

RESULTS OF TRANSPLANTING GONADIAL TISSUE IN DAIRY COWS AND BULLS¹

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INTRODUCTION

Gonadial tissue has been transplanted for the purpose of (1) making physiological and genetic studies, (2) relieving suffering (in human beings), and (3) rejuvenating and stimulating the genital organs. A great deal has been published concerning the transplanting of gonadial tissue in human beings, small laboratory animals, and birds, but few experiments have been reported with larger domestic animals, especially cattle. In the present work gonadial tissue was grafted on 11 cows and 5 bulls to determine whether the extra hormones would so stimulate, rejuvenate, or strengthen the genital organs that they would function normally. The work was done at the United States Dairy Experiment Station at Beltsville, Md.

REVIEW OF LITERATURE

Grunert² has reported the results of experiments with two cows, one of which was 15 years old and the other 12 years old. The 15-year-old cow had calved July 26, 1925; no oestrus was observed, and on June 21, 1926, ovarian tissue was grafted. Oestrus appeared August 8, the cow was bred and became pregnant. The 12-year-old cow had calved November 27, 1925; no oestrus was observed and ovarian tissue was grafted June 21, 1926. Oestrus was noted July 31, but the cow was not bred.

Haring³ in reviewing his observations of grafting gonadial tissue in Switzerland stated that oestrus appeared in so-called sexual infantilism in heifers after grafting and also that libido appeared in bulls, but he made no mention of the effect on fertility.

Stäheli⁴ grafted organs of young heifers onto 51 aged cows that had refused the bull for nearly a year. Forty-six came in season and mated. Of these, 31 conceived, and 15 were mated again and produced a second crop of calves.

Turner⁵ reports that a successful autoplasmic ovarian transplantation was made in a 6-month-old spayed heifer. At 12 months of age the heifer showed signs of heat, but the periods were not regular.

There is nothing in the literature to indicate that any irregularities of reproduction in cattle other than senility and infantilism have been corrected by grafting gonadial tissue.

¹ Received for publication Mar. 9, 1934; issued August, 1934.

² GRUNERT, C. H. ABSENCE OF OESTRUM CORRECTED BY OVARY-TRANSPLANTATION. *Vet. Med.*, 22: 112-113. 1927.

³ HARING, C. M. OVARY AND TESTICLE TRANSPLANTATION IN VETERINARY PRACTICE. *Jour. Amer. Vet. Med. Assoc.* (n.s. 21) 68: 419-428. 1926.

⁴ STÄHELI, ÜBER EIERSTOCKSIMPLANTATIONEN BEI PRAESENILEN. *Schweizer Arch. Tierheilk* 67: [451]-458. 1925.

⁵ TURNER, C. W. SUCCESSFUL OVARIAN GRAFT IN SPAYED HEIFER. *Mo. Agr. Expt. Sta. Bull.* 285: 60, illus. 1930.

EXPERIMENTS WITH COWS

Grafts of ovarian tissue were made on 11 Jersey and Holstein Friesian cows in 1927 and 1928. In 4 of the cows grafts were made twice, at intervals of 7 days. The transplantations of ovarian tissue were made in the longissimus dorsi, serratus posticus, and trapezius muscles. The skin of the animal was cleaned of hair and dirt the day before the grafting. Vertical incisions were made through the skin in some of the animals and horizontal incisions in others. In the first four animals the tissue was implanted in the muscles and in all others it was attached to the roughened surface of the muscles. All operations were performed with the animals secured in the standing position. Chloral hydrate administered through the mouth served as a general sedative, and procaine was used as a local anaesthetic. Catgut sutures served to attach the gonadial tissue, and umbilical tape was used to close the skin incisions.

Ovaries were obtained from young healthy cows and healthy pigs. The cow ovaries were cut into 3 pieces, the larger pig ovaries were cut into 2 pieces, and the smaller ones were left whole. The ovarian tissue was kept in dry sterile glass containers until it was transplanted several hours later.

GENITAL HISTORY AND EXPERIMENTAL RESULTS

Table 1 shows the dates grafts were made on each cow, the source of the ovarian tissue, the number of pregnancies following the grafting, and the post-mortem appearance of the graft.

TABLE 1.—*Birth dates of the experimental animals, dates of grafting, sources of ovarian tissue, number of pregnancies following grafting, and post-mortem findings at grafted site*

Cow no.	Date of birth	Date of grafting	Pregnancy for which cow was being bred when grafted	Kind of ovarian tissue used	Pregnancies following grafting	Date of slaughter	Finding at site of grafting
626	July 11, 1924	Sept. 29, 1927	First	Cow	0	Jan. 27, 1928	Scar.
264	Dec. 20, 1920	do	Third	do	0	Feb. 10, 1928	Degenerating ovarian tissue.
462	Mar. 14, 1917	Oct. 25, 1927	Seventh	do	0	Jan. 24, 1928	Do.
490	Dec. 19, 1922	Nov. 1, 1927	Third	do	2	Sept. 30, 1930	Small scars.
253	Jan. 27, 1919	Jan. 13, 1928	Sixth	Pig	0	June 11, 1928	Scar.
279	Jan. 5, 1923	do	Fourth	do	0	Sept. 21, 1928	Do.
412 ¹	Dec. 30, 1918	Jan. 20, 1928	Sixth	do	1	Feb. 20, 1930	Normal tissue.
		Jan. 13, 1928					
		Jan. 20, 1928					
442	Aug. 20, 1918	Jan. 13, 1928	Seventh	do	0	Dec. 6, 1928	Do.
		Jan. 20, 1928					
		Jan. 13, 1928					
468	Feb. 8, 1919	Jan. 20, 1928	Fifth	do	0	Sept. 24, 1928	Scar.
		Jan. 13, 1928					
613 ¹	Mar. 29, 1924	Jan. 13, 1928	Fourth	do	4	Jan. 6, 1933	Normal tissue.
609	Jan. 16, 1924	Jan. 20, 1928	do	do	1	Nov. 30, 1928	Scar.

¹ These cows were pregnant when subjected to the grafting operation and conceived later as indicated.

Cow 626

Cow 626, a registered Jersey, was 3 years 2½ months old when used in the grafting experiment in 1927.

This heifer had been mated 13 times, without conceiving, over a period of 16 months. During this time her genital organs appeared normal as determined by 15 examinations.

Grafting was done (Sept. 29, 1927) by separating the fibers of the left longissimus dorsi to form a pocketlike depression in which a piece of ovary was attached. In a similar manner two pieces of ovary were attached in a pocket made in the right trapezius cervicalis. The skin sutures were removed October 3, and the wounds were completely healed by October 29.

This animal did not conceive following the operation, although she was mated at four consecutive oestrus. She was slaughtered January 27, 1928, and the organs and tissues throughout the body appeared normal. Only dense scar tissue was found at the sites of the grafts.

Cow 264

Cow 264, a registered Holstein, was 6 years 9 months old when used in the grafting experiment in 1927.

This cow had been bred 11 times for her initial conception, giving birth to a normal calf July 1, 1924, after a gestation period of 293 days. Two matings were required for her second conception, which terminated in the birth of a normal calf on the two hundred and eighty-sixth day. Following this calving, 13 services between February 19, 1926, and August 21, 1927, inclusive, did not result in conception. On seven examinations the genital organs appeared normal, then on February 24, 1927, the uterus seemed to be enlarged as if she had been pregnant for approximately 40 days. Ten examinations between April 8 and September 28, 1927, inclusive, each revealed a soft uterus hanging forward in the abdominal cavity.

