7.20 ± 0.48		28.5 ± 8.9	196.9 ± 60.4
Difference[d]	0.46		2.0	1.3

[a]Values are means ± standard errors.

[b]Volume obtained during each entire ejaculation process.

[c]Total daily volume obtained including volume during ejaculation and volume in previous 24-hour collection vial.

[d]All differences are non-significant.

TABLE XIII

COMPARISON OF EPIDIDYMAL AND EJACULATE SPERM OUTPUT WITHIN BULL GROUPS DURING THE RECOVERY PERIOD[a]

Sperm Type	No. of Samples	Volume (ml)	Sperm Conc. per ml ($X10^7$)	Sperm Output Per Sample[b] ($X10^7$)	Daily Sperm Output ($X10^7$)
Storage Group - 4 Bulls					
Epididymal	123	0.31 ± 0.05	461.9 ± 15.9	154.6 ± 21.1	154.6 ± 21.1
Ejaculate	52	6.75 ± 0.61	30.5 ± 9.1	198.2 ± 52.9	84.0 ± 26.9
Difference					70.6
Non-Storage Group - 4 Bulls					
Epididymal	195	0.22 ± 0.03	295.5 ± 68.6	76.2 ± 25.3	76.2 ± 25.3
Ejaculate	82	7.20 ± 0.48	28.5 ± 8.9	196.9 ± 60.4	75.7 ± 25.3
Difference					0.5
Overall Mean - 8 Bulls					
Epididymal	318	0.27 ± 0.03	378.7 ± 45.3	115.4 ± 21.3	115.4 ± 21.3
Ejaculate	134	6.97 ± 0.37	29.5 ± 5.91	197.6 ± 37.2	79.9 ± 17.2
Difference		6.7**	349.2**	82.2	35.5

[a]Values are means ± standard errors.

[b]Epididymal samples taken daily. Ejaculate samples taken three times per week.

**($P < .01$).

epididymal sperm was partially due to the higher frequency of collection out of the cannulated system and no loss of sperm through the reproductive tract. Amann et al. (1963) reported that the output through cannula was similar to ejaculate output if bulls are ejaculated twice daily. The daily sperm output in the non-storage group was quite similar between the epididymal and ejaculate because of the lower epididymal output. The overall daily epididymal sperm output tended to be higher than the ejaculate because of the influence of the storage group.

In summary, this study showed that fairly large volumes of epididymal sperm can be obtained regularly by this cannulation technique and the daily sperm output is greater than that collected during three times per week ejaculation (converted to daily basis) from the non-cannulated system. The greater degree of swelling and inflammation of the non-storage group bulls caused a marked decrease in epididymal sperm output but the swelling had little effect on the sperm output of the non-cannulated system.

Effects of Cannulation on Testis Weights and Histology

To determine the effects of cannulation on the testis and epididymal tissue, the bulls were castrated at the termination of each trial and the testes weighed and the tissue histologically examined. Table XIV gives a comparison of the weights of the cannulated testis and non-cannulated testis of each bull. The mean difference in testis weights within bull was analyzed for significance by the paired observation "t" test. The mean weight of the cannulated testis was significantly ($P < .01$) lower than that of the non-cannulated testis by 28 g. Assuming that both testes were equal in weight before cannulation, this sug-

TABLE XIV

COMPARISON OF TESTIS WEIGHTS OF CANNULATED AND NON-CANNULATED SYSTEMS WITH CANNULATION INTERVALS

Bull No.	Non-Cannulated Testis Weight (g)	Cannulated Testis Weight (g)	Difference (g)	Cannula Patency Length (Days)	Interval Between Cannulation and Castration (Days)
3	254.1	169.9	84.2	71	78
4	272.0	236.8	35.2	69	78
5	273.0	232.5	40.5	69	69
6	281.0	291.0	10.0	66	69
7	262.3	230.4	31.9	50	64
8	323.0	292.1	30.9	3	20
9	306.3	265.9	40.4	46	55
10	357.9	353.3	4.6	50	57
11	248.8	240.5	8.3	35	35
12	329.2	316.0	13.2	4	36
Means	290.8 ± 11.5	262.8 ± 16.5	27.9**		

**($P < .01$).

gests a degeneration of parenchyma tissue within the testis.

Table XV gives a summary of the tissue histology of the cannulated system. These histological findings supported the degeneration in the cannulated testis. All but one bull showed some degeneration of the germinal cells in the seminiferous tubules in the cannulated testis. The one exception was Bull 12 whose cannula flowed only 4 days and had slight swelling for only 5 days during the 36 day period from cannulation to castration. In contrast, the cannulated testis of Bull 8 showed much degeneration even though this cannula flowed only 3 days; but this bull experienced much swelling for 15 days during the 20 day period between cannulation and castration. These results suggest that degeneration of the germinal cells was the result of the swelling of the scrotum causing a heating effect on the testis rather than degeneration being caused by the continuous flow of the cannula for long periods of time.

This interpretation of the degeneration is supported by the reports on the effects of scrotal insulation and local heating on the testis (Van Demark and Free, 1969; Setchell et al., 1971; Samisoni and Blackshaw, 1971). These workers indicated that the primary spermatocyte stage was the most sensitive to increases in testicular temperature. The main degeneration observed in the present study was in the spermatocyte stage also with vacuole formation in the cytoplasm and nuclei disintegrating. Much variation was observed in the degree of degeneration and the number of tubules without spermatozoa. For example, the cannulated testes of Bulls 9 and 10 had only a few degenerating cells and all tubules had spermatozoa in them while the testis of Bull 8 had many degenerating cells and showed 85 to 90% of the tubules with no spermatozoa. The cannulated testis of seven bulls showed some portion of the semini

TABLE XV

SUMMARY OF TISSUE HISTOLOGY OF THE CANNULATED SYSTEMS

Bull No.	Testis		Cauda Epididymis		Vas Deferens	
	Degenerating Germ Cells[a]	Aspermic Tubules[a]	Shape of Duct[b]	Amount of Sperm in Duct[c]	Thickness of Epith. Lining[d]	Presence of Inflammation[e]
3	many	many	shrunken	small	increased	epith, submucosa, muscular wall.
4	few	some	shrunken	small	increased	epithelium
5	some	some	normal	small	normal	
6	some	some	normal	small	normal	submucosa
7	many	many	normal	variable	normal	
8	many	many	misshapen	small	normal	
9	few	none	granuloma[f]	variable	normal	muscular wall
10	few	none	normal	normal	increased	
11	some	some	shrunken	variable	increased	epithelium
12	none	none	misshapen	large	normal	

[a]Scale of values range from none to many.

[b]Appearance of wall of epididymal duct.

[c]Scale of values range from small to large.

[d]Epithelium was normal or increased in thickness compared to non-cannulated vas deferens.

[e]If inflammation was not observed, no entry was made in table.

[f]Indicates break in epididymal duct.

ferous tubules with no sperm formation.

The effect of cannulation on the cauda epididymal duct on the cannulated side varied (Table XV). In three bulls the duct appeared to be shrunken; in two bulls it was misshapen; and in five of the bulls the duct contained a smaller amount of sperm than in the duct on non-cannulated side. Bull 9 had a granuloma in the cauda which was caused by the duct breaking and an antibody reaction developing due to the sperm leaking into the tissues. This condition was probably caused by stopping the flow of the cannula for 9 days. The reaction of the vas deferens to cannulation was of a greater degree than the reaction observed in the cauda. In four bulls the cannulated vas deferens showed a marked increase in the thickness of the epithelial lining and in five bulls the vas showed signs of inflammation in the lining, submucosa or muscular wall. Six bulls had vas deferens that appeared normal but two of these bulls (8 and 12) had cannulae that flowed only 3 to 4 days and were castrated at 20 and 36 days after cannulation.

During castration the surgical area on the spermatic cord was examined for tissue reactions and in almost every bull a thick fibrous pocket (spermatocoele) containing leukocytes and sperm was located. Most of the leukocytes were neutrophiles and the sperm cells were tailless and capless. In two bulls the vas deferens showed deterioration at the site where sutured to the cannula collar and may have torn loose in two other bulls. Apparently there was a small leaking of sperm around the cannula at sometime which caused the tissue reaction and formation of the spermatocoele.

The histological examination of the non-cannulated systems (testis, cauda epididymis and vas deferens) showed all tissues were normal and

the testes had normal spermatogeneic activity. Bull 4 was the only bull that had a few degenerative cells in the seminiferous tubules. Since the histology showed that the unilateral cannulation had no apparent effect on the contralateral testis and sperm output, the association between ejaculate sperm output and testis weight was determined. The non-cannulated testis weight for the eight bulls was significantly ($P < .01$) correlated with the average ejaculate concentration ($r = .88$) and with average sperm output per ejaculate collected three times per week ($r = .90$). This output correlation is quite comparable to values of 0.84 and 0.91 (Amann and Almquist, 1962c) in dairy bulls and to 0.83 (Swierstra, 1966) in Shorthorn bulls. No correlations were calculated using the cannulated testis weights because of the storage treatment imposed on four bulls and the possible degeneration of testes after storage treatment.

In summary, unilateral cannulation resulted in a significant decrease in the cannulated testis weight and a degeneration of the spermatocytes in the seminferous tubules. The cauda epididymal duct was only slightly affected showing a decrease in size and misshapen form, but the vas deferens showed hyperplasia of the epithelial lining and inflammation in the mucosa and submucosa. A spermatocoele was formed at the cannulation site suggesting a chronic inflammatory reaction. The contralateral testis and epididymal tissue appeared normal and unaffected.

