THE EFFECTS OF EXOGENOUS TESTOSTERONE ON SPERMATOGENESIS OF BULLS

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INTRODUCTION

The relative importance of the bull in a breeding program is increased since a single ejaculate may be used to inseminate many cows in an artificial insemination program. This has brought about an increased interest in problems concerning impaired fertility in the bull.

Although the male sex hormone, testosterone, has been used clinically in the bull and recognized as essential for spermatogenesis in mammals, little is known of the effects of prolonged testosterone injections in the bull.

Androgen appears to be produced primarily by the Leydig cells of the testicle under the influence of pituitary gonadotrophins. It is responsible for the male secondary sex characteristics, and stimulates the germinal epithelium in some unknown way favoring spermatogenesis. In addition, androgen apparently acts on the pituitary to inhibit gonadotrophin production and/or release.

This experiment was designed to study the effects of large doses of testosterone on spermatogenesis in the bull. In laboratory animals, high dosages of testosterone tend to inhibit production and/or release of pituitary gonadotrophins thus reducing Leydig cell production of testosterone and germinal cell production of spermatozoa. Consequently, this experiment should indicate if spermatogenesis and testicular cytology are altered in the bull by the administration of testosterone. Furthermore, spermatogenesis and testicular cytology could be studied by semen evaluation and testicular sections after cessation of testos-

terone injections. Such a study should elucidate some basic endocrine patterns in the bull on which rational experimental and clinical work can be built.

REVIEW OF THE LITERATURE

Previous to adoption of artificial insemination programs by cattle breeders, herd sires with impaired fertility were frequently slaughtered without their owners considering corrective therapy. The increased use of artificial insemination in cattle has stimulated interest in restoring fertility of the valuable bull. The causes of impaired fertility are innumerable and vary from impaired locomotion to loss of ability to produce spermatozoa. Since the male reproductive organs are primarily dependent on endocrine mechanisms for normal function, improvement of fertility by endocrine therapy appears logical. This literature review therefore is primarily concerned with the endocrine aspect of spermatogenesis as a central factor in fertility.

Initiation and Maintenance of Spermatogenesis

In normal mammals, spermatogenesis is generally accepted as being controlled primarily by two pituitary gonadotrophins: follicle stimulating hormone (FSH) and luteinizing hormone (LH). These have been obtained in relatively pure form from pituitaries of the hog, horse, sheep, and man. By use of these hormones in the relatively pure form (Steelman, Segaloff and Andersen, 1959) FSH has been shown to affect spermatogenesis by directly stimulating the germinal epithelium whereas LH (interstitial cell-stimulating hormone) indirectly increases spermatogenesis by increasing Leydig cell function. Simpson, Li and Evans (1944) injected 40-day old rats with FSH to demonstrate the action of FSH and showed

that the target of FSH in the male gonad was primarily the tubule. FSH alone maintained the tubules; the secondary sex glands involuted in the FSH-treated, hypophysectomized male rats even though spermatogenesis was maintained.

Simpson, Li and Evans (1944) hypophysectomized 40-day old male rats and stimulated the Leydig cells of these rats to produce testosterone by injections of LH. An increased activity of the germinal epithelium was also noted in the LH treated rats as substantiated by Randolph et al. (1959). These workers suggested that testosterone production might be the cause of the increased germinal cell activity.

Work by Zuckerman (1939), Hellbaum and Greep (1943) and Ludwig (1950) indicated that FSH directly stimulates germinal epithelium and LH directly stimulates Leydig cells.

Testosterone apparently effects the formation and/or the release of pituitary gonadotrophins in rats (Hellbaum and Greep, 1943). These workers injected immature female rats with dried pituitary glands in aqueous suspension. The glandular suspension from castrated, testosterone-treated male rats resulted in follicle formation, an indication of FSH activity. The glandular suspension from similar rats receiving no testosterone, resulted in corpus luteum formation, an indication of LH activity. Injected serum from castrated, testosterone-treated male rats caused corpus luteum formation whereas injected serum from similar non-treated rats caused follicle formation. All of the foregoing injections were made twice daily for three days, and autopsies were done two days after the last injection. From these results, the workers postulated that testosterone caused a release of LH and inhibited the release of FSH from the pituitary glands of the rat.

Wainman, Reese and Koneff (1942) and Ludwig (1950) showed that testosterone maintained spermatogenesis in the hypophysectomized rat. There was no return of spermatogenesis following hypophysectomy if sufficient time elapsed to allow cessation of spermatogenesis before testosterone injections were begun (Hamilton and Leonard, 1938). The pituitary gonadotrophins are necessary, therefore, to initiate spermatogenesis even though testosterone can maintain spermatogenesis at least for a period following hypophysectomy.

Santamarina and Reece (1957) reported that the lumen of the seminiferous tubules in the bull became well formed at four to five and one-half months of age indicating initiation of the early stages of spermatogenesis. These workers found that normal development of the testis varied more within age and breed groups than between age and breed groups during both pre-natal and post-natal life.

Hooker (1944) traced the development of seminiferous tubules and Leydig cells in bulls from birth to fifteen years of age. This study indicated that the tubule "size" and Leydig cells were near maturity at eight months while tubules reached adult "proportions" at 13 months of age.

Testicular Response to Testosterone Administration

Exogenous testosterone affects both Leydig cells and germinal epithelium of the testes of rat, man and chicken (Moore and Price, 1932; Moore and Price, 1938; Hamilton and Leonard, 1938; Wainman, Reese and Koneff, 1942; Hall and Sykes, 1947; Heckel, Rosso and Kestel, 1951; Maraud and Stoll, 1955; Keetel et al., 1956; Paesi, deJongh and Croes-Buth, 1959). Large doses of testosterone caused less degeneration of the testes than

small doses (Zuckerman, 1938; Hellbaum and Greep, 1943; Ludwig, 1950). This work also indicated that small doses of testosterone inhibited production and/or release of pituitary gonadotrophins without sufficient direct stimulation to maintain spermatogenesis.

Hooker (1944) showed that X-ray irradiation of the testes or loss of tubular function due to cryptorchidism did not cause castration changes of the accessory sex glands or loss of the male sex characteristics in bulls. This worker showed the origin of testicular androgens to be the Leydig cells.