Ovarian tissue was grafted in this cow on the same day (Sept. 29, 1927) and in the same way as in heifer 626, except that a corpus luteum of the ovary was discarded. The skin sutures were removed October 3, and the wounds seemed completely healed October 29.

This animal showed no oestrus after the operation. The uterus showed little improvement as determined by five examinations, and the cow was slaughtered February 10, 1928. The walls of the uterus were found to be oedematous, with adhesions over the anterior parts of the uterus. The lymph glands around the pelvic inlet were enlarged.

Sections from the grafted area of the musculature showed small masses or islands of cells which were recognized as remnants of the ovarian graft. These masses of partially degenerated cells were surrounded by fibrous tissue proliferations and there appeared to be some slight degeneration of the adjacent muscle tissue. There was a noticeable round cell infiltration and also marked vascularization of the entire grafted area (fig. 1).

Cow 462

Cow 462 was a registered Jersey, 10 years 7 months old when used in the grafting experiment in 1927.

The portion of the breeding history of this cow on which there is a complete record is given here. She conceived in one mating, and on June 25, 1922, aborted after a 239-day gestation period. After this abortion she was bred seven times before she conceived, delivering a normal calf October 11, 1923, after a 272-day gestation period. During the next sexual season nine services were required before she conceived, delivering a normal calf June 28, 1925, after a 282-day

gestation period. She was then bred only three times before she conceived, delivering a normal calf August 13, 1926, after a 280-day gestation period. She was then mated eight times without conception.

One month after calving in August 1926 a cyst the size of a baseball was found on the right ovary and a smaller one on the left ovary. Cysts were present until June 1927, when they disappeared leaving the ovaries enlarged. Five examinations during July and early August revealed a soft toneless uterus and enlarged ovaries. During late August, September, and early October, five examinations revealed that the uterus was normal in size and consistency and that there was no change in the ovaries. Throughout the summer the vulva was constantly swollen to approximately the size of that of a cow ready to freshen.

Ovarian tissue was grafted in this animal October 25, 1927. An incision was made in the left longissimus dorsi and two pieces of ovary

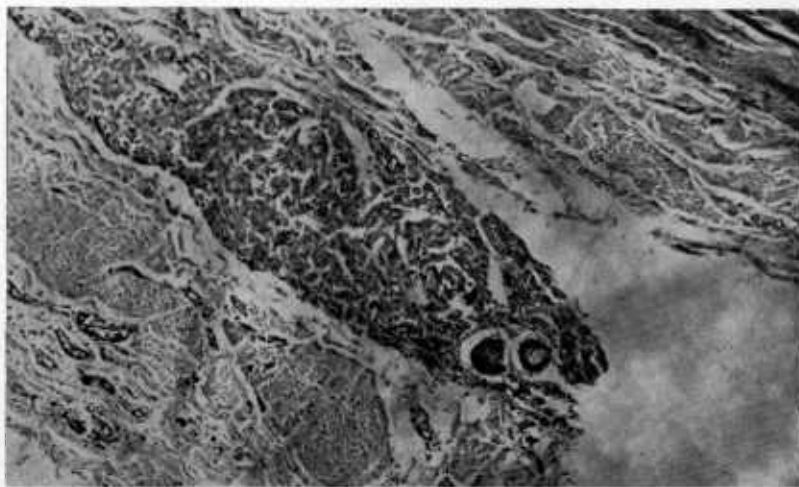


FIGURE 1.—Section from muscle of cow 264 showing remnant of ovarian graft. $\times 90$.

were attached. Another piece was anchored to the roughened surface of the serratus posticus, and three pieces were anchored in a pocket in the right trapezius cervicalis. The skin sutures were removed November 1. At this time a hard swelling about 4 by 7 inches was noticed at the point of the operation on the back and a similar enlargement about 2 by 3 inches in size at the site of the operation on the neck. These enlargements broke November 13 and discharged a thick yellow pus. The wounds were healing normally by November 19. The swelling of the vulva had disappeared by November 1. When healing of the wounds assumed normal appearance the vulva began to enlarge again. There was no change in the condition of the rest of the genital organs. This animal was mated twice after the operation and did not conceive.

Autopsy on January 24, 1928, revealed enlarged dense ovaries with adhesions around them. The walls of the uterus were thick. Cysts were found in the walls of the vagina. Many minute blood vessels were observed in the region of the transplanted tissue. Tissue

resembling small ovarian follicles was found in the trapezius cervicalis and some dried tissue was noted in the region of grafting on the back.

Histologically the grafted area of the musculature showed a small portion of the transplanted ovarian tissue, surrounded by fibrous tissue. There was lymphocytic and round cell infiltration, and a number of multinucleated cells were noted. The grafted area showed extensive vascularization. Hemorrhages were observed toward the center of the area and also degenerated muscle tissue, masses of pus cells, and necrotic material (fig. 2).

Cow 490

Cow 490, a registered Jersey, was approximately 4 years 11 months old when used in the grafting experiment in 1927.

This cow had been bred three times before she conceived May 2, 1924. A normal calf was born 278 days later. Following this calving

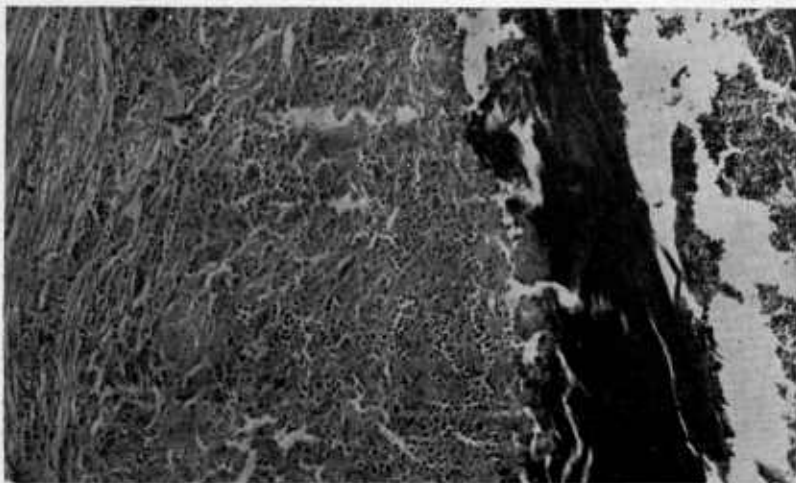


FIGURE 2.—Section from muscle of cow 482 showing remaining portion of transplanted ovarian tissue. $\times 90$.

she conceived to the fifth service on October 5, 1925. She aborted 181 days later. Between the time of this abortion and the transplanting of ovarian tissue this cow was bred 15 times without conception. A metritis followed the abortion, and by April 1927 the ligaments supporting the genital organs had so relaxed that the uterus hung far forward in the abdominal cavity. By September 1927 there was much improvement in the tone of the uterus and the ligaments had strengthened sufficiently to hold the genital organs near their normal position, where they remained until after the grafts were made.

Grafting was done November 1, 1927. An incision was made in the right trapezius cervicalis and pieces of one ovary were attached to this cut surface. In the same manner pieces of another ovary were attached to a cut surface of the left longissimus dorsi. On November 2 both wounds were swollen. On November 8 diffuse swellings extended over the right side of the neck and over most of the left side of the back. The sutures were removed and the lower ends of the

wounds opened. A foul-smelling, brownish liquid ran from the openings. The wounds then healed slowly. In August 1928 an abscess formed at the site of grafting on the neck. It healed rapidly when drained.