Effects of Cannulation on Bull Behavior

When a surgical procedure is performed in the scrotum and a device is attached to the outside, one would logically be concerned about possible adverse reactions of the animal to this experimentation. No ill

effects were observed on the eating and drinking habits of the bulls, and most bulls stayed in a fleshy condition during the experimental period. They were not permitted exercise during this period which might have been advantageous in keeping the cannula patent, but the swelling might have receded faster if exercise had been allowed. The bulls did not exhibit any signs of irritation or aggravation, such as kicking or nervousness because of the device. In fact, the cannula collection vials could be changed daily without a kick board behind the bull for most of the bulls after the first several days. It should be remembered that these bulls were range bulls and were not handled before the acclimatization period. The bulls became adjusted to the collection procedure so well that the cannula would begin flowing in some cases by just the appearance of the ejaculation equipment and technician. Also, occasionally the second bull would masturbate while the first bull was being collected. The electroejaculation of these bulls (at least 3 times per week) did not seem to cause any undesirable effects over a 70 day period. When some bulls were collected daily for 10 days, a slight sensitivity to the rectal probe was noticed. Some sensitivity in the scrotum was also noticed when the scrotum was swollen and after the cannula was clamped. The cauda on the cannulated side distended about 3 to 5 days after clamping the cannula closed and took about 3 days to return to normal after the cannula resumed flowing.

Pre-Storage Period

The data compiled during the 10-day pre-storage period were obtained from only the four bulls of the storage group (Bulls 3, 6, 7 and 9) that recovered sufficiently from the effects of cannulation to have

the storage treatment imposed on them. This pre-storage data was compared to the recovery period data to provide additional evidence for the effects of cannulation. This pre-storage data gives the best estimation of the typical epididymal sperm parameters since these sperm were the least affected by swelling and inflammation.

Evaluation of Sperm Characteristics

Comparisons of the pre-storage and recovery periods within bull for the epididymal and ejaculate sperm characteristics are given in Tables XVI and XVII. In most bulls the pre-storage epididymal and ejaculate sperm characteristics showed improved quality over the recovery data with the epididymal sperm showing the greatest improvement. These data were pooled over bulls within sperm type and compared in Table XVIII. For the epididymal characteristics, the percent live cells was significantly ($P < .01$) higher (76 to 86.5%) in the pre-storage period. The percent and rate of motility tended to be higher and the percent abnormals and aged acrosomes tended to be lower. These results would indicate that the epididymal sperm increased in quality after the recovery period; and they support the previous results of the adverse effects of cannulation. The pre-storage ejaculated sperm characteristics showed only slight improvement with a trend for increased percent motility and decreased percent abnormals. This would suggest that the ejaculate was only affected slightly by cannulation and swelling.

Table XIX compares the epididymal and ejaculated sperm characteristics during the pre-storage period. The percent motility of sperm of all four bulls was lower in the epididymal than in the ejaculate sample, but only in Bull 6 was the difference statistically significant ($P < .01$).

TABLE XVI

COMPARISON OF PRE-STORAGE AND RECOVERY PERIODS FOR EPIDIDYMAL SPERM CHARACTERISTICS OF BULLS OF THE STORAGE GROUP[a]

Period	No. of Samples	Motility (%)	Rate of Motility	Live (%)	Abnormal (%)	Aged Acrosome (%)
Bull 3						
Recovery	3	63.3 ± 8.82	2.83 ± .33	77.3 ± 6.74	18.3 ± 2.33	29.3 ± 12.67
Pre-Storage	10	61.5 ± 2.79	3.00 ± .11	89.3 ± 0.50	12.0 ± 1.07	25.5 ± 2.57
Diff.		1.8	0.17	12.0***	6.3*	3.8
Bull 6						
Recovery	9	60.6 ± 4.12	2.33 ± .22	70.2 ± 5.59	11.4 ± 2.27	48.3 ± 6.02
Pre-Storage	10	56.5 ± 2.69	3.10 ± .15	81.1 ± 2.06	15.0 ± 2.11	55.4 ± 3.28
Diff.		4.1	0.77***	10.9	3.6	7.1
Bull 7						
Recovery	9	37.2 ± 5.34	1.72 ± .17	77.9 ± 3.23	20.8 ± 5.0	60.0 ± 6.61
Pre-Storage	10	78.5 ± 2.59	2.20 ± .15	86.1 ± 1.83	12.9 ± 0.82	44.2 ± 2.88
Diff.		41.3***	0.48	8.2*	7.8	15.8*
Bull 9						
Recovery	13	63.8 ± 1.8	3.23 ± .12	78.5 ± 1.45	21.8 ± .99	45.9 ± 5.35
Pre-Storage	10	67.0 ± 2.0	2.90 ± .22	89.4 ± 0.72	17.0 ± .72	30.5 ± 4.63
Diff.		3.2	.33	10.9***	4.8***	15.4

[a]Values are means ± standard errors.

*(P < .05).

**(P < .02).

***(P < .01).

TABLE XVII

COMPARISON OF PRE-STORAGE AND RECOVERY PERIODS FOR EJACULATE SPERM CHARACTERISTICS OF BULLS OF THE STORAGE GROUP[a]

Period	No. of Samples	Motility (%)	Rate of Motility	Live (%)	Abnormal (%)
Bull 3					
Recovery	3	66.7 ± 6.67	3.17 ± .17	76.3 ± 8.29	21.7 ± 8.97
Pre-Storage	10	69.0 ± 2.96	3.25 ± .08	70.0 ± 4.92	12.9 ± 1.43
Diff.		2.3	.08	6.3	8.8
Bull 6					
Recovery	9	64.4 ± 3.17	3.22 ± .12	75.3 ± 2.28	10.8 ± 1.30
Pre-Storage	10	72.0 ± 2.81	3.60 ± .10	77.3 ± 3.56	7.5 ± 0.65
Diff.		7.6	.38*	2.0	3.3*
Bull 7					
Recovery	9	70.6 ± 2.69	3.56 ± .06	86.8 ± 1.71	21.2 ± 2.27
Pre-Storage	10	83.5 ± 1.07	3.50 ± 0	84.4 ± 1.65	16.3 ± 1.37
Diff.		12.9***	.06	2.4	4.9
Bull 9					
Recovery	13	66.2 ± 2.95	3.35 ± .09	83.5 ± 1.20	16.6 ± 0.90
Pre-Storage	9	71.1 ± 2.98	3.39 ± .07	87.1 ± 2.34	16.3 ± 1.26
Diff.		4.9	.04	3.6	0.3

[a]Values are means ± standard errors.

*(P < .05).

**(P < .02).

***(P < .01).

TABLE XVIII

COMPARISON OF PRE-STORAGE AND RECOVERY PERIODS FOR SPERM CHARACTERISTICS OF STORAGE GROUP[a]

Period	No. of Samples	Motility (%)	Rate of Motility	Live (%)	Abnormal (%)	Aged Acrosome[b] (%)
Epididymal						
Recovery	34	56.2 ± 6.38	2.53 ± .33	76.0 ± 1.94	18.1 ± 2.34	45.9 ± 6.33
Pre-Storage	40	65.9 ± 4.70	2.80 ± .20	86.5 ± 1.95	14.2 ± 1.12	38.9 ± 6.77
Diff.		9.7	.27	10.5***	3.9	7.0
Ejaculate						
Recovery	34	67.0 ± 1.31	3.33 ± .09	80.5 ± 2.79	17.6 ± 2.53	
Pre-Storage	39	73.9 ± 3.26	3.40 ± .08	79.7 ± 3.84	13.3 ± 2.10	
Diff.		6.9	0.07	0.8	4.3	

[a]Values are means ± standard errors.

[b]Ejaculate aged acrosome values omitted due to technique error.

*($P < .05$).

**($P < .02$).

***($P < .01$).

TABLE XIX

COMPARISON OF EPIDIDYMAL AND EJACULATE SPERM CHARACTERISTICS WITHIN BULL AND FOR GROUP FOR THE PRE-STORAGE PERIOD[a]

Sperm Type	No. of Samples	Motility (%)	Rate of Motility	Live (%)
Bull 3				
Ejaculate	10	69.0 ± 2.96	3.25 ± .08	70.0 ± 4.92
Epididymal	10	61.5 ± 2.79	3.00 ± .11	89.3 ± 0.50
Diff.		7.5	.25	19.3***
Bull 6				
Ejaculate	10	72.0 ± 2.81	3.6 ± .10	77.3 ± 3.56
Epididymal	10	56.5 ± 2.69	3.1 ± .15	81.1 ± 2.10
Diff.		15.5***	0.5**	3.8
Bull 7				
Ejaculate	10	83.5 ± 1.07	3.5 ± 0	84.4 ± 1.65
Epididymal	10	78.5 ± 2.59	2.2 ± .15	86.1 ± 1.83
Diff.		5.0	1.3***	1.7
Bull 9				
Ejaculate	9	71.1 ± 2.98	3.39 ± .07	87.1 ± 2.34
Epididymal	10	67.0 ± 2.00	2.90 ± .22	89.4 ± 0.72
Diff.		4.1	.49	2.3
Group Mean				
Ejaculate		73.9 ± 3.26	3.4 ± .08	79.7 ± 3.84
Epididymal		65.9 ± 4.70	2.8 ± .20	86.5 ± 1.95
Diff.		8.0	0.6*	6.8

[a]Values are means ± standard errors. *(P < .05). **(P < .02). ***(P < .01).

The mean percent motility of 66 percent for the group for epididymal sperm was higher than the 41 percent reported by Igboeli and Foote (1968) for four bulls and the 32 percent reported by Amann and Almquist (1962b) on 47 bulls. This data agrees with Zeltobrjuh et al. (1966) who reported that epididymal sperm were lower in motility than ejaculated, and is contrary to the report of Bennett and Dott (1966) that epididymal sperm decreases in motility when mixed with seminal plasma.