The effect of testosterone administration on Leydig cells has not been clearly defined. Zuckerman (1938) reported azoospermia and no noticeable interstitial cell changes in monkeys due to testosterone injections. Noland and Burris (1956) reported interstitial cell alteration in boars fed methyl testosterone. Ludwig (1950) noted depression of Leydig cells indicated by a reduction in cell size, and of spermatogenesis shown by decreased number of germinal cells in some rats in response to testosterone administration.

Prolonged testosterone administration inhibits spermatogenesis in man and rat (Wainman, Reese and Koneff, 1942; Ludwig, 1950; Heller et al., 1950; Heckel, Rosso and Kestel, 1951; Heckel and McDonald, 1952; Schultz, 1956; Charney, 1956). Moore and Price (1932) demonstrated a permanent tubular degeneration in young male rats due to testosterone administration; but in mature male rats the degeneration was temporary followed by a return of functional tubular cells as substantiated by Heller et al. (1950) and Heckel and McDonald (1952). There are several possible ways that testosterone might bring about its effects on spermatogenesis. Large doses may inhibit release of both FSH and LH from the

Pituitary gland and thus inhibit factors favoring spermatogenesis. Howard et al. (1950) indicated that testosterone favors release of the water soluble "X" hormone produced by Sertoli cells which in turn reduced FSH release and stimulated pituitary LH release. Therefore, spermatogenesis was inhibited since FSH is the pituitary gonadotrophin directly affecting spermatogenesis.

General agreement is not apparent for the dosage of testosterone required to depress spermatogenesis as indicated by the range of dosage in man (Heckel and McDonald, 1952; Schultz, 1956; Charney, 1956). The dosage of testosterone necessary to depress spermatogenesis has not been established in the bull.

Testicular Response After Testosterone Administration

The response of the testicle to withdrawal of exogenous testosterone appears to be more consistent than the response during testosterone administration. Following testosterone withdrawal in man and rat there is a tendency for spermatogenesis to increase above pre-injection levels (Moore and Price, 1932; Heller et al., 1950; Heckel, Rosso and Kestel, 1951; Heckel and McDonald, 1952; Schultz, 1956; Charney, 1956). This is known as the testicular rebound phenomenon. There is no information available in the literature concerning this phenomenon in the bull.

The sudden cessation of testosterone administration releases from inhibition the pituitary gonadotrophins which stimulate Leydig cells and seminiferous tubules. The testicular rebound phenomenon is based on the theory that release of pituitary gonadotrophins will stimulate spermatogenesis to higher levels. Charney (1956) reported an improvement in 18.5 percent of 92 cases of infertile men including spermatozoa production

in two of nine men that had azoospermia. Heckel and McDonald (1952) reported that spermatogenesis returned to above normal levels in 23 of 36 men. Keetel et al. (1956) reported a rebound in spermatogenesis following testosterone therapy in ten of 17 subfertile men.

Heller et al. (1950) reported improved spermatogenesis in "several" men within six months and complete recovery in five men within 17 months following testosterone therapy. Heckel and McDonald (1952) indicated a variable lag in man's response to testosterone withdrawal varying up to "several months." Johnsen (1958) indicated that it takes about 48 days for the germinal epithelium of a bull testicle to produce spermatozoa; the delay in the response of the testicle to withdrawal of testosterone would therefore depend upon the stage of regression of the germinal epithelium.

Ambient Temperature Effects

One of the more important environmental factors affecting the level of fertility in the bull is the ambient temperature. Phillips et al. (1934) and Erb, Andrews and Hilton (1942) reported a decrease in fertility of bulls in hot weather with the lowest percent fertile matings in August and the highest percent fertile matings in April giving an inverse relationship of fertility to ambient temperature.

Casady, Meyers and LeGates (1953) concluded from controlled ambient temperatures that "under chamber conditions, spermatogenesis in the young dairy bull may be impaired when the animal is continuously exposed to temperatures exceeding 85°F. for periods exceeding five weeks." This work also indicated that range bulls were more resistant to heat than bulls raised under more sheltered conditions.

Work by Ogle (1934) indicated that rats have a partial ability to adapt to both increased or decreased ambient temperature. Impaired fertility occurred however, in both sexes maintained at 88° to 92°F. and 75 percent relative humidity even after a period of adaptation had taken place.

Dutt and Hamm (1955) reported the insulating effects of body wool on unshorn rams exposed to 90° Fahrenheit. Fertility was reduced as evaluated by the percent motile and abnormal spermatozoa. Shorn rams had little change in fertility under the same conditions.

Semen Evaluation

The evaluation of semen by its ability to fertilize ova is the ultimate test of its viability. This criterion is too expensive and time consuming in the bull; therefore, another criterion is necessary that is more practical. Although no single other criterion of semen evaluation is completely satisfactory, volume, number of spermatozoa per milliliter, total number of spermatozoa per ejaculate and motility are the most valuable characteristics presently available (Harvey and Jackson, 1945; Bishop et al., 1954; Bratton et al., 1956; Bialy and Smith, 1957; Lindley, et al., 1959).

Gassner, Hill and Sulzberger (1952) found no correlation between spermatozoa count and fructose content of bull semen and concluded fructose level is a better measure of testosterone production than a method of semen evaluation. Fructolysis may indicate individual spermatozoa viability or number of viable spermatozoa (Gassner, Hill and Sulzberger, 1952; Hopwood, Rutherford and Gassner, 1956). Bialy and Smith (1958) found that vesticular fluid reduced the number of protoplasmic droplets on bull spermazoa thereby altering morphology.

Testicular Biopsy and Cytology

Any procedure such as testicular biopsy which may alter the tubular continuity may alter spermatogenesis. Whenever the continuity of the testicular protective coverings are disturbed an avenue for infection is created. This is true under practical conditions involving testicular biopsy in the bull. The Vim-Silverman testicular biopsy needle as adapted to the bull (Knudsen, 1958; McDonald and Hudson, 1960) is safer in this respect than the open biopsy (Hill et al., 1955; McEntee, 1958; Santamarina, 1958). Work by Heller et al., (1950), Knudsen (1958) and McDonald and Hudson (1960) with the needle biopsy gave valuable information on Leydig cells, germinal epithelium and basement membrane without unduly affecting spermatogenesis.