The uterus became slightly relaxed a month after the grafting and remained so until conception took place at the sixth mating on October 5, 1928. The cow gave birth to a normal calf 277 days later. Only one service was required for the next conception, which terminated normally at the end of a 281-day gestation period on September 23, 1930.

On autopsy September 30, 1930, the uterus was found partly involuted. Only small scars were observed at the sites of the transplanted ovarian tissue.

Cow 253

Cow 253, a registered Holstein, was approximately 9 years old when used in the grafting experiment in 1928.

This cow had given birth to her first calf July 17, 1921. She then conceived to the second mating and calved normally at the close of a 281-day gestation March 19, 1923. Following this calving she conceived, to the sixth mating, on January 26, 1924, and aborted 270 days later. After this abortion she conceived, to the third mating, May 6, 1925, and a normal calf was born 287 days later. The next conception occurred, to the first mating, on June 22, 1926, and a dead calf was delivered 293 days later. Between the time of this calving and the transplanting of ovarian tissue this cow was bred nine times without conception. No abnormalities were found in the genital organs during this time.

Grafting was done on January 13, 1928. Three pieces of ovarian tissue were attached to the roughened surface of the right trapezius thoracalis, and one whole ovary was placed in a pocket in the lower part of the wound. The wound seemed to be clean and healing when the sutures were removed on January 23, but on January 30 a slight discharge of pus was noticed coming from the lower end of the wound and a piece of foul ovary was removed February 6. After its removal the wound healed rapidly.

Only one oestrus (May 1, 1928) was observed after ovarian tissue had been grafted in this cow. On autopsy (June 11, 1928) adhesions were found around the ovaries. Scar tissue only was found at the place of grafting.

Cow 279

Cow 279, a registered Holstein, was 5 years old when used in the grafting experiment in 1928. Conceiving on April 14, 1924, to the first mating, this cow gave birth to a dead calf 278 days later. Only one service (July 9, 1925) was needed for the second conception, which terminated in the birth of a normal calf 275 days later. She was bred only once (July 19, 1926) for the third conception, which terminated in an abortion 198 days later. Between this abortion and the time of grafting ovarian tissue 10 matings did not result in conception. This abortion was followed by metritis, and by December 1927 the ligaments supporting the genital organs had relaxed to such an extent that the uterus hung forward in the abdominal cavity.

Grafting was done on this cow at the same time (Jan. 13, 1928) and in the same manner as on cow 253. There was a little swelling around the wound January 16. The skin sutures were removed January 23 and complete healing had taken place by February 7. Seven days after the first grafting three pieces of ovarian tissue and one whole ovary were grafted to the medial surface of the left trapezius thoracalis. The skin sutures were removed January 30 and the wound was completely healed by February 7.

This cow did not conceive at two matings following the operations. The uterus remained forward in the abdominal cavity until the animal was slaughtered September 21. On autopsy congestion around the pelvic inlet was noted. The walls of the uterus were thin and soft and the ovaries were enveloped in thick connective tissue. Only scar tissue was found at the sites of grafting.

Cow 412

Cow 412, a registered Jersey, was approximately 9 years old when used in the grafting experiment in 1928.

The early records of the activities of the genital organs of this cow are incomplete. She gave birth to normal calves October 21, 1923, November 26, 1924, February 14, 1926, and May 16, 1927. The genital organs seemed normal after the last calving. She was mated again on December 23, 1927.

Grafting was done at the same time (Jan. 13, 1928) and in the same manner as on cow 253. There was diffuse swelling over the grafted area on January 16, but it had practically disappeared by January 19. The skin sutures were removed January 23 and complete healing had taken place by February 7. Seven days after the first grafting three pieces of ovarian tissue were grafted to the roughened surface of the left trapezius thoracalis. The skin sutures were removed January 30 and the wound was completely healed by February 7.

An examination on February 14 showed that conception had occurred 21 days before the first grafting operation. This pregnancy terminated in the birth of a normal calf after a gestation period of 281 days. The next conception occurred from one mating on December 12, 1928, and a normal calf was born 285 days later. Three services following this calving did not result in conception. The genital organs seemed to be normal when examined during this sexual season.

On autopsy February 20, 1930, the walls of the uterus were found to be gelatinous and the fallopian tubes closed. Only connective tissue was found at the sites of grafting.

Cow 442

Cow 442, a registered Jersey, was approximately 9 years 5 months old when used in the grafting experiment in 1928.

The early records of the activities of the genital organs of this cow are incomplete. On December 3, 1920, she had aborted after a 224-day gestation period. Following this abortion she conceived to the first mating and gave birth to a normal calf February 1, 1922, after a 272-day gestation period. She then conceived to the second mating,

delivering a weak calf April 30, 1923, after a 273-day gestation period. Conception took place again when the cow was mated the second time. This pregnancy continued 261 days, terminating in the delivery of a normal calf. Following this calving she conceived to the fourth mating and aborted 201 days later on July 3, 1925. Then she conceived to the second mating and aborted 237 days later on July 26, 1926. She was then bred six times before the grafting operation, but did not conceive.

Involution of the uterus seemed complete 40 days after her abortion in 1926. By the end of another 30 days the uterus was large, its walls were thick, and there was a white discharge when the organ was massaged. This discharge continued until December 17, or approximately 5 months after the abortion. The uterus seemed to have returned to its normal nonpregnant size by March 3, 1927. It remained so until after the ovarian grafts were made. Breeding was not attempted until April 13, 1927, and six matings thereafter failed to produce conception.

The grafting of ovarian tissue was done (Jan. 13 and 20, 1928) in the same manner as described for cow 412. Healing was complete by February 7. An examination on February 14 revealed that the ligaments supporting the genital organs had relaxed to such an extent that the uterus hung forward in the abdominal cavity. The uterus remained in this position until August, when it assumed its normal position. On November 15 adhesions were found around the ovaries. The cow was mated twice after the grafting of ovarian tissue and did not conceive.

Autopsy on December 6 revealed adhesions around the ovaries and fallopian tubes. Only connective tissue was found at the points of grafting.

Cow 468

Cow 468, a registered Jersey, was approximately 8 years 11 months old when grafted with ovarian tissue in 1928.

The records of the early activities of the genital organs of this cow are incomplete. On January 5, 1924, she delivered a normal calf, after a 276-day gestation period. She then conceived to the fourth service and delivered a normal calf July 20, 1925, after a 283-day gestation period. The next conception occurred with only one mating, and a dead calf was born 277 days later, on September 1, 1926. Involution of the uterus seemed normal after this calving. Later the uterus enlarged and discharged pus. One year later it returned to its normal nonpregnant size. Between the 1926 calving and the death of the cow only one oestrus appeared. This was on March 8, 1927. After that the right ovary and fallopian tube became enlarged.

The grafting of ovarian tissue was done (Jan. 13 and 20, 1928) in the same manner as described for cow 412. Healing was complete by February 7. Following this there was no apparent change in the uterus and no oestrus occurred. The right ovary and fallopian tube were removed surgically March 17. Abscesses appeared in the udder, right flank, and the throat region in August.

Autopsy on September 24 revealed abscesses in the udder and under the skin in the throat region, and fibrous proliferations (fibroplastic peritonitis) were noted throughout the abdominal cavity and

over the genital organs. The fluids of the body cavities were gray in color. Only scar tissue was found at the sites of grafting.

