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Experimantal procedures for the study of physiology of the farm animals

T. Seenappa

To the Graduate Council:

I am submitting herewith a thesis written by T. Seenappa entitled "Experimantal procedures for the study of physiology of the farm animals." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Husbandry.

George M. Merriman, Major Professor

We have read this thesis and recommend its acceptance:

H.J. Smith, J.D. Smalling

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

December 1, 1964

To the Graduate Council:

I am submitting herewith a thesis written by T. Seenappa entitled "Experimental Procedures for the Study of Physiology of the Farm Animals." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Husbandry.

George M. Mervinson
Major Professor

We have read this thesis and recommend its acceptance:

Harold J. Smith

John D. Smalley

Accepted for the Council:

Helton A. Smith
Dean of the Graduate School

EXPERIMENTAL PROCEDURES FOR THE STUDY OF PHYSIOLOGY OF THE FARM ANIMALS

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

T. Seenappa

December 1964

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CHAPTER I

INTRODUCTION

Experimental techniques involving surgery, hematology, and related procedures have been essential in the development of our knowledge in animal physiology. These techniques have been catalogued for basic physiology and are presented in many laboratory manuals. Techniques in applied physiology of the large domestic animals have also, in most cases, been intensively studied. However, these techniques, their usefulness and their limitations are reported sporadically in diverse sources. It is often difficult and time consuming for an investigator to assemble all pertinent information on a given technique in physiological research.

The present work, therefore, was designed and completed to accomplish the following goals: (1) catalogue and collect information on the availability, usefulness, and limitations of experimental techniques useful in research and teaching concerned with large-animal physiology, (2) describe in detail techniques which have been adapted for use at this experimental station.

These goals have been accomplished by literature review, by application of specific techniques, and by presentation of a selected case history.

I. RECTAL PALPATION FOR DETERMINATION OF PREGNANCY IN THE COW

Early determination of pregnancy in the cow is of great economic importance in practical dairying and beef production and in programs of control of reproductive diseases. Hafez (1962) reported that there are at least three major methods used for diagnosis of pregnancy in farm animals. These are clinical diagnosis, biological assay, and chemical diagnosis. Cowie (1948) reported that in contrast to the woman and the mare there is as yet no established chemical or hormonal test in cattle. He also found that gonadotropins do not appear to be excreted in the urine of the cow; estrogens can be detected in the urine by biological methods, but only after the third month when accurate diagnosis can readily be made by clinical methods. It was stated by Cowie (1951) that the Cuboni test is of no value during any stage of pregnancy in the cow and the only reliable methods of bovine pregnancy diagnosis are those of a clinical nature. Salisbury and Van Demark (1961) found in recent years that there have been great improvements in the clinical methods of diagnosis. The clinical methods include examination of vulva, vagina, and cervix and the palpation of ovaries and the uterus through the wall of the rectum.

Palpation of the genital organs of the cow per rectum for determination of early pregnancy is a widely accepted method in the livestock industry according to Rice et al. (1957) and Williams (1939). The reputation of a practicing veterinarian may stand or fall on his accuracy in diagnosis. This is particularly true in determination of

pregnancy in cattle. Salisbury and Van Demark (1961) found the accuracy of rectal palpation technique dependent largely on the training, skill, and experience of the veterinarian. The condition of the uterus, the ovaries, and the uterine arteries and the presence or absence of a fetus or fetal membrances in the uterus are the criteria on which the diagnosis is based. Results of a diagnosis can be related to the client without delay. Merriman (1963) found that rectal examination for the determination of pregnancy in beef cows is simple and safe. It is also relatively rapid, requiring one to two minutes to secure and examine each cow when adequate equipment is available.