McDonald and Hudson (1960) obtained by needle biopsy a sample composed of the cross-section of 30-40 seminiferous tubules without unduly affecting spermatogenesis or causing testicular degeneration in eight bulls.

An open biopsy of the bull testicle may cause complications such as adhesions, hemorrhages or infections (Hill, et al., 1955; McEntee, 1958; Santamarina, 1958). Open testicular biopsy (Santamarina, 1958) by Santamarina and Reece (1957) on a calf did not alter development of the germinal epithelium.

MATERIALS AND METHODS

Seven Hereford range bulls, selected for uniformity were purchased from a Newkirk, Oklahoma, herd. Two bulls were discarded because of temperament, and one was discarded due to complete lack of libido. The data presented in this study were obtained from the four remaining bulls, which ranged in age from 21 to 26 months at the beginning of the experiment.

Housing and Care

The bulls were kept in a one-half acre exercise lot. A 16 by 16 foot enclosed shed with two open doors on the east side and an evaporative cooler provided year-round protection. The summer daytime temperature inside the shed did not exceed 85°F., hence the effects of high temperatures on spermatogenesis were probably minimized (Ogle, 1934; Phillips, et al., 1943; Casady, Meyers and LeGates, 1958).

The bulls were fed a ration of prairie hay and protein-vitamin-mineral supplement at a rate to permit normal growth.

Experimental Periods

The experiment was begun January 10, 1958, following an eight week trial period. During the trial period the bulls became accustomed to the environment, personnel and ejaculation into the artificial vagina. The first experimental period was a 17 week control period which was followed by 18 weeks during which androgen injections were given. The final

37 week post-injection period was divided into two periods of 11 and 26 weeks respectively to facilitate interpretation of data. The experiment was concluded May 25, 1959, by slaughter and tissue collection.

Semen Collection

Semen was obtained from the bulls in an outdoor collecting chute by use of a teaser cow and an artificial vagina. The artificial vagina was of usual design except that the funnel continued through the artificial vagina as an additional lining inside the water jacket. A quilted cover with zipper was placed over the exposed portion of the funnel for further protection against the weather elements.

The temperature of the water in the artificial vagina was adjusted for each bull within a range of 41° to 44°Centigrade.

Since these range Hereford bulls were not halter broken very little teasing was possible. After the bull entered the alley leading to the cow, the person who collected semen stood between the bull and the cow attempting to hold the bull back at least two minutes. However, the bulls usually mounted and ejaculated soon after reaching the cow. Weekly ejaculations were obtained for semen evaluation.

Semen Evaluation

Motility, volume, color, density and libido were recorded as soon as possible after collection of each sample. Density, motility, and libido were rated 0 to 4, the highest denoting the most favorable quality. These are arbitrary ratings and do not necessarily agree with all workers (Phillips, et al., 1943; Bratton, et al., 1956; Lindley, et al., 1959). Volume was recorded to the nearest one-fourth milliliter. The

semen samples were taken to the central laboratory where spermatozoa counts were made according to the method of Smith and Mayer (1955); the diluent was three percent sodium citrate containing one milliliter formalin and 0.6 grams of eosin-B stain in each 100 milliliters. The eosin-B in the diluent facilitated making an accurate count and identifying abnormal spermatozoa. A spermatozoon was recorded as abnormal if it had a curled or curved tail, a double, tailless or pyriform head, or protoplasmic droplets.

The number of spermatozoa per ejaculate was calculated as the product of spermatozoa per milliliter times volume.

The sample of semen was gently but thoroughly mixed before each procedure to prevent error due to settling.

Androgen Injections

Two-hundred and fifty milligram doses of testosterone propionate in cottonseed oil was administered intramuscularly (Leathem, 1943; Bernstorf, 1957) three times weekly for 18 weeks to each bull. This dosage was calculated and adjusted from the literature concerning the dose in man and converted as nearly as possible to the bull (Heckel and McDonald, 1952; Schultz, 1956; Charney, 1956), according to weight.

Testicular Size

Measurements of the testicles to the nearest 1/16 inch were taken monthly. An average of at least two measurements of the length and diameter, including the skin coverings, was recorded for each testicle.

The volume of the testicle was calculated by the formula for a prolate spheroid; $4/3\ pi\ ab^2$ where a is the major semiaxis and b the minor

semiaxis. This method assumes that any error due to shape was the same for each testicle.

Histological Studies

A needle biopsy was taken from the left testicle of each bull at the beginning of the injection period, and from the right testicle at the end of the injection period according to the method of McDonald and Hudson (1960). Sections were taken at slaughter from the center and periphery of each testicle and from areas of adhesions. The tissues were immediately placed in Bouin's solution and returned to the Department of Pathology for processing and staining with hematoxylin and eosin.

Health of Experimental Animals

All animals were apparently free of clinically detectable disease before and during the experiment except on two occasions. Two bulls experienced an acute febrile anemic condition diagnosed as anaplasmosis. One bull showed symptoms beginning March 20, and was fully recovered by April 10, 1958, and the second bull showed symptoms on September 4, was not ejaculated on September 5, and was apparently normal September 18, 1958. Excepting for omitting the collection of one ejaculate as indicated, no effect on spermatogenesis was thought to exist.

RESULTS AND DISCUSSION

Data were collected from four Hereford bulls over a period of 72 weeks. Two hundred and seventy-three semen samples were evaluated for volume, spermatozoa per milliliter, spermatozoa per ejaculate, motility, and percent abnormal forms. Twelve periodic measurements were taken of testicular size, from which the volume was calculated.

There was a control period of 17 weeks at the beginning of the experiment followed by an 18 week period of thrice weekly injections of 250 milligrams of testosterone per bull (Table I and Figure 1).

The data collected during the 37 week post-injection period are plotted in Figure 1 and shown in Table I. Examination of the parameters plotted in Figure 1 indicates that semen quality (especially number of spermatozoa per ejaculate) declined markedly for approximately 11 weeks after discontinuance of the injections, then returned rather quickly to normal or above normal levels. Therefore, it seemed desirable to separate the data of this 37 week period into weeks one through 11 and 12 through 37 for further consideration (Table I).