Cow 613

Cow 613, a registered Jersey, was approximately 3 years 9 months old when used in the grafting experiment in 1928.

She conceived to her first mating, July 11, 1925, but the pregnancy terminated in an abortion 164 days later. Five matings then occurred before conception again took place, June 2, 1926. This pregnancy terminated in an abortion 264 days later. The genital organs seemed normal following the abortion. She was bred nine times, after which an examination of the semen of the bull showed inactive spermatozoa. The cow was then mated with a normal young bull on December 24, 1927.

Grafting was done (Jan. 13, 1928) in the same manner as described for cow 253. The skin sutures were removed January 23 and the wound was completely healed by February 7.

An examination on February 14 revealed that conception had taken place 20 days before the grafting operation. On October 4 a normal calf was born, after a 285-day gestation period. Four conceptions occurred after this calving. The first two required only one mating each. The third resulted from the ninth service, and the fourth required only one mating. The second pregnancy ended in the delivery of a dead calf, and the other pregnancies produced normal calves.

Autopsy January 6, 1933, showed the uterus in a normal involuting condition from the last calving on December 17, 1932. Only connective tissue was found at the site of the transplanted ovarian tissue.

Cow 609

Cow 609, a registered Jersey, was approximately 4 years old when used in the grafting experiment in 1928.

She had conceived to her first mating, April 23, 1925, but aborted 212 days later. Then conception resulted from one mating on February 4, 1926, and she aborted 120 days later. She was bred the third time October 18, 1926, but aborted 229 days later. She was then bred nine times but failed to conceive. No abnormalities were found in the genital organs during this time.

Grafting was done on January 20, 1928. The surface of the left trapezius thoracalis was roughened and five pieces of ovaries were attached. The skin sutures were removed January 30 and healing was complete by February 7.

The cow conceived at the sixth mating following the transplanting of ovarian tissue. A fetus was found in the uterus on autopsy November 30, 1928. The genital organs appeared normal. Only scar tissue was found at the site of the ovarian grafts.

EXPERIMENTS WITH BULLS

The technic of grafting testicular tissue in the five bulls differed but little from that of grafting ovarian tissue in the cows. Testicular tissue obtained from the testicles of young bulls and pigs was attached to the roughened surface of the trapezius muscle, and quinine urea

hydrochloride was used as a local anaesthetic. The testicles from the young bulls and pigs were used as soon as possible after they were collected. In the meantime they were kept in dry sterile glass containers.

SERVICE RECORD AND EXPERIMENTAL RESULTS

Table 2 shows the service records of the experimental bulls, when mated to fertile cows, before and after the implantation of testicular tissue.

TABLE 2.—Service records of experimental bulls mated to fertile cows, before and after the bulls had received transplanted gonadal tissue

Bull no.	Date of birth	Dates of grafting	Date of slaughter	Year	First quarter		Second quarter		Third quarter		Fourth quarter	
					Services	Conceptions produced	Services	Conceptions produced	Services	Conceptions produced	Services	Conceptions produced
1.....	July 29, 1917	{Dec. 9, 1927 Jan. 23, 1928 May 3, 1928	{Nov. 20, 1928	{1926	10	5	13	4	22	3	15	4
				{1927	14	3	30	7	36	1	14	0
				{1928	3	0	1	0				
2.....	Apr. 30, 1915	{Dec. 9, 1927 Jan. 23, 1928	{Mar. 2, 1928	{1926	13	3	10	4	12	5	12	4
				{1927	9	2	1	1	13	0	21	0
				{1928	3	0						
3.....	Apr. 21, 1911	Feb. 24, 1928	July 9, 1928	{1926	3	1	2	2	3	0	5	3
				{1927	7	1	3	2	2	1	5	1
				{1928	7	0	1	0				
4.....	Aug. 18, 1921	do	Sept. 12, 1929	{1926	18	6	8	5	4	3	10	5
				{1927	10	3	15	6	14	4	18	4
				{1928	17	4	22	9	7	1	10	1
5 ¹	June 9, 1926	May 3, 1928	July 9, 1928	{1929	16	5	5	3	4	0		

¹ See discussion in text, p. 276.

BULL 1

Bull no. 1 was a registered Jersey born July 29, 1917.

The activity of the spermatozoa of this bull, as seen through the microscope, was never greater than 50 percent during 1926 and the first 9 months of 1927. No activity was found during the last 3 months of 1927 and during 1928. The spermatozoa stained poorly and many heads were without tails. In October 1928 only 25 percent appeared normal morphologically. Spermatozoa numbered from 1 million to 166 million in a cubic centimeter.

On December 9, 1927, two pieces of bull testicle, which retained the greater part of the epididymis, were fastened to the roughened surface of the left trapezius thoracalis. On December 12 a dark yellow discharge came from the lower part of the wound. The skin sutures were removed and the wound cleaned. On December 17 the wound was found gaping open. There appeared to be firm live testicular tissue in the upper part of it. The lower part of the incision held a small amount of debris, otherwise healing was progressing favorably. By January 13, 1928, the wound appeared to be healed.

No change was apparent in the usefulness of this bull following this grafting, but there was evidence of increased sexual desire. His passive disposition also changed to one of aggression.

On January 23, 1928, two pieces of testicle, about 2 inches in diameter, edges one-fourth inch thick with the parenchymatous tissue bulging to about one-half inch thickness, were attached to the