The rate of motility of epididymal sperm was lower than the ejaculate sperm in all bulls with the differences significant in Bulls 6 and 7 and in the group means. This would suggest that the mixing of seminal plasma with the epididymal sperm stimulates the sperm into vigorous movement. Many theories have been expressed on what causes the motility of epididymal sperm such as dilution, oxygen, substrate, etc. Observations in this study suggest that dilution combined with an increase in oxygen and a slight increase in temperature causes an increase in rate of epididymal sperm motility.

The percentage of live sperm in the epididymal sample was slightly higher than in the ejaculate sample in all bulls with Bull 3 showing a significantly higher value. The difference in the group mean of the epididymal and ejaculate sperm (86.5 vs 79.7%) was non-significant. White et al. (1959) reported that the epididymal and ejaculated sperm in rams was similar in percent live; and Bennett and Dott (1966) found that seminal plasma when mixed with epididymal sperm of bulls did not change the percent live. However, Faulkner et al. (1968) and Alexander et al. (1971) reported that when the seminal vesicles were removed in bulls a decrease in percent live sperm resulted indicating a need for this glandular secretion.

Table XX gives the comparisons of epididymal and ejaculate sperm abnormal morphology during the pre-storage period. The abnormal morphology was categorized into percentages of freeheads, abnormal heads and midpieces, gross abnormal tails and abnormal tail ends. The epididymal sperm was quite similar to the ejaculate in all the above classes. A few significant differences were found in the various classes but no trend was established over bulls within class. The group mean percentages of the different classes for the epididymal and ejaculate sperm were very similar with no significant differences between them. This indicates that the type of abnormal morphology is not influenced by the seminal plasma. The mean epididymal values for the various classes are comparable to the values reported by Amann and Almquist (1962b) for 47 normal dairy bulls.

The difference in percent total abnormals between the epididymal and ejaculate sperm varied among the bulls with Bull 6 showing a significantly ($P < .01$) higher percent for the epididymal sperm while Bull 7 showed a significantly ($P < .05$) lower percent. The group means were quite similar indicating that there was probably little difference in abnormal morphology between the epididymal and ejaculated sperm. This statement is true only when the distal cytoplasmic droplets are considered normal for epididymal sperm in the cauda. A range of from 20 to 50 percent of the epididymal sperm had distal droplets in this study which agrees with reports by White et al. (1959), Amann and Almquist (1962b) and Bennett and Rowson (1963). Faulkner et al. (1968) and Bennett and Dott (1966) indicated an increase in sperm abnormalities after seminal vesiculectomy and mixing epididymal sperm with seminal plasma, respectively. However, these reports do not agree with the

TABLE XX

COMPARISON OF EPIDIDYMAL AND EJACULATE SPERM ABNORMAL MORPHOLOGY WITHIN BULL DURING THE PRE-STORAGE PERIOD[a]

Sperm Type	No. of Samples	Freeheads (%)	Abnormal Heads and M.P. (%)	Gross Abn. Tails (%)	Abn. Tail Ends (%)	Total Abnormal (%)	Aged Acrosome[b] (%)
Bull 3							
Epididymal	10	1.5 ± .22	4.1 ± .55	1.8 ± .29	4.6 ± 0.90	12.0 ± 1.08	25.5 ± 2.57
Ejaculate	10	2.2 ± .25	4.9 ± .64	1.9 ± .28	3.9 ± 1.11	12.9 ± 1.43	
Diff.		0.7	0.8	0.1	0.7	0.9	
Bull 6							
Epididymal	7	1.4 ± .37	5.7 ± .42	3.1 ± .63	1.0 ± 0.22	11.3 ± 0.92	57.6 ± 4.10
Ejaculate	7	1.3 ± .18	3.9 ± .51	1.0 ± .22	0.6 ± 0.09	6.7 ± 0.36	
Diff.		0.1	1.8*	2.1	0.4	4.6***	
Bull 7							
Epididymal	10	2.3 ± .42	2.6 ± .27	3.3 ± .33	4.7 ± 0.87	12.9 ± 0.82	44.2 ± 2.88
Ejaculate	10	2.2 ± .25	4.7 ± .56	4.7 ± .62	4.7 ± 1.04	16.3 ± 1.37	
Diff.		0.1	2.1***	1.4	0	3.4*	
Bull 9							
Epididymal	10	4.2 ± .59	4.6 ± .40	2.8 ± .25	5.4 ± 0.93	17.0 ± 0.71	30.5 ± 4.63
Ejaculate	9	3.7 ± .53	4.3 ± .50	1.8 ± .22	6.6 ± 1.20	16.3 ± 1.25	
Diff.		.5	0.3	1.0**	1.2	0.7	
Group Mean							
Epididymal		2.4 ± .65	4.3 ± .64	2.8 ± .33	3.9 ± 0.99	13.3 ± 1.28	38.9 ± 6.77
Ejaculate		2.4 ± .50	4.5 ± .22	2.4 ± .81	4.0 ± 1.25	13.1 ± 2.26	
Diff.		0	0.2	0.4	0.1	0.2	

[a] Values are means ± standard errors.

[b] Ejaculate aged acrosome values omitted due to technique error.

* ($P < .05$). ** ($P < .02$). *** ($P < .01$).

present study or the work of Amann and Almquist (1962b) who reported, in an exhaustion study, that the cauda epididymal sperm was similar to the ejaculate sperm.

The group percent aged acrosomes in the epididymal sperm was about 40 percent and consisted of mostly roughened and disintegrating caps. Since the acrosome has been reported as being very labile (Awa, 1970; Saacke, 1970; Wells and Awa, 1970), this rather high percent may have been due to the long lasting effects of post-surgical swelling on the acrosome state. In summary, this study indicated that in the epididymal samples the percent and rate of sperm motility tended to be lower and the percent live sperm slightly higher than in the ejaculate samples, but the percent abnormals were similar.

Evaluation of Sperm Output

The sperm output data for the pre-storage period was compared to that of the recovery period within bull within sperm type. Only small nonsignificant differences were found between the two periods within bull for the epididymal and ejaculate sperm output. The pre-storage and recovery data for all bulls in the group were pooled and compared in Table XXI. The daily sperm volume, concentration and output in the epididymal sample showed very little change between the recovery and pre-storage periods. Because of this similarity, the comparison of this study with other studies was discussed under the recovery period. This similarity indicates that cannulation had little effect on the epididymal sperm output for the storage group bulls. However, the sperm concentration and sperm output per ejaculate during the pre-storage period tended to be lower than during the recovery period. This difference is probably the

TABLE XXI

COMPARISON OF PRE-STORAGE AND RECOVERY PERIODS FOR SPERM OUTPUT OF THE STORAGE GROUP[a]

Period	No. of Samples	Total Volume (ml)	Sperm Conc./ml ($\times 10^7$)	Sperm Output/sample ($\times 10^7$)
Epididymal				
Recovery	123	0.31 ± .046	461.9 ± 15.9	154.6 ± 21.1
Pre-Storage	40	0.30 ± .036	491.8 ± 49.4	150.2 ± 17.4
Diff.[d]		0.01	29.9	4.4
Ejaculate				
Recovery[b]	52	6.74 ± .61	30.5 ± 9.1	198.2 ± 52.9
Pre-Storage[c]	39	6.64 ± .39	19.2 ± 5.3	117.3 ± 26.1
Diff.[d]		0.1	11.3	80.9

[a]Values are means ± standard errors.

[b]Samples collected three times per week.

[c]Samples collected daily.

[d]All differences non-significant.

result of the bulls being collected three times per week during the recovery period and collected daily during pre-storage. As frequency of collection increases, the sperm output per ejaculate decreases (Almquist and Cunningham, 1967; Amann, 1969). Therefore, this difference in output cannot be attributed to the effects of cannulation.

Table XXII gives the comparisons of epididymal and ejaculate daily sperm output during the pre-storage period. Of course, the epididymal sperm volume was considerably lower and its concentration considerably higher than the ejaculate for all bulls because of the lack of seminal plasma in the epididymal samples. The daily sperm output of the epididymal samples were higher than the ejaculate in all but one bull for the pre-storage period. The group mean epididymal daily output was non-significantly greater than the daily ejaculate output by 33 x 10^7 sperm. This means that the output through the non-cannulated system was only 78% of the output via the cannula. This suggests that some sperm are lost from the normal reproductive tract even with daily collections possibly by urination or masturbation. The pre-storage epididymal daily sperm output for these four bulls of 150 x 10^7 is higher than the mean recovery period output of all eight bulls (115 x 10^7), but is still considerably lower than reports of output from dairy bull cannulations. The ejaculate daily sperm output doubled was 2.34 x 10^9, which was higher than the calculated daily output (1.6 x 10^9) using the three times per week collection data. This suggests that the three times per week collection frequency is an underestimate of sperm output and is not often enough to get a good estimate of sperm production. This is in agreement with the recommendations of Amann and Almquist (1961) that daily collections be used to estimate sperm production. This daily sperm output is

TABLE XXII

COMPARISON OF EPIDIDYMAL AND EJACULATE SPERM OUTPUT WITHIN BULL AND FOR GROUP FOR THE PRE-STORAGE PERIOD[a]

Sperm Type	No. of Samples	Daily Total Vol. (ml)	Sperm Conc./ml ($\times 10^7$)	Daily Sperm Output ($\times 10^7$)
Bull 3				
Epididymal	10	0.23 ± .028	405.1 ± 39.8	99.4 ± 19.9
Ejaculate	10	7.0 ± .81	8.6 ± 1.41	59.5 ± 11.3
Diff.				39.9
Bull 6				
Epididymal	10	0.40 ± .023	435.4 ± 20.5	173.9 ± 14.8
Ejaculate	10	6.6 ± .83	23.7 ± 3.85	150.6 ± 26.5
Diff.				23.2
Bull 7				
Epididymal	10	0.27 ± .025	627.9 ± 25.8	171.0 ± 18.2
Ejaculate	10	7.4 ± .64	12.7 ± 3.17	88.0 ± 20.8
Diff.				83***
Bull 9				
Epididymal	10	0.31 ± .017	498.6 ± 19.8	156.6 ± 10.8
Ejaculate	9	5.6 ± .42	31.9 ± 6.21	171.0 ± 37.1
Diff.				14.4
Group Mean				
Epididymal	40	0.30 ± .036	491.7 ± 49.4	150.2 ± 17.4
Ejaculate	39	6.64 ± .389	19.2 ± 5.29	117.3 ± 26.1
Diff.		6.34***	472.5***	32.9

[a]Values are means ± standard errors.