Three obvious advantages were reported by Merriman (1964) for early determination of pregnancy by this method: First, early examination of cows in a herd allows separation of non-pregnant cows from the herd for special management or feeding. Second, pregnancy diagnosis facilitates early detection of herd-wide sterility problems. Third, the accurate prediction of parturition date may enable the herdsman to separate the pregnant cows and provide them with special care and close observation during parturition. Casida (1960) reported that rectal palpation is also employed to determine ovarian conditions associated with variations in the estrous cycle; to detect recognizable causes of sterility; to modify ovarian structures; and to inseminate artificially.

II. RUMEN FISTULA IN CATTLE

Cattle and sheep with rumen fistulae perform important functions in research, teaching, and veterinary practice. Stoddard *et al.* (1951)

reported that the history of rumen fistulation in domestic animals dates to 1883, as evidenced by the work of Flourens in sheep. Later, other domestic animals were used by Colin (1886) and by Schalk and Amadon (1928) for the study of rumen physiology. Quin et al. (1938) reported that various other German and French investigators employed rumen fistulae in their studies.

During the last three decades, fistulation techniques have contributed not only to studies in rumen microbiology but also to general studies in nutrition research. Rumen fistulation in domestic animals generally has no ill effects on the animal's behaviour or physiology. The technique, with openings of various sizes, has been used in all types of animals, old, young, well nourished, and emaciated by Schalk and Amadon (1928).

Rumen-fistulated steers and sheep are used extensively for studying digestion. A permanent fistula affords access to the rumen for sampling the rumen contents and studying the physiology and microbiology of the rumen without sacrificing the animal. The fistulated animals furnish a ready supply of the microorganisms for laboratory studies. A number of organisms which have the ability to digest cellulose have been isolated and are being propagated in pure culture in vitro. Mixed cultures taken from fistulated animals are also used under standardized conditions in the laboratory to digest samples of forages as a laboratory method to measure the digestibility of forages. Recently, a technic for establishing rumen fistulae has been developed by Roberts (1962) and Noorday et al. (1963), as a therapeutic measure for feed lot cattle.

III. SPLENECTOMY IN CATTLE

The spleen is one of the most interesting structures in the body in that it has been known to veterinarians since the earliest history of medicine. Foote et al. (1951) reported that the spleen comprises the largest single collection of lymphoid and reticulo-endothelial cells. The physiology of the spleen is not well understood, but it is generally accepted that it is not indispensable to the functioning of normal animals. Dukes (1955) observed that no permanent ill effects arise from its surgical removal; other tissue cells (reticulo-endothelial cells which are scattered widely throughout the body) readily take over the spleen's function. Roberts (1955) reported that, among its other functions, this gland filters bacteria, forms antibodies, produces many lymphocytes, and probably produces most of the monocytes. Under certain pathological conditions, Gates (1953) found that the spleen manufactures blood in some adult animals. In human medicine, Roberts (1955) observed that the spleen is primarily involved in several pathological conditions. In these instances its removal is often followed by recovery of the patient. Hence splenectomy in human medicine has been used as therapy.

However, in veterinary medicine, the chief use of splenectomy has been the experimental increase in susceptibility to disease and the production of test antigens. Gates (1953) reported that experimental splenectomies on animals were first performed in the seventeenth century. Apparently the dog was the first animal to be used for this purpose. Zambeccari also made observations on splenectomized dogs as evidenced by

Pool et al. (1923). Dekock and Quinlan (1926) reported successful splenectomy operations on horses, sheep, cattle, and goats. Mohler, then chief of the Bureau of Animal Industry (1931) stated that several splenectomies were performed on bovines during the years 1929-31, in connection with anaplasmosis investigation. Reese (1933) stated that in investigations dealing with experimental anaplasmosis in calves it became necessary to remove spleens prior to infection in order to increase susceptibility. Gates (1953) reported that during the ten-year period from 1943 to 1952, splenectomized calves were used for production of anaplasmosis antigen for use in the complement fixation test and other diagnostic work at the animal disease station, Beltsville, Maryland.

Merriman (1962 and 1963) splenectomized eight bull calves between six to twelve weeks of age and used them for anaplasmosis inoculation tests in comparing the accuracy of complement fixation and capillary-agglutination tests for anaphasmosis.