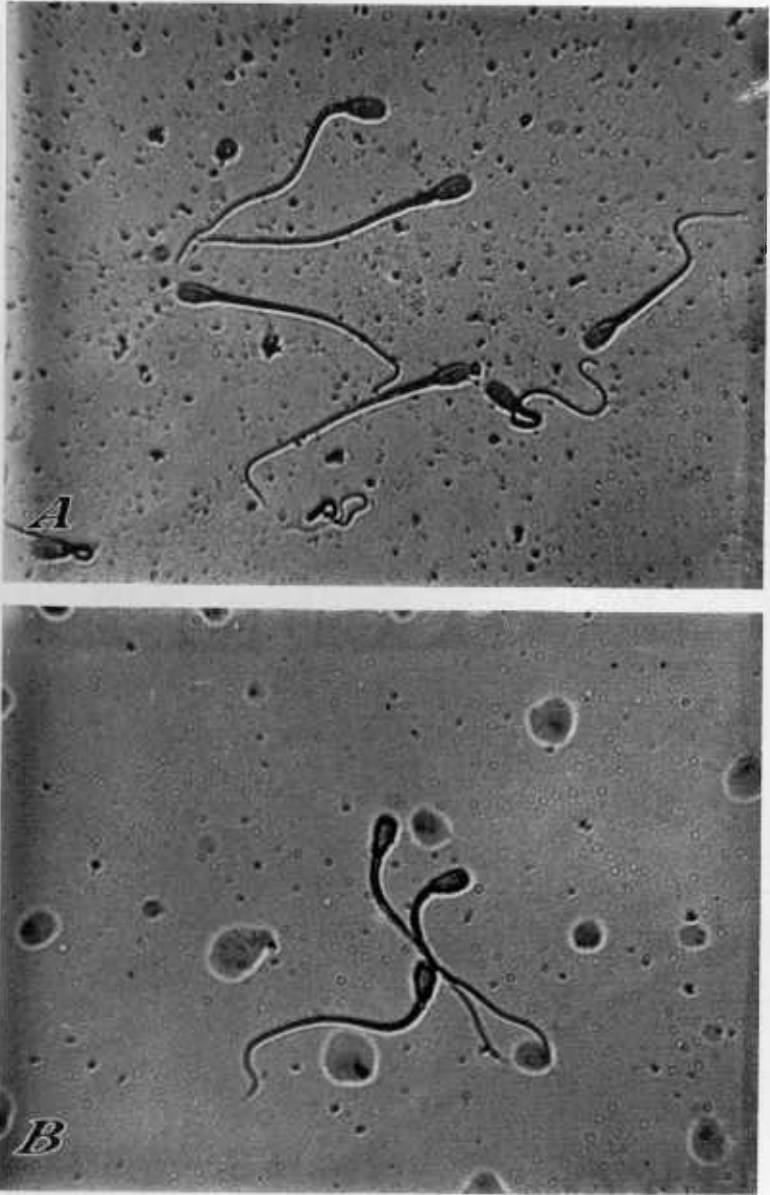


FIGURE 3.—Spermatozoa collected from bull no. 1: *A*, 27 days before grafting; *B*, 152 days after first grafting and 6 days after third grafting. $\times 400$.

roughened surface of the right trapezius thoracalis. These pieces of testicle were as warm as the surrounding tissue where they were attached. The skin sutures were removed February 7 and the wound

seemed to be healing normally. On February 24 some dried tissue protruded through the skin and was removed. No change in the usefulness of the bull occurred.

On May 3, a third grafting was tried. Three pieces of pig testicle were attached to the roughened surface of the right trapezius thoracalis and six pieces were attached to the roughened surface of the left trapezius thoracalis. The skin sutures on the left side were removed May 14. The wound had a disagreeable odor but appeared dry and clean. The skin sutures on the right side were removed May 26. Both wounds healed by first intention.

The spermatozoa of this bull did not change in activity, staining ability, or morphology, following these graftings (fig. 3). No conception occurred from the use of this bull after September 1927.

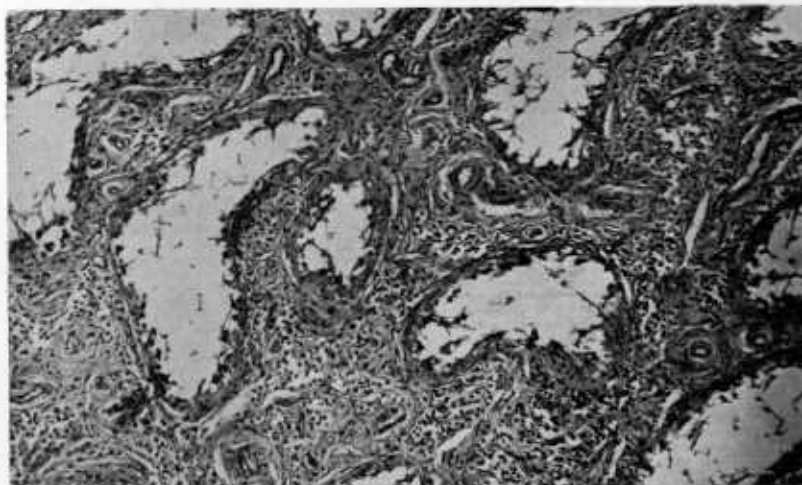


FIGURE 4.—Section from testicle of bull no. 1. $\times 90$.

Following each operation increased appetite and aggressiveness were noted.

This bull was slaughtered November 20, 1928, and the lumbar vertebrae were found to be fused into one piece. The sixth and seventh thoracic vertebrae were fused with a bony growth extending into the thoracic cavity. The sixth, seventh, and eighth ribs had sharp bony growths projecting into the thoracic cavity. Small abscesses were found where these sharp projections had injured the lungs. The genital organs were normal in shape but appeared atrophied. Scar tissue only was found at places of grafting.

Sections from the testes of this bull showed extensive atrophic changes throughout. There was almost a complete absence of tubular epithelium in the various sections examined, and in many of the tubules the epithelial structure had been replaced by concentric layers of connective tissue, which was gradually closing the lumen of the tubules. In some parts of the testes there was almost complete fibrosis, many of the tubules having been completely obliterated. A number of the remaining tubules contained deposits of a brownish pigment. Sections from the epididymis showed sloughing of the tubular epithelium and also extensive fibrous changes (fig. 4).

BULL 2

Bull no. 2 was a registered Jersey born April 30, 1915. Early in 1926 microscopical examinations of the semen of this bull revealed a

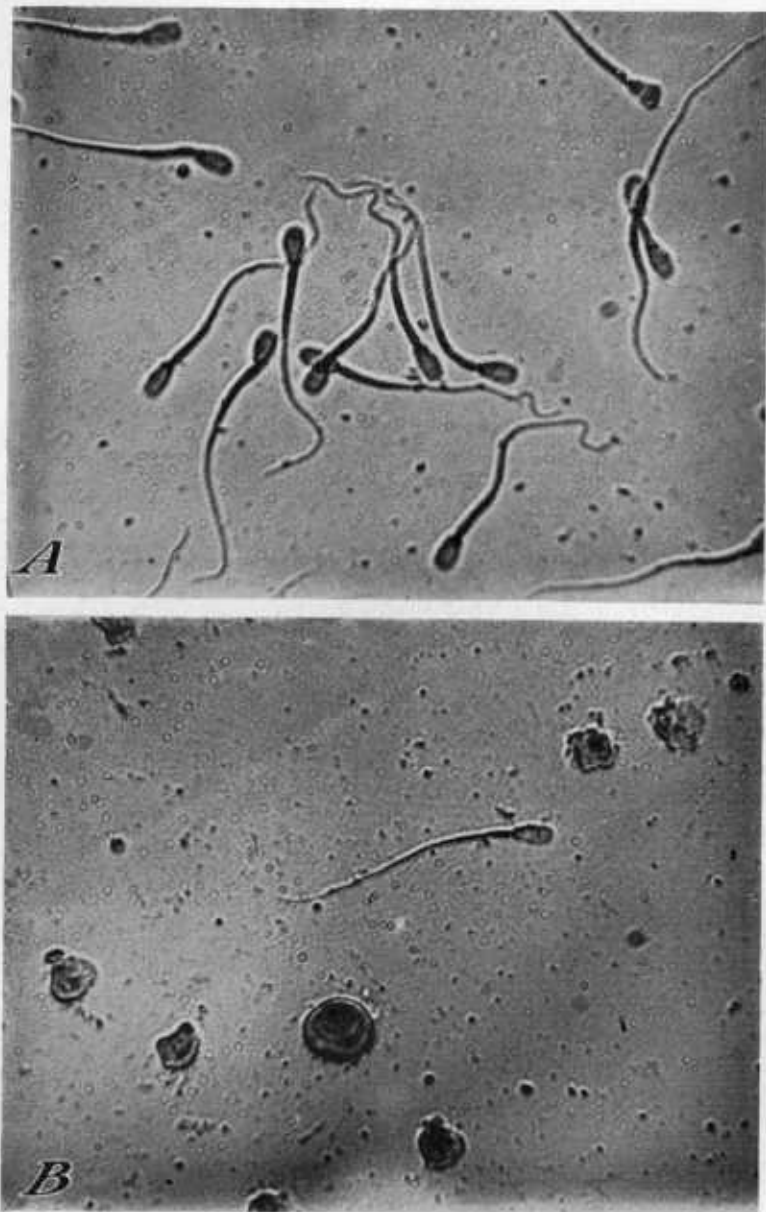


FIGURE 5.—Spermatozoa collected from bull no. 2: *A*, 513 days before grafting; *B*, 59 days after first grafting and 14 days after second grafting. $\times 460$.