*($P < .05$).

**($P < .02$).

***($P < .01$).

greater than the 1.71×10^9 output reported by Almquist and Cunningham (1967) for five 1½-year-old Angus and Hereford bulls collected six times per week. But this output was less than the 3.9×10^9 output of 11 mature Angus and Hereford bulls collected six times per week (Almquist, 1969) and less than the 3.5×10^9 output of eight 2½-year-old Hereford bulls collected nine times per week (Hupp et al., 1962).

In conclusion, the small volume of very concentrated epididymal sperm from the cannulae provided a greater daily sperm output than that obtained by daily ejaculation from the non-cannulated system.

Post-Storage Period

The 6-day storage treatment was imposed on four bulls to determine the effects of in vivo storage on the characteristics and output of epididymal sperm. This was accomplished by comparing the pre-storage data with the post-storage data within bulls. Since post-storage data could be obtained on only two bulls, this limited the accuracy of determining the storage effects. Two other bulls had periods of in vivo storage of epididymal sperm so some additional evidence was obtained on storage effects.

Evaluation of Sperm Characteristics

The sperm characteristics for each day of the post-storage period were compared to the mean pre-storage characteristics to determine the nature of the changes in the sperm cell and when they occurred. By comparing the number of days required for the sperm cell to pass through the epididymis with the post-storage day when the change was observed, one can determine fairly accurately the region in the epididymis where the

change occurred. The epididymal transport time of a sperm cell in the bull has been reported at 8 to 11 days by Noyes (1968) in a review on sperm transport. This transport time can be shortened by increasing the frequency of ejaculation according to Koefoed-Johnson (1960). Therefore, the transport time in the epididymis on the cannulated side was estimated at 7 days because of the daily ejaculations.

Table XXIII gives the comparison of each post-storage day to the pre-storage mean for the epididymal and ejaculate sperm characteristics of Bulls 3 and 6. The blank cells in this table mean that no samples could be obtained. Figures 21 and 22 give a graphic illustration of the epididymal sperm characteristics for the pre- and post-storage days. The percent motility of the epididymal sperm of Bull 3 showed a highly significant ($P < .01$) increase on days 1 and 2 post-storage and a significant decrease on day 9 while in Bull 6 a slight increase was observed on day 4. This bull exhibited some swelling in the scrotum on the cannulated side on days 8 and 9 post-storage which may have caused the poorer quality sperm on these days. These results suggest a tendency for sperm stored 6 days in the cauda to increase in motility during the first 4 days post-storage. The rate of motility showed a significant decrease on days 3, 4 and 9 post-storage in Bull 3 while no significant change from the pre-storage mean was found in Bull 6.

The epididymal percent live sperm (Figure 22) of Bull 3 showed a significant ($P < .01$) increase on day 2 and day 7 and a significant decrease on days 3, 4, 5, 6, 8 and 9. Bull 6 showed a decrease in percent live on day 1 and an increase on day 5. The results of Bull 3 suggest that storage in the distal region of the cauda may have caused an enhancement of the percent live sperm but storage in other areas of the

TABLE XXIII

COMPARISON OF SPERM CHARACTERISTICS FOR EACH DAY POST-STORAGE TO THE PRE-STORAGE MEANS[a]

		Epididymal			Ejaculate		
		Motility (%)	Rate of Motility	Live (%)	Motility (%)	Rate of Motility	Live (%)
Bull 3							
Pre-Storage	n=10	61.5 ± 2.79	3.0 ± .11	89.3 ± .50	69.0 ± 2.96	3.3 ± 0.1	70 ± 4.92
Post-Storage[b]	Day						
	1	90**	3.5	90	60	3.5	44*
	2	90**	3.0	94**	70	2.5*	75
	3	60	2.0**	84**	70	2.5*	70
	4	60	1.5**	82**	--	----	---
	5	70	2.5	81**	80	4.0*	63
	6	70	3.0	84**	--	----	---
	7	70	3.0	94**	70	3.5	76
	8	65	3.0	66**	--	----	---
	9	40**	2.0**	81**	60	3.0	29**
Bull 6							
Pre-Storage	n=10	57.0 ± 2.81	3.1 ± .15	81.2 ± 2.24	72 ± 2.81	3.6 ± 0.1	79.3 ± 3.88
Post-Storage[b]	Day						
	1	50	2.5	66*	75	3.5	76
	2	65	3.5	81	75	3.5	57*
	3	--	---	---	70	3.5	61***
	4	70***	3.5	77	70	3.5	39**

TABLE XXIII (Continued)

	Epididymal			Ejaculate		
	Motility (%)	Rate Motility	Live (%)	Motility (%)	Rate of Motility	Live (%)
Day						
5	60	2.5	93*	70	3.5	92
6	60	3.5	86	70	3.5	86
7	--	---	---	65	3.5	--
8	60	3.0	90	60	3.5	89
9	60	3.5	91***	70	3.5	82
10	60	3.5	90	70	3.5	85

[a]Values are means ± standard errors.

[b]Missing values indicate no samples obtained.

*($P < .05$).

**($P < .01$).

***($P < 0.1$).

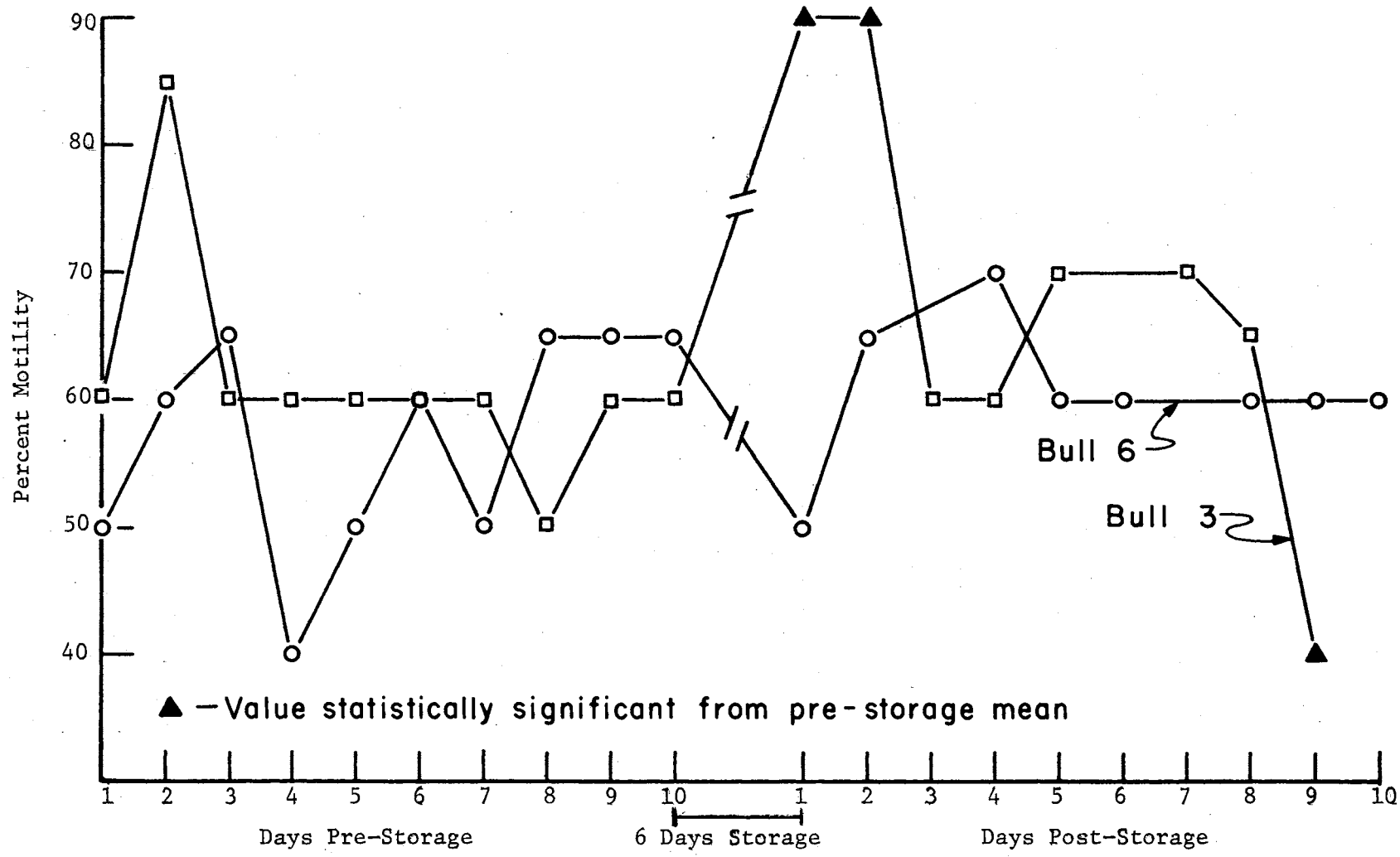

Figure 21. Percent Motility of Epididymal Sperm Pre- and Post-Storage for Bulls 3 and 6