Semen Evaluation

The semen volume averaged 4.06 milliliters per ejaculate during the control period (17 weeks), 4.43 milliliters during the period of testosterone injections (18 weeks), and 4.00 milliliters during the post-injection period (37 weeks). These values do not indicate any appreciable change in semen volume due to the experimental procedure. However,

TABLE I. ANALYSIS BY EXPERIMENTAL PERIOD OF WEEKLY SEMEN VOLUME, SPERMATOZOA/ML., TOTAL SPERMATOZOA/EJACULATE, MOTILITY RATING, AND ABNORMAL FORMS FOR FOUR HEREFORD BULLS

Period	Control (17 weeks) Jan. 10, 1958-May 1, 1958					Injection (18 weeks) May 8, 1958-Sept. 5, 1958					Post Injection (37 weeks) Sept. 12, 1958-May 2, 1959					Post Injection (weeks 1-11) Sept. 12, 1958-Nov. 21, 1958					Post Injection (weeks 12-37) Nov. 26, 1958-May 23, 1959				
	N/	Mean	s**	Range	C*	N/	Mean	s**	Range	C*	N/	Mean	s**	Range	C*	N/	Mean	s**	Range	C*	N/	Mean	s**	Range	C*
Volume(ml.)	64	4.06	1.70	1.50 to 8.25	42	67	4.43	2.01	1.50 to 8.50	45	142	4.00	2.04	1.00 to 9.50	51	41	3.29	2.20	1.00 to 9.50	67	101	4.29	1.91	1.50 to 9.50	45
Spermatozoa/ml.($X10^6$)	64	916.10	525.90	40.00 to 2450.00	57	67	634.60	454.70	40.00 to 2240.00	72	142	816.55	595.80	10.00 to 2690.00	73	41	355.40	366.50	10.00 to 1240.00	103	101	1003.80	569.50	60.00 to 2690.00	57
Total Spermatozoa/Ejaculate ($X10^6$)	64	3885.30	3234.30	140.00 to 1295.00	83	67	3240.90	3242.60	80.00 to 13760.00	101	142	3589.93	3508.60	30.00 to 14820.00	97	41	1139.30	1331.30	30.00 to 6110.00	117	101	4584.80	3598.90	160.00 to 14820.00	76
Motility Rating	64	2.94	-	0-4	-	67	2.87	-	0-4	-	142	2.65	-	0-4	-	41	2.00	-	0-4	-	101	2.92	-	0-4	-
Abnormal Forms (%)	64	17.09	-	4-60	-	67	18.54	-	5-45	-	142	23.75	-	7-96	-	41	32.59	-	12-96	-	101	20.16	-	7-46	-

Unilateral Biopsy May 1, 1958

Unilateral Biopsy Sept. 5, 1958

/ N = Number of Observations
** s = Standard Deviation
* C = Coefficient of Variation

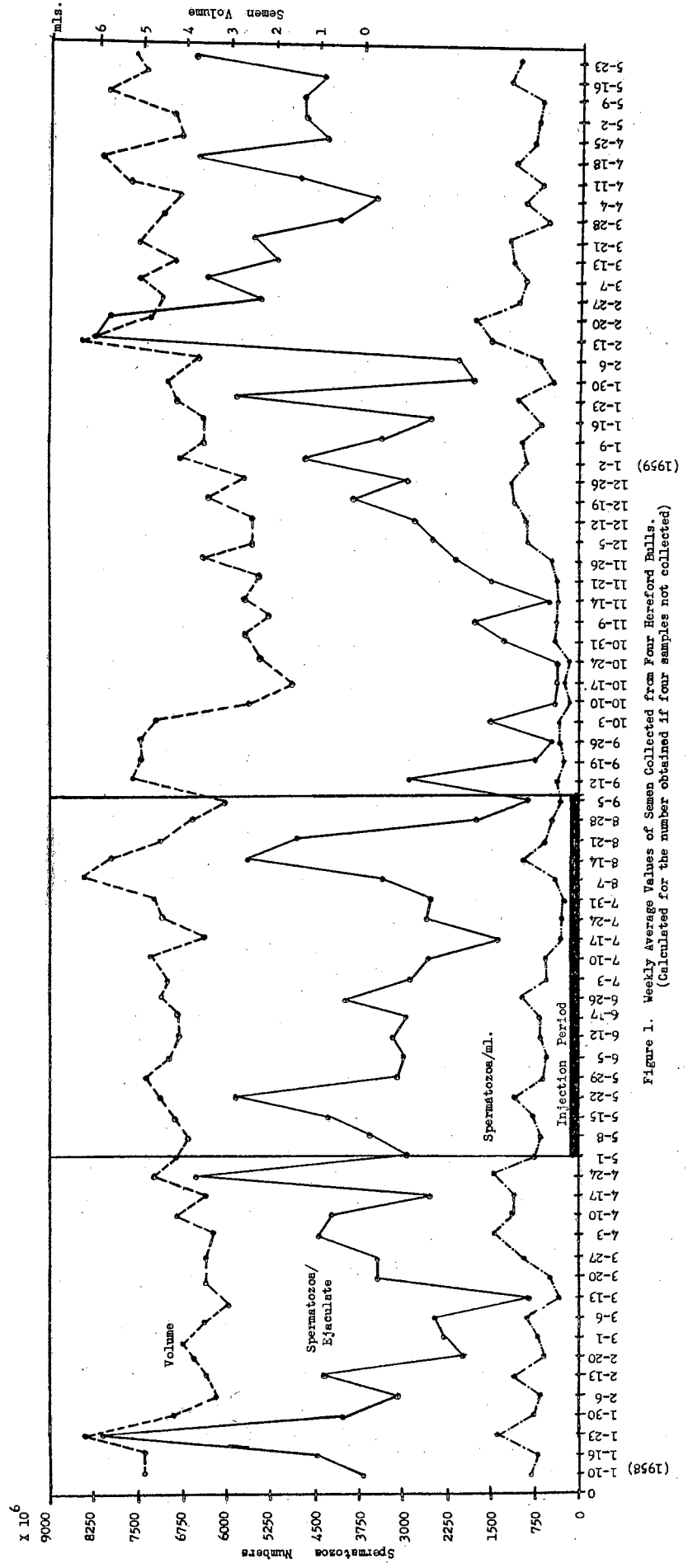

Figure 1. Weekly Average Values of Semen Collected from Four Hereford Bulls.
(Calculated for the number obtained if four samples not collected)

attention should be drawn to the mean semen volume of 3.29 milliliters during the first 11 week post-injection period due to a rather pronounced drop five weeks after cessation of injections. This decrease may reflect the dependency of the secretory process of the accessory glands on injected testosterone. A lag phase resulted before endogenous testosterone could again affect accessory gland secretions.