90-percent activity of spermatozoa. This activity decreased until it was 40 percent by June 1927; no activity was noted after August 1927. Morphologically the spermatozoa appeared normal, even

when there was no activity. The number varied from 3 million to 476 million in a cubic centimeter.

On December 9, 1927, two pieces of bovine testicle were attached to the roughened surface of the left trapezius thoracalis. Very little of the epididymis remained on these pieces of testicle. On December 17 the skin sutures were removed. On December 21 the lower end of the incision was enlarged and oozing pus. On December 31 some tissue came from the wound. By January 13, 1928, the wound appeared to be healed. There was no change in this bull after the operation, and no change was noticed in his spermatozoa.

In a second transplanting on January 23, 1928, three pieces of bovine testicle were attached to the roughened surface of the right trapezius thoracalis. None of the epididymis was used. The skin sutures were removed on February 7. On February 24 a piece of decomposing testicle was protruding through the skin. It was re-

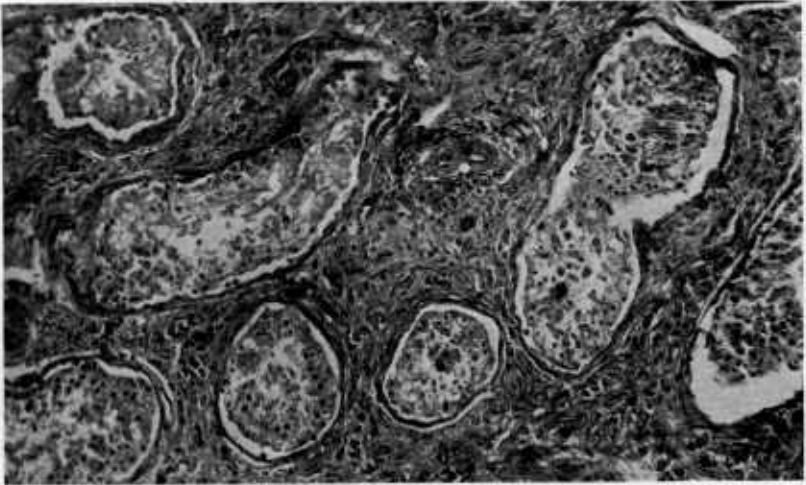


FIGURE 6.—Section from testicle of bull no. 2. $\times 90$.

moved. The spermatozoa of this bull remained inactive, but his appetite and aggressiveness increased (fig. 5). For several years he had been unable to mount a cow because of injured rear legs and he had had to walk up on the breeding crate and maneuver into position before he could serve the cow. After the second grafting operation he mounted cows in the normal way.

On autopsy March 2, 1928, gonitis and inflammation of the hip joint with bone growth was found in both rear legs. The genital organs were normal in shape but appeared atrophied. Only scar tissue was found at the sites of grafting.

Gross sections of the testes revealed a tumor formation which histologically was found to be of the nature of a teratoma. Several of these new growths, which could be outlined fairly well in both testes on sectioning, largely replaced the testicular tissue in the areas involved. The small growths were composed of large polygonal cells, showing an alveolar arrangement in some areas, while in other parts the cells assumed more or less of an adenomatous appearance.

The tumor cells were undergoing rapid degenerative changes and on the whole stained very poorly. Outside of the growths proper, masses of the tumor cells could be seen infiltrating the interstitial

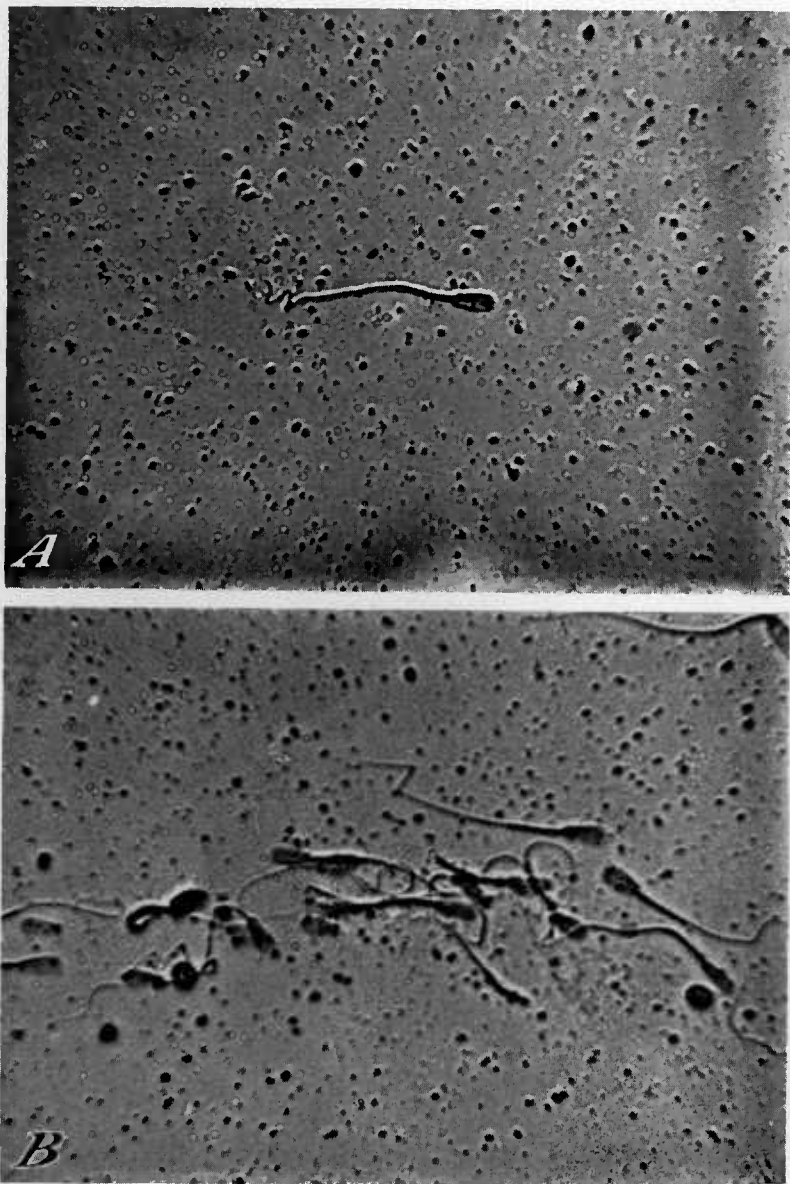


FIGURE 7.—Spermatozoa collected from bull no. 3. *A*, 9 days before grafting; *B*, 55 days after grafting. $\times 460$.

tissue which was greatly increased throughout the testes. Many tubules had undergone complete atrophy and the remaining tubules showed varying stages of degeneration and desquamation of the

tubular epithelium. A few spermatozoa were noted in a limited number of the tubules (fig. 6).

BULL 3

Bull no. 3 was a registered Holstein born April 21, 1911. Microscopical examination of the semen of this bull revealed a 95-percent activity of spermatozoa early in 1926. The activity had decreased to 40 percent by February 1928. Morphologically the spermatozoa seemed normal until 1928, when the heads appeared narrow and long. The number of spermatozoa in a cubic centimeter varied from 1 million to 135 million.