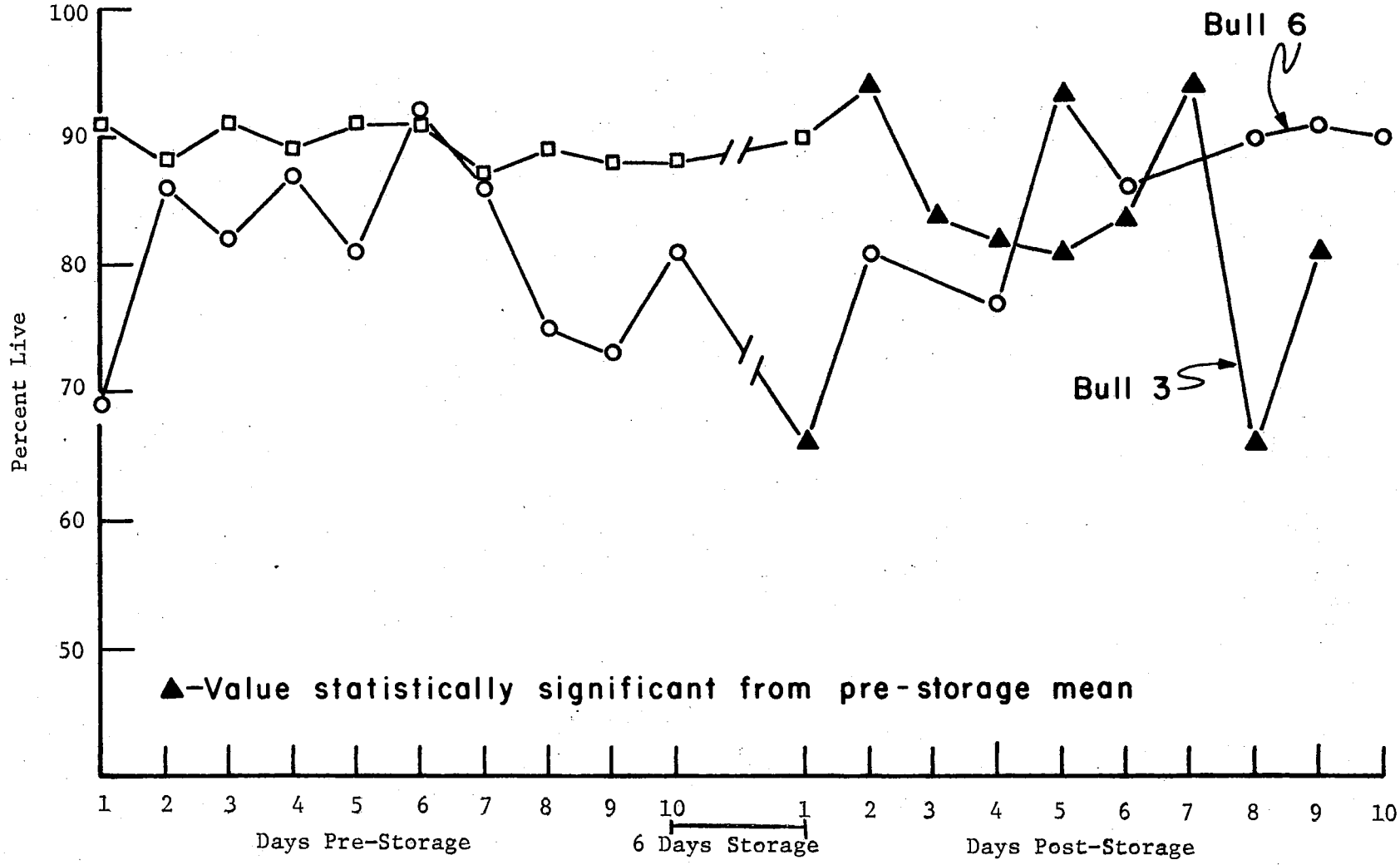

Figure 22. Percent Live Epididymal Sperm Pre- and Post-Storage for Bulls 3 and 6

epididymis was detrimental. However, the results of Bull 6 do not support this reasoning. No significant changes were observed for the percent motility of the ejaculate sperm in Bulls 3 and 6. The rate of motility in Bull 3 showed a decrease on days 2 and 3 and an increase on day 5 while no change was found in Bull 6. The percent live of Bull 3 decreased significantly on day 1 and 9. The ejaculate percent live of Bull 6 showed decreases on days 2, 3 and 4 post-storage. It appears from these results that there is much variation in the reaction of bulls to this storage treatment.

The comparisons of epididymal sperm abnormalities for each post-storage day to the pre-storage means are given in Table XXIV. The percent freeheads did not change with storage in either bull. The percent abnormal heads and midpieces showed a tendency to decrease slightly in both bulls. Figures 23 and 24 graphically show the changes in percent abnormal tails for Bulls 3 and 6, respectively. The percent gross abnormal tails (mainly bent) in Bull 3 increased significantly on days 2, 3 and 7. The percent abnormal tail ends (hooked or coiled at tip) increased significantly from 4.6 percent pre-storage to 11 percent on days 2 and 3. These data would indicate that storage of 6 days in the cauda caused a detrimental effect on the sperm tail. For Bull 6 (Figure 24), the percent gross abnormal tails (bent and looped) showed a tendency to increase with a peak on day 9. The percent abnormal tail ends (mainly coiled at tip) showed a considerable increase especially on days 5 and 9. These data support Bull 3 for the adverse effects of storage on the sperm tail. Figure 25 shows the total abnormal epididymal sperm for both bulls. Bull 3 showed the highest percent abnormals during the early portion of the pre-storage period with a decline while Bull 6

TABLE XXIV

COMPARISON OF EPIDIDYMAL SPERM CELL ABNORMALITIES FOR EACH POST-STORAGE DAY TO MEANS OF THE PRE-STORAGE PERIOD[a]

		Freeheads (%)	Abn. Heads and Midpieces (%)	Gross Abn. Tails (%)	Abn. Tail Ends (%)	Total Abnormal (%)	Aged Acrosome (%)
Bull 3							
Pre-Storage	n=10	1.5 ± .22	4.1 ± .55	1.8 ± .29	4.6 ± .90	12.0 ± 1.08	25.5 ± 2.6
Post-Storage[b]	Day						
	1	2	3	2	2	9	17
	2	1	4	7**	11*	23*	33
	3	2	1*	4*	11*	18*	28
	4	1	3	3	4	11	23
	5	2	2	2	4	10	30
	6	2	3	2	6	13	14
	7	1	4	9**	2	16	36
	8	2	3	2	1	8	25
	9	2	2	2	1	7	27
Bull 6							
Pre-Storage	n=10	1.4 ± .37	5.7 ± .42	3.1 ± .63	1.0 ± .22	11.3 ± .92	57.6 ± 4.1
Post-Storage[b]	Day						
	1	1	4***	2	1	8	78*
	2	2	4***	2	4**	12	73***

TABLE XXIV (Continued)

	Freeheads (%)	Abn. Heads and Midpieces (%)	Gross Abn. Tails (%)	Abn. Tail Ends (%)	Total Abnormal (%)	Aged Acrosome (%)
Day						
3	----	----	----	-----	-----	-----
4	2	3*	2	9**	16***	63
5	1	2**	6***	22**	31**	75***
6	2	3*	4	11**	20**	51
7	----	----	----	-----	-----	-----
8	2	4***	6***	9**	21**	54
9	3***	4***	12**	18**	37**	53
10	1	3*	5	14**	23**	27*

[a]Values are means ± standard errors.

[b]Missing values indicate no samples obtained.

*(P < .05).