The mean number of spermatozoa per milliliter of semen ($X10^6$) dropped from 916.10 during the control period to 634.60 during the injection period and returned to 816.55 during the post-injection period (Table I). It appears that the injection of testosterone had a depressing effect on production of spermatozoa (Figure 1). Breakdown of the post-injection period reveals a mean number of spermatozoa per milliliter of 355.40 during the first 11 weeks and 1003.80 during the last 26 weeks. This indicates that the depressing effect caused by injected testosterone may have been manifested to an even greater degree during this early post-injection period due to lack of exogenous testosterone as well as lack of FSH, LH, and endogenous testosterone. The question immediately raised is whether the injections temporarily physiologically hypophysectomized the bulls insofar as gonadotrophic hormones are concerned, which in turn reduced endogenous testosterone production. The value during the last 26 week period (1003.80) is similar to the control period (916.10) which indicates the depression of spermatogenesis was a reversible process.

The mean spermatozoa per ejaculate ($X10^6$) during the control period was 3885.30, during the injection period 3240.90, and during the 37 week post-injection period 3589.93 (Table I). Further examination of the post-injection period reveals a drop to 1139.30 in the first 11 weeks and a return to 4584.80 in the final 26 week period. The change in values from

the control period to the first 11 weeks following injections of testosterone was more marked in the total spermatozoa per ejaculate than in the spermatozoa per milliliter. There is little doubt that spermatogenesis was affected by testosterone beyond the period of injection. A review of Figure 1 reveals a rise in the spermatozoa per ejaculate following the 11 week post-injection period indicating that the depression had been reversed and recovery had occurred.

Motility rating during the control period was 2.94. The injection of testosterone had little effect on motility as shown by Table I; the average during this period was 2.87. The post-injection 37 weeks showed a drop to 2.65. During the first 11 weeks following injection, the motility value was 2.00 compared to 2.92 in the last 26 weeks. The motility values declined at the same time that the numbers of spermatozoa declined. There is the possibility that the reduced number of spermatozoa account for the apparent lowering of motility.

The 17.09 percent abnormal spermatozoa during the control period increased to 18.75 percent during the injection period (Table I), and 23.75 percent during the post-injection period. During the first 11 weeks of the post-injection period the abnormal forms rose to 32.59 percent and dropped during the final 26 weeks to 20.16 percent. This deterioration of semen quality during the immediate post-injection period coincides with the changes in other quality factors. The injection of testosterone was followed by a decrease in semen volume, spermatozoa per milliliter, spermatozoa per ejaculate and motility, and an increase in abnormal forms.