IV. VASECTOMY IN BULLS AND RAMS

Vasectomy refers to surgical excision of part or all of the vas deferens or to ligation of the vas deferens, and is performed in the male to cause permanent sterility. If the operation is performed bilaterally, the animal is rendered infertile; however, libido is not reduced. Warwick (1924) reported that vasectomy has long been practiced upon laboratory animals, such as rabbits and guinea pigs.

Several methods of sterilizing the male have been reported by Belling (1961). In the bovine species these include: amputation of

penis, chemical sterilization, and creation of adhesion of the penis to the lower abdominal wall, thus preventing protrusion of the penis. It is generally agreed that the technique of vasectomy is comparatively simple and practical.

One of the foremost problems in artificial breeding of dairy and beef cattle is the detection of cows in heat. Tharp (1955) found that the vasectomized bull proved a satisfactory means of identifying cows in estrus. Anderson (1955) reported that the use of vasectomized rams in sheep breeding programs is becoming increasingly popular in the United States and Australia. Smith et al. (1959) reported that placing rams with ewes during the transition from the anestrous to the estrous season has been found to stimulate the first heat period and facilitate early lambing.

V. MUSCLE BIOPSY

Muscle biopsy refers to the removal and examination of a piece of muscle tissue from the living body for purposes of diagnosis. In veterinary practice there are several medical and surgical conditions in which examination of biopsy specimens is helpful or even essential in the establishment of a diagnosis. Besides this, Spurlock et al. (1962) found the technique is of value for analyzing muscles of live meat animals and for providing an accurate evaluation of the more important characteristics of their carcasses. Wilson et al. (1954) employed the technique to study the effects of fattening on the tenderness of beef.

According to Albertin (1954) and Jubb and Kennedy (1963), muscle biopsy technique is also employed for the following purposes: (1) determination of chemical composition of muscle, (2) muscle metabolism studies, (3) diagnosis of white muscle disease, and (4) diagnosis of muscle dystrophy.

In human medicine, muscle biopsy has proven helpful. Adams (1962) found it sometimes has yielded critical information in the following clinical circumstances: (1) differential diagnosis of the several types of chronic, atrophic, and dystrophic diseases, (2) in ascertaining the state of nerve and muscle after injury, (3) in confirming the diagnosis of infectious diseases such as Trichinosis, Toxoplasmosis, Cysticercosis, and Sarcosporidiosis, and (4) in certain metabolic diseases such as amyloidosis and glycogen storage. Bowden et al. (1945) reported that muscle biopsy, besides giving information about the state of the muscle fibers, is also a form of nerve biopsy, because nerve fibers, Schwann tubes or motor end plates are found among the muscle fibers.

VI. SEMEN COLLECTION TECHNIQUE (ELECTRO-EJACULATION)

The world-wide use of artificial insemination has increased the value of each ejaculate of semen from valuable sires. The artificial insemination technique involves proper collection of semen, examination and processing of semen, timely insemination, and care of equipment. The ideal semen collection technique is one which is satisfactory to the male, requires simple equipment, is easy to operate, and facilitates

collection of a pure specimen of normally ejaculated semen free from contamination of dirt and bacteria.

The history of semen collection dates back to the fourteenth century. Perry (1952) reported that during the year 1331, an Arabian chief stealthily collected semen from a stallion of his enemy chieftain and inseminated his prized mare. Spallanzani (1780) an Italian physiologist, inseminated a bitch with the semen artificially collected from a dog. Similar works are reported by Hunter (1799) in the human, and by Plannis (1876), and Albrecht (1894) in dogs. Ivanoff (1899), a renowned Russian worker, did the pioneer work in collection of semen and artificial breeding in farm animals. In this country, similar work was done by Lewis (1921) in equines and by Perry (1930) and Henderson (1938) in other farm animals.