**(P < .01).

***(P < 0.1).

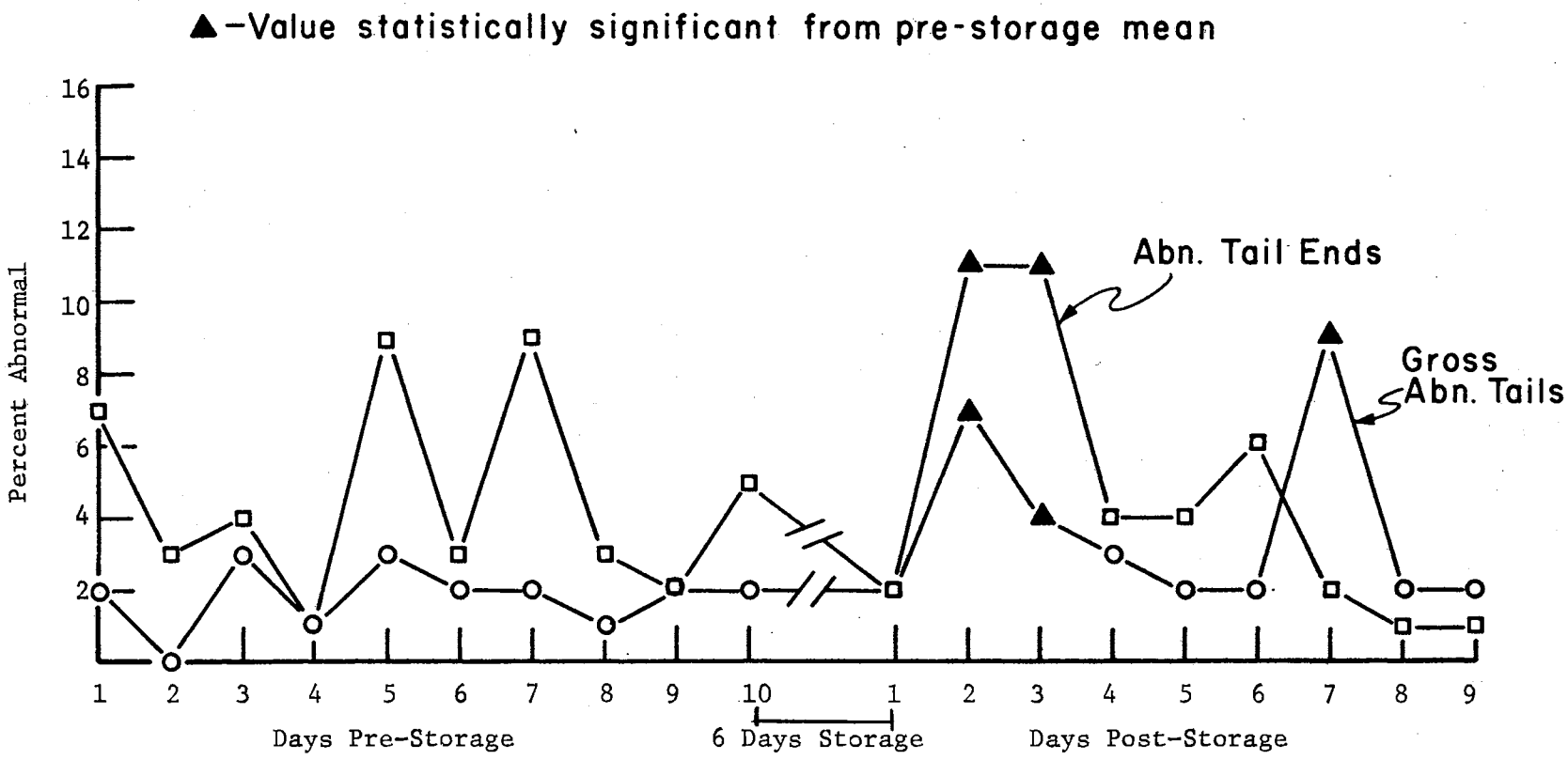

Figure 23. Percent Abnormal Tails of Epididymal Sperm Pre- and Post-Storage for Bull 3

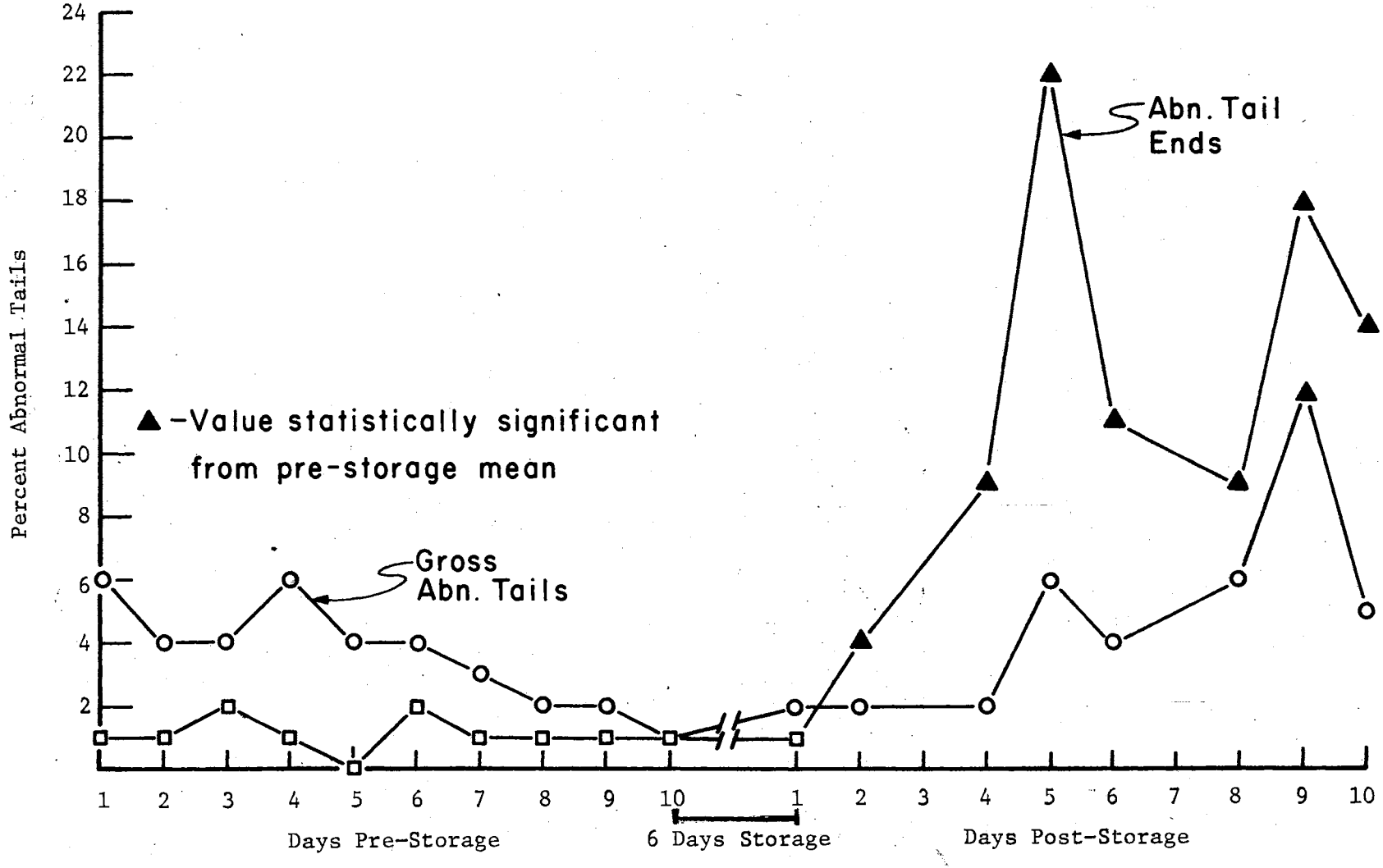

Figure 24. Percent Abnormal Tails of Epididymal Sperm Pre- and Post-Storage for Bull 6

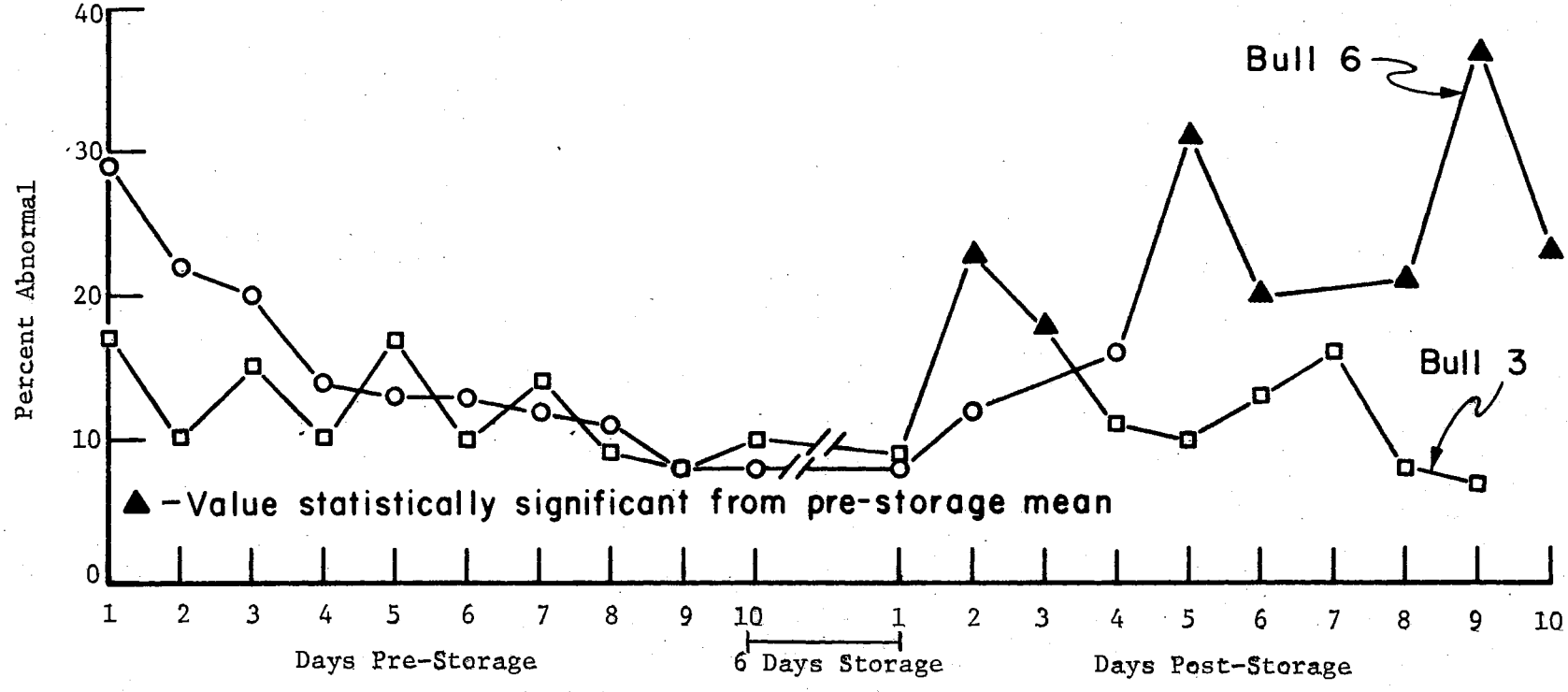

Figure 25. Percent Total Abnormal Epididymal Sperm Pre- and Post-Storage for Bulls 3 and 6

showed a gradual increase in abnormals with the highest percent in the latter portion of the period. From these results, it is difficult to pin point what region of the epididymis caused the increase in abnormal tails, but a definite increase was found in both bulls.