Testicular Volume

The decrease in testicular size during testosterone administration

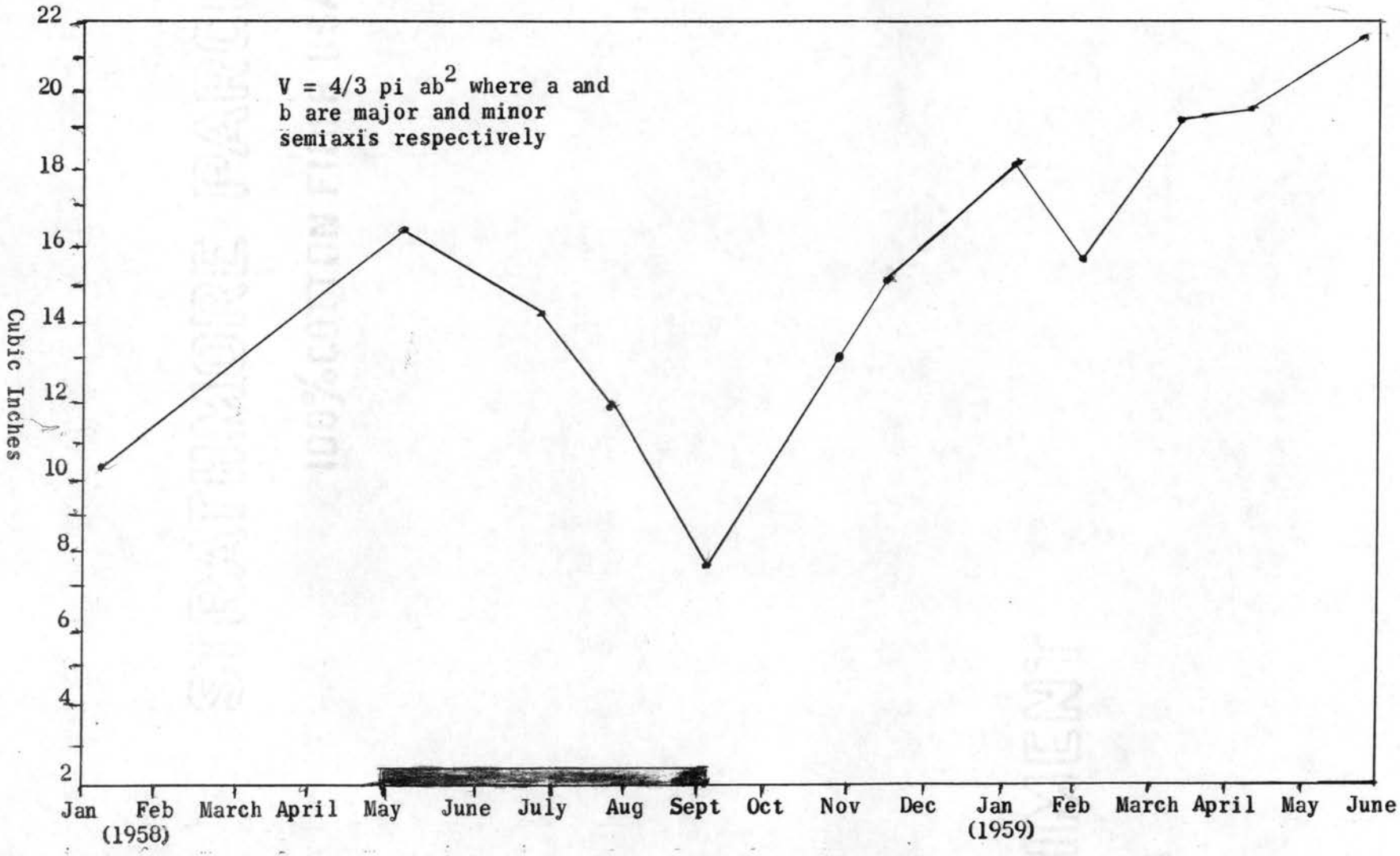

Figure 2. Average Testicular Volume for Four Bulls.

was one of the most profound effects noted (Figure 2). There was an increase in testicular volume for three and one-half months following withdrawal of testosterone administration until pre-injection size returned. Spermatogenesis decreased along with the decrease in testicular size during the injection period but a return in spermatogenesis (Figure 1) did not immediately accompany the restoration of testicular volume. Spermatogenesis recovery occurred when testicular volume returned to near pre-injection levels about December 15, 1958. Delay in the return of spermatozoa to the semen can be partially accounted for by the time it takes for a germinal cell to develop a spermatozoon and for it to transverse the various tubules before it is found in the semen sample; about a 70 day period (Johnsen, 1958). Added to this delay would be any delays in response to testosterone withdrawal due to factors such as residual testosterone.

Testicular Biopsy

Biopsy samples taken at the end of the injection period (September 5, 1958) revealed marked cytological changes when compared to the biopsy sections taken at the end of the control period (May 1, 1958). The seminiferous tubules had lost most of the germinal epithelial cells and had a less distinct lumen. The lumen of the tubule had reduced numbers of spermatozoa attached to the Sertoli cells. There appeared to be an increase in the number and size of the Sertoli cells. The increase in number of Sertoli cells may be relative due to the decrease in germinal epithelial cells.

The Leydig cells were reduced in size and number at the termination of the injection period. Connective tissue was increased in amount in

the intertubular spaces. The remaining Leydig cells had pycnotic nuclei, very few granules and were approximately one-half the size of those from pre-injection sections. The cytological changes in the Leydig cells could be due to the effects of the exogenous testosterone. Perhaps the dosage given blocked pituitary LH production and/or release, thereby allowing the LH dependent Leydig cells to decrease in size and numbers. Small deposits of calcium were noted in the intertubular spaces in some testicles at biopsy. Barker (1956) found calcification of the testicles in old bulls. Perhaps a similar phenomenon was observed in these experimental bulls.

The testicular sections taken at the termination of the experiment showed an increase in size and number of Leydig cells and germinal epithelial cells when compared to the post-injection biopsy. The tubules were filled with active germinal cells with mitotic figures. The Sertoli cell number and size was similar to that of the pre-injection section. The Leydig cells were enlarged, contained vacuoles and granules indicating hormone production, and were increased in number as compared to the pre-injection section. Hooker (1944) reported similar changes occurring with maturity in the bull but to a lesser degree. The calcium deposits noted in the pre-injection and post-injection biopsies were not found in the final sections.

Testicular volume and cytology in the final period of the experiment indicated that testosterone injections did not cause permanent depression of the germinal epithelium or the Leydig cells. It is interesting to observe that the Sertoli cells responded to testosterone injections by increasing in size and possibly number whereas Leydig cells and seminiferous epithelium decreased in size and number. Since these bulls may have been

temporarily physiologically hypophysectomized by the testosterone injections, this may mean that Sertoli cell function is not dependent on gonadotrophins whereas Leydig and seminiferous cells may be so dependent.

The cytological changes which occurred during and following testosterone therapy were reflected in the testicular volume (Figure 2). The loss of germinal cells and the reduction of Leydig cells in both size and number probably accounts for the reduced volume of the testicle. It should be noted that during the recovery period following testosterone therapy that return of testicular volume preceded return of spermatozoa production. This is logical when one considers the cytological basis of spermatogenesis; that is, an increase of cellular elements in the tubules as spermatogenesis returns would increase the testicular volume.

The volume increase during the last five months of the experiment may be due to the normal testicular growth of bulls of this age.

Comparison of Two Corresponding Seasons

Two periods, one before injections and one after injections, were compared to determine if a "rebound phenomenon" occurred in these experimental bulls such as reported in rats and men. Due to the effects of seasonal variations in semen quality as reported by Lindley et al. (1959), and Erb, Andrews and Hilton (1942) two equal periods, one year apart, were compared in this experiment. The first period was the control period beginning January 10, 1958, and ending May 1, 1958; the second period was during the post-injection phase beginning January 9, 1959, and ending May 2, 1959. It is assumed that weather effects were similar for both periods, the main variable (other than testosterone injection) was one year of age increase (Lindley et al., 1959).

There was an increase in average volume of semen from 4.06 milliliter per ejaculate during the control period to 4.60 milliliter one year later (Table II and Figure 3). Lindley et al., (1959) likewise found a tendency (but not a significant one) for a volume increase with age.

The number of spermatozoa ($X10^6$) per milliliter was increased from the 916.10 during the control period to 1006.46 one year later (Table II). Figure 4 shows an erratic distribution of this increase causing one to assign little importance to it other than the expected increase with age of young bulls (Lindley et al., 1959).

The total spermatozoa ($X10^6$) per ejaculate during the control period was 3885.30 as compared with 4900.61 one year later (Table II). Figure 5 shows the two periods had wide variations within periods; however, the values one year later have more peaks than the control period. Since total spermatozoa per ejaculate is the product of the previously discussed volume and spermatozoa per milliliter, it follows that the increase should be assigned to the same factor, namely, effect of age on spermatogenesis (Hooker, 1944 and Lindley et al., 1959.) The motility value of 2.94 during the control period is not greatly different from 3.02 one year later.