Several techniques for the collection of semen have been reported in the literature. According to Rice et al. (1957), the earlier methods were: (1) recovery of semen from the vagina, (2) massage technique, (3) breeder's bag, (4) use of sponge in vagina, (5) placing rubber liner in the vaginal canal, (6) collection of semen by means of an urethral fistula, and (7) semen collection from the epididymis. The above methods were given up in favor of the use of the artificial vagina and electro-ejaculation.

The technique of electro-ejaculation is a useful procedure for collection of semen from bulls which are either unwilling or unable to serve an artificial vagina. Hafez (1962) reported that electrical stimulation is one of the methods used to collect semen from old and

injured bulls. Nilsson et al. (1959) reported that more than 25 per cent of the bulls used for artificial insemination were culled out for physical reasons and half of these might have been used satisfactorily if semen could have been collected by electro-ejaculation. Herman and Madden (1963) stated that electro-ejaculation could be used very successfully in the bull and ram. Smalling (1964) was of the opinion that electrical stimulation is the preferred method for collection of semen from beef bulls.

The advantage of routine electro-ejaculation is that the collection of semen is easily performed with the minimum of reaction from the stimulated animal. Thus, a technique is sought in which the stimulation results in erection of the penis and the ejaculation of a dense and motile semen sample, but which at the same time does not affect or injure the bull.

CHAPTER II

REVIEW OF LITERATURE

I. RECTAL PALPATION FOR DETERMINATION OF PREGNANCY IN THE COW

Williams (1939) found that palpation of genital organs per rectum was most valuable in clinical diagnosis of early pregnancy in cattle. Wagner (1946) claimed 100 per cent efficiency for the rectal method applied 60 to 120 days after conception. Similar reports are made by Rice et al. (1957), Casida (1960), and Salisbury and Van Demark (1961). These authors examined the cows at 60 to 130 days of gestation. Hafez (1962) preferred an examination on the thirty-seventh day of gestation for accurate diagnosis.

Strerath (1944) claimed possible diagnosis of early pregnancy from an examination of the corpus luteum ten days after service.

Moore (1947) reported that in sixty to ninety days of pregnancy, the gravid uterine horn is three to five inches in diameter, the cotyledons are felt, and the fetus measures about 4.5 inches in length. His findings are in accord with the reports made by Williams (1939) and Hancock (1962).

Hancock (1962) reported that the middle uterine artery on the gravid side shows a characteristic fremitus after ninety days post-conception. Williams (1939) found the uterine artery of the non-pregnant cow measures three to four millimeters in diameter, but during pregnancy

it attains a diameter of 1 to 1.5 centimeters. Moore (1947) reported a diameter of three-eighth inch, and a prolonged, soft pulse in this artery 120 days after conception. Hancock also found that middle uterine arteries are most useful for diagnosis of pregnancy in late gestation when the uterus and its contents are frequently out of reach.

Wisnicky et al. (1948) described a method whereby pregnancy in cattle could be accurately diagnosed between the thirty-fifth and forty-ninth days of gestation by palpating the amniotic vesicle per rectum. The uterine horns are palpated between the thumb and first two fingers, throughout their entire length. The anmiotic vesicle can be detected as a small, turgid, slightly oblong, balloon-like structure which slips away from between the thumb and finger as pressure is applied. It is claimed that with experience the age of the fetus, correct to within a day, can be estimated when examination is carried out between the thirty-fifth and forty-ninth days. A similar method was reported by Hafez (1962). But Fosgate et al. (1954) reported that rupture of the amniotic vesicle would result in fetal death when the cow was examined per rectum at the thirty-fifth day post-conception. A similar hazard of early pregnancy diagnosis caused by palpation of the amniotic sac at thirty-seven days post-conception was reported by Rowson et al. (1963). Fetal loss of 5.5 per cent was reported by Barret et al. (1948) and 6.8 per cent by Ball et al. (1963) due to the use of this method of pregnancy diagnosis.