Four pieces of pig testicle were attached to the roughened surface of the left trapezius thoracalis February 24, 1928. The skin sutures were removed March 9 and the wound healed by first intention.

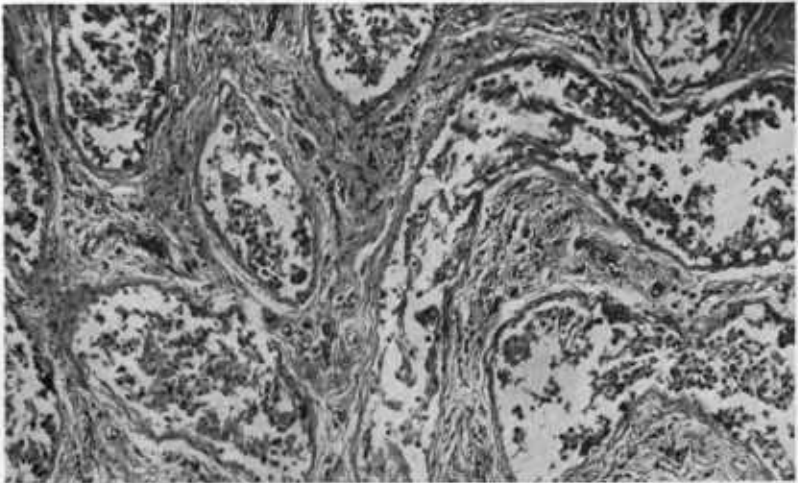


FIGURE 8.—Section from testicle of bull no. 3. $\times 90$.

This bull improved in appetite and vigor. The activity of his spermatozoa increased 10 percent (fig. 7). When he was slaughtered July 9 the genital organs appeared normal. A growth was found in the thymus. Only scar tissue was found at the point of grafting.

Sections from portions of the testes showed extensive fibrous proliferation with consequent atrophy and obliteration of many of the seminiferous tubules. In those areas showing only limited fibrous changes there was degeneration and desquamation of the tubular epithelium. In some tubules there had been complete destruction of the epithelium, leaving only the basement membrane. Calcareous material was noted in a few of the tubules, and a number contained pigment deposits. Some tubules showed a limited number of spermatozoa. In the epididymis there was likewise destruction of the tubular epithelium, the lumen of the tubules being filled with the detached cells and cellular debris. There was also a noticeable increase in the interstitial tissue (fig. 8).

BULL 4

Bull no. 4 was a grade Holstein born August 18, 1921. Microscopical examination of this bull's semen showed a 90-percent activity

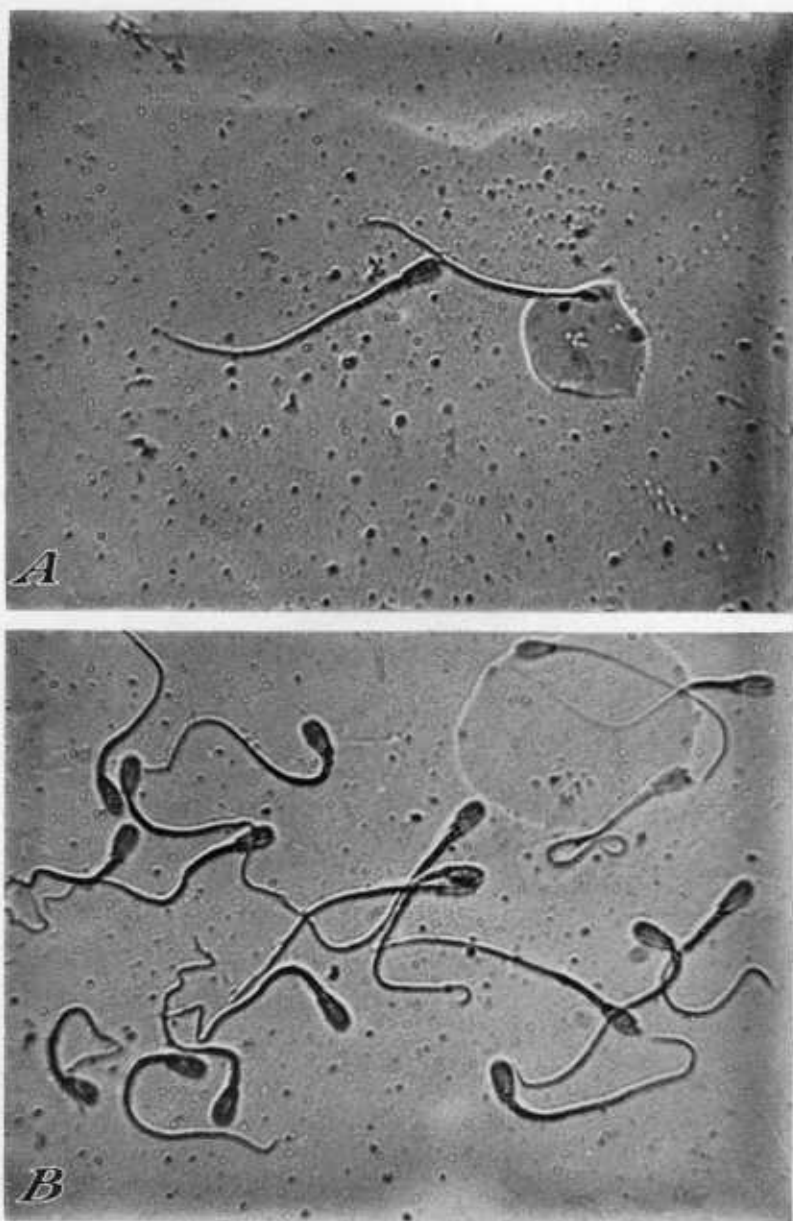


FIGURE 9.—Spermatozoa collected from bull no. 4: *A*, 41 days before grafting; *B*, 52 days after grafting. $\times 460$.

of spermatozoa during 1926 and 1927. In January 1928 the activity was zero. Morphologically the spermatozoa appeared normal.

On February 24, 1928, four pieces of pig testicle were grafted to the roughened surface of the left trapezius thoracalis. The skin sutures were removed March 9, and the wound healed rapidly after one piece of testicle was taken out. The spermatozoa of this bull again showed an activity of 90 percent (fig. 9); table 2 shows his service record following the grafting.

This bull was slaughtered September 12, 1929, and no gross abnormalities were found. A scar was observed at the place of grafting, but no testicular tissue was found.