The percentage abnormal forms of spermatozoa increased from 17.09 during the control period to 19.48 one year later (Table II). Since this subjective criterion of semen quality showed so little change, little importance should be assigned to it.

Since the variations in parameters between bulls and within bulls were so large in this experiment, a statistical analysis would not indicate true differences as they may exist. Important biological trends can be observed during the course of the experiment.

TABLE II. COMPARISON OF CORRESPONDING SEASONAL PERIODS BEFORE AND AFTER TESTOSTERONE INJECTIONS

Period	Jan. 10, 1958 to May 1, 1958					Jan. 9, 1959 to May 2, 1959				
	N+	Mean	s**	Range	C*	N+	Mean	s**	Range	C*
Volume (ml.)	64	4.06	1.70	1.50 to 8.25	42	65	4.60	1.49	2-9.5	32
Spermatozoa/ ml. ($X10^6$)	64	916.10	525.90	40.00 to 2450.00	57	65	1006.46	535.20	110 to 2690	53
Spermatozoa/ Ejaculate ($X10^6$)	64	3885.30	3234.30	140.00 to 12950.00	83	65	4900.61	2716.70	220 to 14060	55
Motility Rating	64	2.94	-	0-4	-	65	3.02	-	0-4	-
Abnormal Forms (%)	64	17.09	-	4-60	-	65	19.48	-	7-46	-

\+ N = Number of Observations
** s = Standard Deviation
* C = Coefficient of Variation

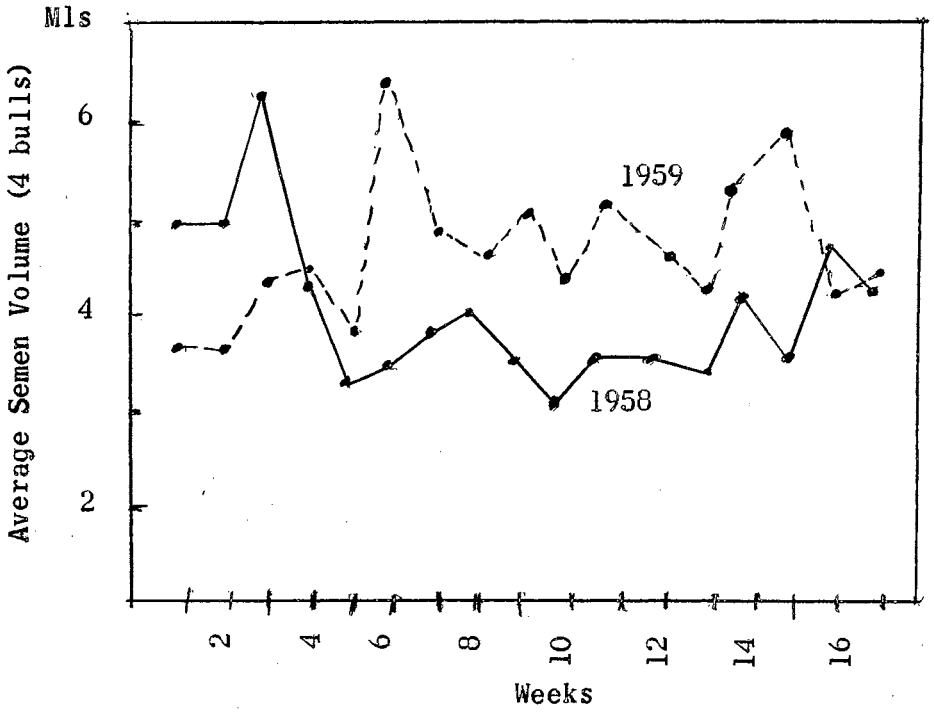

Figure 3. Comparison of Pre-injection Period (Jan. 10-May 1, 1958) with Corresponding Seasonal Post-injection Period (Jan. 9-May 2, 1959).

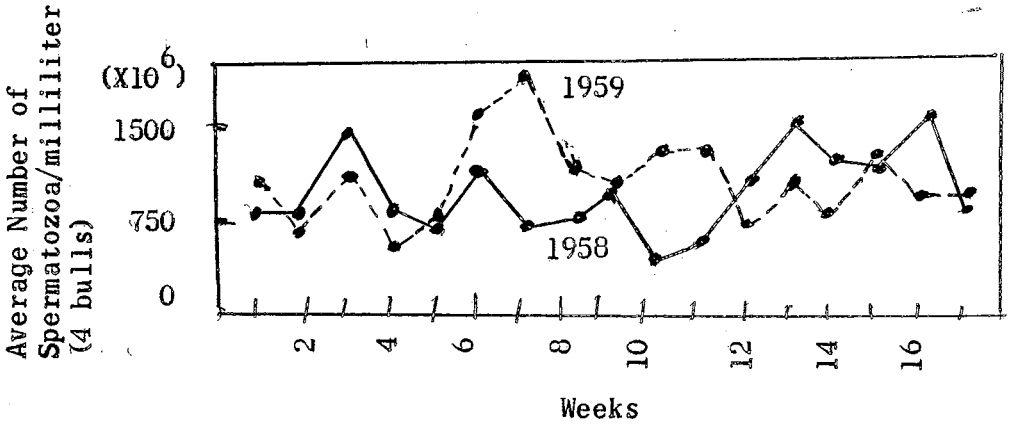

Figure 4. Comparison of Pre-injection Period (Jan. 10-May 1, 1958) with Corresponding Seasonal Post-injection Period (Jan. 9-May 2, 1959).