Salisbury and Van Demark (1961) stated that accurate diagnosis even by skilled personnel is possible only after sixty days or more of pregnancy in cattle.

During a period of 7-1/2 to 13-1/2 weeks post-conception, Lamond et al. (1963) examined per rectum at weekly intervals ten Hereford cows, which conceived on the same day. There was a tendency for uterine diameter to increase in this period but there was considerable variation between cows at any one time and especially during the period of 8-1/2 to 11-1/2 weeks following conception. Additional scoring of length of uterine body or more frequent examinations might improve the precision of the assessment of stage of gestation.

Hancock (1962) examined the accuracy of the rectal diagnosis of pregnancy by statistical analysis of 503 examinations of 239 cows during 358 pregnancies where the date of the fertile service was accurately determined from calving records. Pregnancy was confirmed from calving records in 380 of 388 cases (97.9 per cent) in which a positive diagnosis was made. All but one of seventy-two negative diagnoses were apparently correct as per the calving record. Within the total data there was a significant correlation between the estimated and actual duration of pregnancy ($r = 0.87$). Within four classes separated according to the actual duration of pregnancy at the time of examination, the correlations were: 29 to 56 days, $r = 0.55$; 57 to 87 days, $r = 0.52$; 85 to 112 days, $r = 0.42$; and 113 to 140 days, $r = 0.37$.

Merriman (1963) made pregnancy determinations per rectum in two herds belonging to the Tennessee Experiment Station and obtained accuracies in line with those encountered in his routine clinical examinations. In one herd, all fifty-one cows declared pregnant were retained and all calved, resulting in 100 per cent accuracy of determination. In the other herd, only seventy-four of the eighty-one

cows declared pregnant were retained. All seventy-four calved. Of the remaining seven, five were sold for slaughter within sixty-nine days of examination, one aborted, and one died during gestation. Consequently, the degree of accuracy there was above 90 per cent. In the combined groups the author predicted parturition dates for 119 cows. These predictions were somewhat better for one herd than the other, but in all cases 68 per cent of the calving dates were within thirty days of the prediction and only 6 per cent of the predictions were in error by more than sixty days.

II. RUMEN FISTULA IN CATTLE

Operative Technique

Flourens in 1883 and Colin in 1886, fistulated sheep and oxen by crude surgery. They made classical studies on the physiology of the rumen, but made no attempts to close the fistular openings by mechanical means.

The operative technique for rumen fistula in cattle was pioneered in this country by Schalk and Amadon (1928). Their technique has since been refined.

Similar techniques for rumen fistulae were developed by many workers, including Quin et al. (1938); Phillipson et al. (1939); Watts (1948); Balch et al. (1948); and Weiss (1953). Improved operative techniques have been developed by Anderson and Wise (1944) for rumen fistulae in young dairy calves, to study the various phases of the physiology of milk digestion. Nichols (1953) designed a technique for

establishing a rumen fistula in sheep closed by a flap of abdominal wall. Fistulae of this type afford ready access to the rumen, usually are not messy, and do not require closure.

Michael et al. (1954) simplified the classic operative technique by using a type of rumen retractor made up of an aluminum ring.

Permanent rumen and intestinal fistulae in cattle and sheep with some modifications and simplifications were developed by Dougherty (1955); Binns et al. (1959); and Balch et al. (1962). Some of these workers used a two-stage technique in which they allowed skin and muscles to heal before the rumen was incised.

One-stage surgical technique was employed by Schnautz (1957) for satisfactory rumen fistulae in cattle. Many advantages have been claimed over the two-stage operative technique. The author was of the opinion that the exposed portion of the rumen wall should be removed immediately to secure a more satisfactory fistula margin. Further, he stated that this technique is simple, heals rapidly, and has less tendency to constrict than that of the two-stage method.

Mendel (1961) followed a slight modification of Schnautz's technique. Feed and water were withheld on the morning of the operation. He found that any further fasting produced a rumen that was too empty to be handled properly.