Sections from the testes of this animal showed a limited number of the seminiferous tubules with the epithelium intact which contained a fair number of spermatozoa. On the whole, however, there was rather advanced degeneration and desquamation of the epithelium in a very large percentage of the tubules, while in some areas there

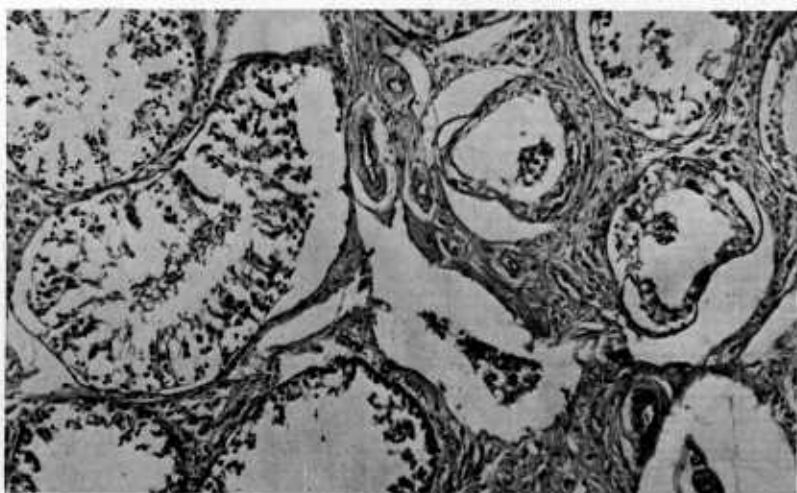


FIGURE 10.—Section from testicle of bull no. 4. $\times 90$.

was practically complete destruction of the tubular epithelium in whole groups of tubules. There was considerable cellular infiltration with a noticeable increase in the interstitial tissue in some of the sections examined (fig. 10).

BULL 5

Bull no. 5 was a registered Holstein born June 9, 1926. He was placed in a herd when a year old, and in September 1927 it was noticed that the cows bred to him did not conceive. Spermatozoa could not be found by a careful microscopic examination of semen September 27, 1927. Examinations between September 27, 1927, and May 3, 1928, revealed pieces of heads of spermatozoa, odd-shaped heads without tails, and some tails with pieces of heads, round heads, and odd-shaped heads attached. The last examination before grafting revealed only a few pieces of spermatozoa.

On May 3, 1928, four pieces of pig testicle were attached to the roughened surface of the right trapezius thoracalis and six pieces were attached to the roughened surface of the left trapezius thora-

calis. Much fat was encountered in this animal. By May 12 the grafted area on the left side of the animal was enlarged to half the

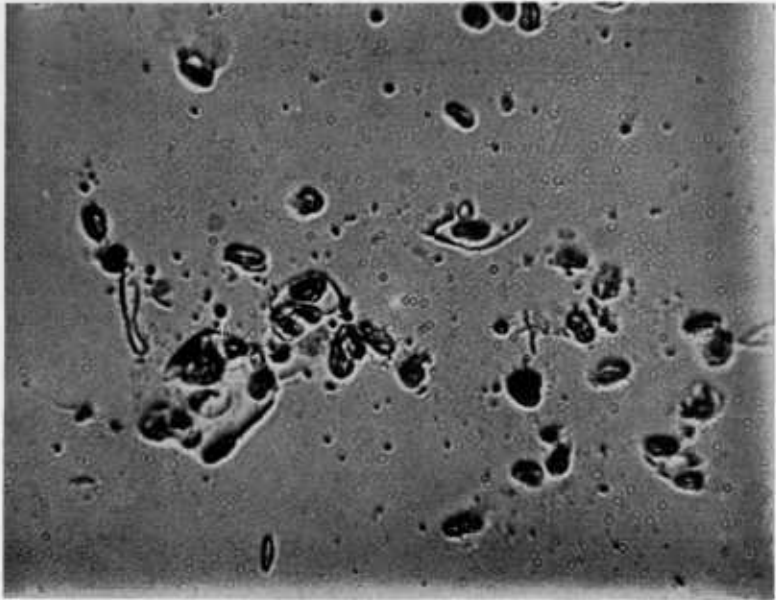


FIGURE 11. Spermatozoa from bull no. 5, collected 28 days after grafting. $\times 430$. Spermatozoa collected before grafting were similar in appearance, but fewer in number.

size of a football. On May 14 the skin sutures on this side were removed and the enlargement was found to be a blood clot. By

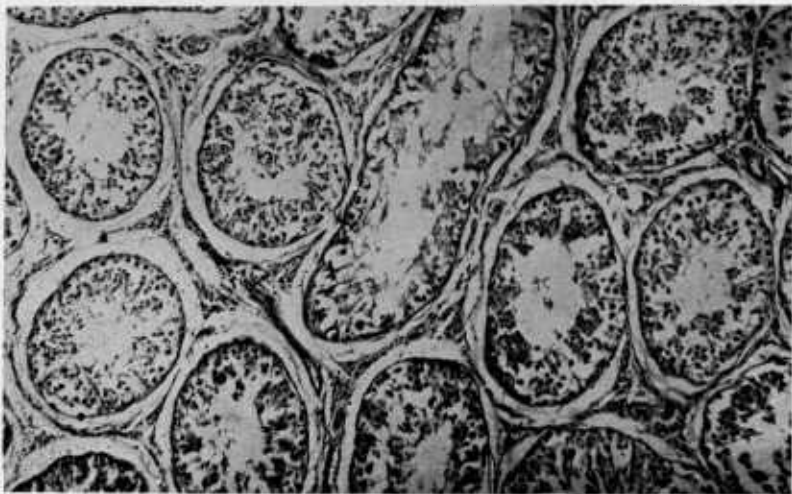


FIGURE 12. Section from testicle of bull no. 5. $\times 90$.

May 16 the incision spread open about 4 inches with the blood clot protruding. On May 26 the skin sutures on the right side were removed and the wounds then healed rapidly.

A microscopical examination of the semen of this bull on May 31, 1928, showed a few whole spermatozoa moving sluggishly; also many pieces of spermatozoa, round heads, and heads of varying shapes were found (fig. 11). Fewer pieces were observed in June, and on June 29 no spermatozoa or pieces of spermatozoa could be found.

On July 9, 1928, when this bull was slaughtered, all organs and tissues appeared normal. Only scar tissue was found at the sites of grafting.

Sections from the testes of this bull showed varying stages of degeneration of the epithelium of the seminiferous tubules. The changes were more advanced in some areas than in others. A certain percentage of the tubules showed either partial or complete desquamation of the tubular epithelium, the lumen of the tubules being filled with the desquamated cells and granular material. Only limited numbers of spermatozoa were noted even in those tubules which were practically normal in appearance. The epididymis also showed some destruction of the tubular epithelium in this case (fig. 12).

SUMMARY

Experiments were made to determine the possibility of correcting faulty reproduction in dairy cattle by the implantation of gonadial tissue.

Ovarian tissue removed from cows shortly after they were killed was grafted on 2 cows that had been affected with metritis, on 1 that had been affected with ovarian cysts, and on an apparently normal heifer. Ovarian tissue obtained from pigs in the same manner was grafted on 3 cows that had been affected with metritis, on 2 apparently normal cows, and on 2 pregnant cows.

The results show that the transplanted ovarian tissue had no appreciable effect on the oestral cycle or on the production of ova. Apparently no damage was done to pregnant cows. A temporary change was noticed in the appearance of the vulva of one cow.

Testicular tissue obtained from a young live bull was grafted on 2 senile bulls. Testicular tissue obtained from live pigs was grafted on 2 senile bulls (one of these also had bovine testicle grafts), on 1 bull that was 6 years 6 months old and temporarily sterile, and on 1 young bull showing aspermia.

The results show that the transplanted testicular tissue had no appreciable effect on the spermatozoa of senile bulls, although it did seem to increase their appetite, body vigor, and aggressiveness. The spermatozoa of the 6½-year old bull became active and fertile following this treatment. This was not necessarily due to the transplanted tissue, however, since a change in feed and management has produced the same results in other bulls. In the young bull showing aspermia, an increase in pieces of spermatozoa was noticed, but there was not enough improvement to enable the bull to become fertile.

In both the bulls and the cows healing was much more rapid where gonadial tissue from the pig was used.