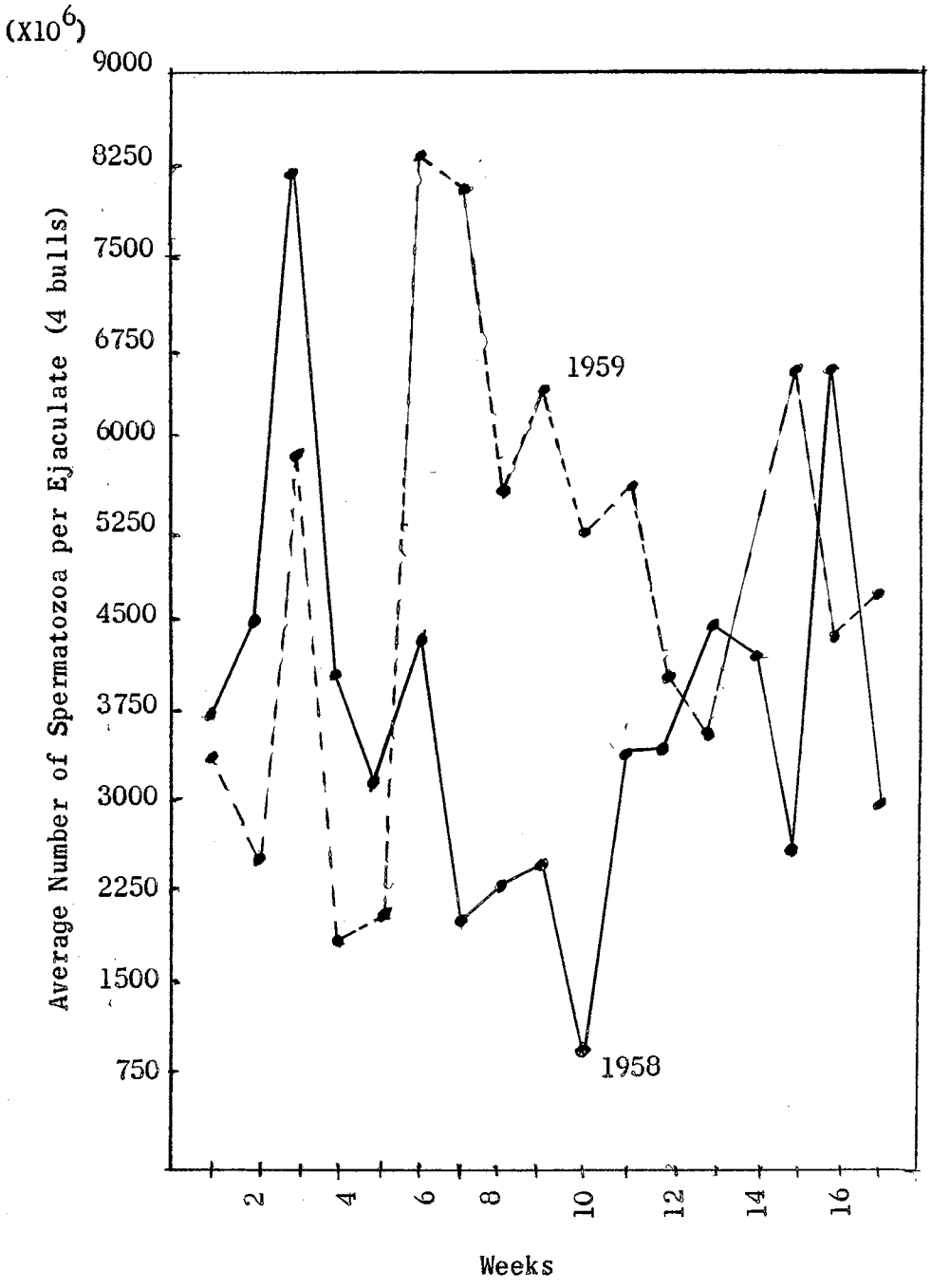

Figure 5. Comparison of Pre-injection Period (Jan. 10-May 1, 1958) with Corresponding Post-injection Period (Jan. 9-May 2, 1959).

SUMMARY

Weekly semen samples from four Hereford bulls were evaluated over a 72 week period and periodic measurements taken of the testicular size. On the eighteenth experimental week, thrice weekly injections of 250 milligrams of testosterone propionate were begun and continued through week 35 at which time testosterone therapy was discontinued; a 37 week recovery period followed. Needle biopsies were taken of the testicles previous to and at the conclusion of testosterone therapy; testicular sections were secured at slaughter at the close of the experiment.

Although there were no great variations in average semen volume or number of spermatozoa per milliliter between the control, and either injection or post-injection periods, biological trends can be seen. Within the post-injection period, however, there were important decreases in semen volumes and numbers of spermatozoa per milliliter during the first 11 weeks followed by a rise to slightly higher than pre-injection levels. Total spermatozoa per ejaculate is the product of the above factors, and consequently varied to a greater degree during the early and later parts of the post-injection period.

The motility rating and percentage abnormal forms of the spermatozoa were within normal ranges and varied so little from period to period, that no importance can be attached to the fluctuations.

The average volume of the testicles gradually decreased 50 percent during the injection period and after cessation of injections returned

to a volume that was slightly above the pre-injection level. This correlated with the cytological findings wherein there were reduced size and number of both Leydig cells and germinal epithelial cells at the end of testosterone therapy. The increased testicular size in the post-injection period is correlated with the increased number of germinal cells and number and size of Leydig cells observed in the final testicular sections.

The injection of testosterone propionate for a period of 18 weeks reduced spermatogenesis, the number and size of Leydig cells, and the number of germinal epithelial cells; however, the Sertoli cells increased in size. Testicular volume began to return to pre-injection levels immediately after cessation of injections whereas spermatogenesis continued to deteriorate for several weeks before semen examination indicated recovery was occurring.

Comparison of corresponding seasonal periods before and after testosterone injections indicated a slight increase in most semen quality factors but such increase may well be due to the effect of increasing age of the bulls. It should be considered that spermatogenesis and testicular size and cytology had been severely depressed during the intervening period.

It therefore appears that spermatogenesis could not be completely depressed by massive doses of testosterone for 18 weeks. Instead, it is probable that the injections, while partially maintaining spermatogenesis, may have effectively blocked gonadotrophin production and/or release. Cessation of injections may have left the bull temporarily physiologically hypophysectomized which permitted a period of low endogenous testosterone production. This low testosterone level coupled with low

gonadotrophin levels resulted in further deterioration of the semen picture. Within several months, gonadotrophin and testosterone levels increased followed by improved spermatogenesis.

Exogenous testosterone caused depression of spermatogenesis and testicular size and cytology. The processes so affected were reversible since spermatogenesis and testicular size returned to pre-injection levels within six months. It appears that the procedures used in this experiment may be used in research or therapy without causing permanent impairment of spermatogenesis. Further work is necessary before the findings of this experiment can be fully evaluated.

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