This increase in bent and looped tails due to storage is in agreement with the report of Amann and Almquist (1962b). They suggested that the gross appearance of sperm degeneration is first noticed by bent and broken tails and then tailless heads. The present study did not find an increase in freeheads as reported by Amann and Almquist (1962a) and Barker and Amann (1971) using vasectomies in bulls and Salamon (1967) using epididymal ligations in rams. This was probably because of the much shorter storage period in the present study and no inflammation reaction was involved as in the vasectomies.

The percent aged acrosomes on the epididymal sperm showed little change in Bull 3, but in Bull 6 the percent increased early post-storage and then decreased. Awa (1970) found a decrease in aged acrosomes when ejaculation frequency increased.

Table XXV gives the comparison of the ejaculate sperm abnormalities post- vs. pre-storage. Only small changes were observed in Bull 3 for all abnormal classes, but in Bull 6 larger changes were found in the percent of abnormal tail ends (days 5 to 10). This caused an increase in the total abnormal sperm during these days which can be seen in Figure 26. These results would suggest that storage may have also occurred in the non-cannulated system or some other effects caused this increase in abnormals.

During the preliminary trial, Bull 1 had the cannula clamped for 4 days to determine the initial effects of storage. On day 2 of post-

TABLE XXV

COMPARISON OF EJACULATE SPERM CELL ABNORMALITIES FOR EACH POST-STORAGE DAY TO MEANS OF THE PRE-STORAGE PERIOD[a]

		Freeheads (%)	Abn. Heads and Midpieces (%)	Gross Abn. Tails (%)	Abn. Tail Ends (%)	Total Abnormal (%)
Bull 3						
Pre-Storage	n=10	2.2 ± .25	4.9 ± .64	1.9 ± .28	3.9 ± 1.11	12.9 ± 1.43
Post-Storage[b]	Day					
	1	2	2***	2	2	8
	2	1	8***	3	7	19***
	3	2	5	3	3	13
	4	-	----	-	-	----
	5	1	3	2	3	9
	6	-	----	-	-	-----
	7	1	3	2	1	7
	8	-	----	-	-	-----
	9	2	7	2	0	11
Bull 6						
Pre-Storage	n=10	1.3 ± .18	3.9 ± .51	1.0 ± .22	0.6 ± .09	6.7 ± .36
Post-Storage[b]	Day					
	1	1	3	0	1	5
	2	1	5	1	1	8

TABLE XXV (Continued)

	Freeheads (%)	Abn. Heads and Midpieces (%)	Gross Abn. Tails (%)	Abn. Tail Ends (%)	Total Abnormal (%)
Day					
3	2	3	0	2***	7
4	1	4	1	0	6
5	1	5	2	7**	15**
6	1	4	3*	6**	14**
7	--	-	--	----	----
8	3*	4	3*	10**	20**
9	1	4	2	8**	15**
10	3*	4	1	7**	15**

[a]Values are means ± standard errors.

[b]Missing values indicate no samples obtained.

*($P < .05$).

**($P < .01$).

***($P < 0.1$).

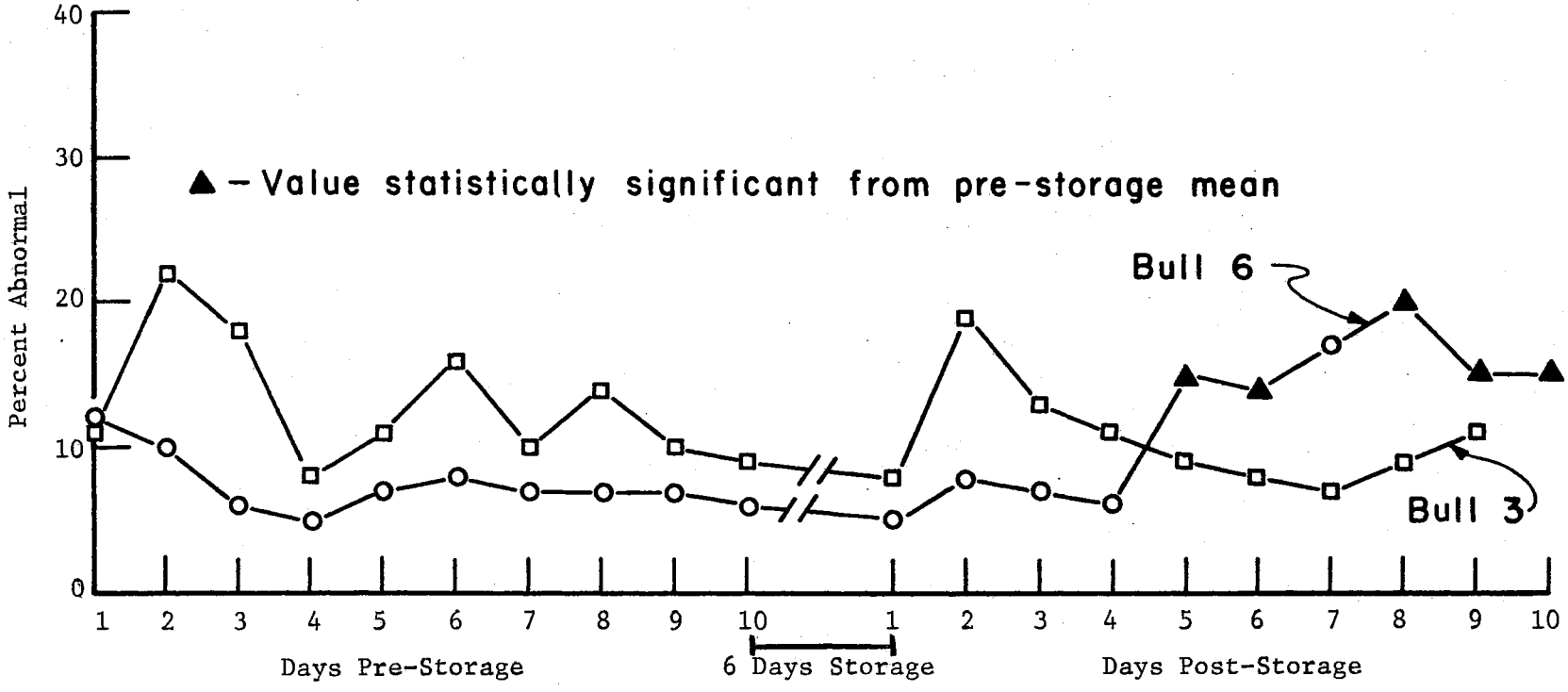

Figure 26. Percent Total Abnormal Ejaculate Sperm Pre- and Post-Storage for Bulls 3 and 6

storage many leukocytes and many tailless heads (35%) were found. Also macrophage cells in the process of engulfing sperm cells were found. This condition is believed to exist when inflammation and infection are involved in the epididymis. On day 5 an increase (from 12 to 21%) in percent gross abnormal tails (mainly looped tails) was observed.

Since the cannula of Bull 12 stopped flowing 4 days after cannulation and no major swelling occurred prior to castration on day 36 post-cannulation, the sperm cells in the cauda were stored for 32 days. At castration the cauda epididymal sperm showed a very high percent live (93%) with a small percentage of freeheads (2%) and abnormal heads and midpieces (4%). However, the percent gross abnormal tails (looped) were significantly higher (12 vs. 0%) than in the sample obtained before storage. The percent abnormal tail ends (coiled) were significantly higher also (13 vs. 3%). The sperm in the caput epididymis on the cannulated side also showed these increases with 20 percent gross abnormal tails and 15 percent abnormal tail ends. Therefore, these results support the previous results that storage causes an increase in bent and looped tails and coiled and hooked tail ends. This condition of coiled tail ends has not been reported in the literature and the reason may be because it has been overlooked due to the difficulty of observing it.

At castration, sperm samples were obtained from the cauda and caput epididymis of both testis. The following is a brief summary of the comparison of sperm characteristics between these two regions of the epididymis. For the cannulated systems, the percent motility in the caput was zero or near zero in all bulls but the average in the cauda was 44 percent. The percent live sperm was similar with the caput showing 76 percent and the cauda showing 71 percent. Because of the

swelling in the scrotum and previous treatment effects on some bulls prior to castration, the differences in percent abnormals varied greatly between bulls. The main types of abnormalities were tailless heads and bent and looped tails. It is believed that the swelling caused the tailless heads and storage caused the more minor tail abnormalities. The percent of aged acrosomes was similar or slightly higher in the caput than in the cauda.

For the non-cannulated systems, the caput sperm showed no motility in all but one bull and the percent live was again similar (71 to 75%) to that of the cauda. The percent abnormals were slightly higher (27 to 21%) in cauda sperm of the storage group bulls, but in the non-storage group the caput sperm had a higher percent abnormals (40 to 21%). The same types of abnormalities occurred in the non-cannulated as the cannulated systems indicating some of the same factors influenced both systems. The percent of aged acrosomes was higher in the caput than in the cauda (89 vs. 61%). In conclusion, the sperm in the caput have almost no motility and are similar to the cauda sperm in percent live. The higher percent of aged acrosomes in the caput sperm may have been caused by the greater effects of heat in the caput from the swelling prior to castration.

Evaluation of Sperm Output

Amann (1962) concluded from a detailed study on dairy bulls that sperm production is continuous and not affected by ejaculation frequency or vasectomy. Therefore, when the cannula was clamped for 6 days in the present study, the rate of sperm entering the epididymis should have continued. This reasoning is valid because the cauda epididymis on the can-

nulated side increased in size during the storage period. One may also think that the sperm output post-storage should be increased by the amount that was accumulated during storage but this is not the case as indicated in the present study.

Table XXVI shows the comparisons of sperm output for the pre- and post-storage periods within bull and pooled over bulls. In Bull 3 the epididymal sperm volume, concentration and daily output showed some increase, but all differences were non-significant. The difference in daily output between the pre- and post-storage periods was only 33×10^7 sperm which represented a recovery of only 55 percent of the cells that should have accumulated during the 6-day storage period. The increase in epididymal sperm output post-storage of Bull 6 was also quite small (30.4×10^7) and represented a recovery of only 26 percent of the estimated sperm that were stored. The ejaculate daily sperm output post-storage of both bulls was very similar to the pre-storage output. These results support the theory of sperm resorption in the epididymis as reported by Amann and Almquist (1962a), Barker and Amann (1971), Salamon (1967), and other workers. The present study agrees with the report of Amann and Almquist (1962a) that the rate of resorption in the cauda was dependent on the number of sperm present because Bull 6 had a higher sperm production and a smaller percent of the sperm were recovered than for Bull 3.

The comparison of the pooled means for the two periods indicated no significant differences in sperm output although the post-storage epididymal output was slightly greater. This increase in output indicated a resorption rate of 80 percent during the 6-day storage period. This study showed that a large percent of the sperm cells are resorbed when stored *in vivo*.

TABLE XXVI

COMPARISON OF PRE- AND POST-STORAGE SPERM OUTPUT WITHIN BULLS AND POOLED[a]

Period	No. of Samples	Epididymal			Ejaculate		
		Total Daily Volume (ml)	Conc./ml (X 10^7)	Daily Sperm Output (X 10^7)	Daily Volume (ml)	Conc./ml (X 10^7)	Daily Sperm Output (X 10^7)
Bull 3							
Pre-Storage	10	.230 ± .028	405.1 ± 39.8	99.4 ± 19.9	7.0 ± 0.81	8.6 ± 1.44	59.5 ± 11.3
Post-Storage	9	.286 ± .022	463.1 ± 33.7	132.4 ± 15.1	6.9 ± 0.94	7.3 ± 3.06	48.4 ± 17.2
Diff.[b]		.056	58.0	33.0	0.1	1.3	11.1
Bull 6							
Pre-Storage	10	.395 ± .023	435.4 ± 20.5	173.8 ± 14.8	6.6 ± 0.83	23.7 ± 3.85	150.6 ± 26.5
Post-Storage	10	.410 ± .066	412.7 ± 55.8	204.2 ± 38.4	8.6 ± 1.05	16.5 ± 3.03	143.8 ± 37.1
Diff.[b]		.015	22.7	30.4	2.0	7.2	6.8
Pooled Mean	Bulls						
Pre-Storage	4	.30 ± .036	491.8 ± 49.4	150.2 ± 17.4	6.6 ± 0.39	19.2 ± 5.29	117.3 ± 26.1
Post-Storage	2	.35 ± .060	437.9 ± 25.2	168.3 ± 35.9	7.8 ± 0.85	11.9 ± 4.59	96.1 ± 47.7
Diff.[b]		0.05	53.9	18.1	1.2	7.3	21.2

[a]Values are means ± standard errors.

[b]All differences are non-significant.

CHAPTER V

SUMMARY

Twelve Angus bulls, 2 to 3 years of age, were utilized in six consecutive paired trials to evaluate the morphology and output of epididymal sperm collected via a unilateral indwelling cannula in the vas deferens and to determine the effects of *in vivo* storage on epididymal sperm. Each trial began with a 2 to 3 week acclimatization period in a temperature controlled room. After this period, each bull was taken to surgery and the left vas deferens was surgically cannulated with a polyethylene-Silastic cannula. This cannula flowed into a glass collection vial attached to the posterior neck of the scrotum by a polyethylene supporting device. After surgery and a 5 hour surgical recovery period, the bulls were moved to an environmental chamber (maintained at 70° F, 50% relative humidity) where they remained for the entire experimental period.

The first two bulls were used in a preliminary trial to determine the procedures for the remaining five trials. The results of this preliminary trial indicated that during electroejaculation a fresh sample of epididymal sperm could be obtained (via the cannula) as well as a sample of ejaculate sperm (via the penis). Also, these two bulls experienced an initial post-surgical swelling of the scrotum which caused a deleterious effect on the epididymal sperm for a 30 to 40 day period. Therefore, a recovery period was deemed necessary to allow the sperm

cells to return to normal.

Trials two to six were conducted under a standardized procedure. After cannulation of the pair of bulls, the cannulae were allowed to flow continuously and the collection vials were changed daily to determine the output of epididymal sperm. Each bull was electroejaculated three times per week during the recovery period and the samples of epididymal and ejaculate sperm were evaluated for their morphology and output. When the quality of the epididymal sperm of a bull returned to the pre-cannulation level, the bull was electroejaculated daily for 10 days to determine the normal morphology and output of epididymal sperm. Then the cannula was clamped shut for a 6-day period to accomplish in vivo storage of epididymal sperm. After this storage period, the clamp was removed and 10 daily collections were obtained, evaluated and compared to the pre-storage values to determine the effects of storage. At the termination of each trial, the bulls were castrated and the testes weighed. Tissues from the vas deferens, cauda epididymides and testes were obtained and studied for histological changes.

Ten of the 12 cannulations were considered successful with an average cannula patency length of 66 days (range 50 to 81 days). The major difficulty experience with this surgical technique was the initial post-surgical swelling and inflammation which was exhibited by 11 of the 12 bulls for about a 10 day period after cannulation. This condition caused an increase in the percentage of abnormal epididymal sperm (mainly tailless) and a decrease in the percent of live sperm, but had little effect on the morphology of the ejaculate sperm. Much variation was observed between bulls for the effects of swelling on the sperm characteristics. However, there was a trend for the bulls with the

greatest degree of swelling to show the greatest adverse effects on the epididymal sperm. Strict aseptic surgical procedures were practiced by the two veterinarians and several modifications of the cannula sterilization and surgical materials were tried, but the swelling could not be prevented. It appears that more experience is needed in the surgical technique to reduce the trauma of cannulation and a superior cannula sterilization procedure should be developed.

During the recovery period, the mean volume of epididymal semen obtained from eight bulls during ejaculation was 0.13 ± .025 ml with a mean total daily volume of 0.27 ± .031 ml. The mean concentration of the 318 epididymal samples was 379 ± 45.3 x 10^7 per ml and the total daily output was 115 ± 21.3 x 10^7. From the eight bulls and 134 ejaculate samples, the mean volume, sperm concentration per ml and sperm output per ejaculate was 6.97 ± .37 ml, 29.5 ± 5.91 x 10^7 and 197.6 ± 37.2 x 10^7, respectively. The daily sperm output via the cannula was 35.5 x 10^7 sperm greater (non-significant) than the ejaculate output obtained during three times per week ejaculation and converted to a daily basis. A similar difference was also found during daily ejaculation for four bulls in the pre-storage period.

The epididymal sperm of only four of the eight bulls recovered sufficiently from the effects of cannulation so that the storage treatment procedures could be imposed on them. Since these four bulls showed the least effects of swelling, their 10-day pre-storage data gave the best estimate of the typical epididymal sperm characteristics. In comparing the epididymal and ejaculate sperm characteristics, only the epididymal sperm rate of motility was significantly ($P < .05$) lower than the ejaculate (2.8 vs. 3.4). The percent motility of the epididymal

sperm tended to be lower and the percentage of live sperm showed a trend for being higher than the ejaculate sperm, but the percent and types of abnormal sperm morphology were quite similar.

In vivo storage of epididymal sperm was imposed on four bulls but the cannulae of only two of the bulls resumed flowing after the clamps were removed. The results from these two bulls suggest that 6 days of in vivo storage of epididymal sperm causes a significant ($P < .05$) increase in the percentage of abnormal sperm (12 to 23% in Bull 3 and 11 to 37% in Bull 6). The main abnormalities observed were bent and looped tails and hooked and coiled tail ends. The percentage of tailless heads and abnormal heads showed little change due to storage. The effects of storage on the percentage of live sperm varied between bulls, but there was a trend for the percent motility to increase with storage.

The daily epididymal sperm output during the 10-day post-storage period increased for each bull about 30×10^7 sperm from the pre-storage levels. This increase in output for Bulls 3 and 6 accounted for only 55 percent and 26 percent, respectively, of the estimated sperm reservoirs due to storage. These results would indicate epididymal resorption of a large percentage of the stored sperm.

The effects of cannulation was observed on the testis weight and in the histological findings. The mean weight of the testis on the cannulated side was significantly less (28g) than that of the contralateral testis. Nine of the 10 bulls showed some degeneration of the germinal cells in the seminiferous tubules in the cannulated testis. It appears that this degeneration was a result of the swelling of the scrotum which caused a heating effect on the testis. The effects of cannulation on the cauda epididymis were minor with only a change in the appearance of

the duct (shrunken or misshapen). However, the cannulated vas deferens showed signs of inflammation and a thickening of the epithelial lining. Also a spermatocoele was formed at the site of cannulation suggesting a chronic inflammatory reaction.

The results of this study show the difficulties involved with the surgical technique of cannulation of the vas deferens for studying epididymal sperm. This study could serve as a good pilot study for an extensive investigation on the effects of the epididymis on the maturation and aging of sperm. If the effects of swelling and inflammation can be overcome, this cannulation technique has a great potential for use in research studies involving the epididymis or epididymal sperm.

In conclusion, the limited results of this study indicate that the typical epididymal sperm characteristic are similar to those of the ejaculate sperm and the output via the cannula is probably slightly greater than through the normal reproductive tract. The results on the in vivo storage of epididymal sperm for 6 days suggest that storage causes an increase in abnormal sperm tails and an increase in the rate of resorption of the sperm cells by the epididymis.

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