Short (1962) established an operative technique for rumen fistulae in white tailed deer, with a view to comparing certain aspects of the nutrition of the white tailed deer and the domestic cow.

Cannulation

Schalk and Amadon (1928) reported that if the large fistula is not closed in some manner, the animal would develop cachexia, depraved appetite, and consequent loss of body condition. The desirability of closing rumen fistulae is well known and has been discussed by Phillipson and Innes (1939). Closure of the fistula is particularly important in studies of rumen microflora, since the presence of air and loss of ingesta produce undesirable environment for the organisms.

Most of the mechanical devices which have been described in the literature for closing fistulae fall into one of three types:

1. a flap of leather or rubber inside the rumen, attached to a block of wood or cork protruding through the fistula and fastened to a flat piece of wood on the outside.
2. pneumatic plugs which are inserted into the fistula and inflated.
3. a cannula of metal or plastic held in place by flanges or clamps on both the inside and the outside of the rumen wall.

There are several advantages and disadvantages in each of the devices cited above.

The leather or rubber flap closures used by Schalk and Amadon (1928) and Diernjofer (1928) were reasonably effective but reportedly caused stretching of the fistulae and subsequently fell from the opening. They were also rather difficult to remove and replace for sampling.

Pneumatic closures were developed and used by Phillipson et al. (1939), Cole et al. (1942), Balch et al. (1948), and Johnson et al. (1959).

Pneumatic plugs are subject at times to damage and leakage. In pastured subjects the plugs have caught on fences and been pulled free as noticed by High (1964). Molds for their manufacture are rather costly.

An unsuccessful effort was made by Quin et al. (1938) to close the fistula in sheep by means of cannulae made of aluminum or chromium plated brass. The cannulae were unsatisfactory because of corrosion and electrolysis.

Phillipson and Innes (1939) devised an ebonite cannula by fitting the cannula with an outer clamp. Several of these cannulae were used in sheep.

Recently, plastic cannulae have been most commonly used. They display advantages over the pneumatic plugs as evidenced by the work of Stoddard et al. (1951) and Yarns et al. (1963). Mendel (1961) found that the outer edge of the cannula must not be too long.

At the Kansas State Veterinary College, cannulae made from gun rubber (plastisol) are being used for cannulation of dairy cattle. Fray (1964) found the cannulae made from gun rubber are much superior to those made from plastic.

III. SPLENECTOMY IN CATTLE

The surgical techniques for performing splenectomy in larger domestic animals were reported early by Dekock and Quinlan (1926) and Reese (1933).

According to Davis (1952) the splenectomy could be performed successfully on calves of any age; but those only a few days old

served best. Paravertebral nerve blocking was preferred by the author; he found no case of shock following surgery. The incision was made parallel to the last rib and sufficiently large to admit the operator's hand. The spleen was excised gently by blunt dissection with the fingers. The splenic vessels and nerve were clamped and ligated. Great care was exercised to rupture the splenic artery an inch or two away from the spleen. Muscle and peritoneum were closed with chromic gut and the skin was closed with chromic gut or silk.

Gates (1953) described his technique which had been perfected in the course of over 200 splenectomies in cattle. General anesthesia was obtained by intravenous administration of 40 per cent chloral hydrate at the rate of 0.15 ml. per pound body weight. Only four animals died from the effects of the operation--two from hemorrhage and two from chloral hydrate poisioning.

Studart (1960) reported a technique of chemical splenectomy in mice. Colloidal preparations of 10 per cent ethyl palmitate (ethpalm) were injected into mice in a dose of 25 mg. of ester per twenty grams body weight. Twenty-four hours later the reticulo-endothelial phagocytic function was measured by the carbon-clearance techniques. Studart observed that suppression of phagocytic function and destruction of the spleen was due to the effect of the drug. Bone marrow and lymphoid tissue of the thymus were unaffected.

Merriman (1962 and 1964) splenectomized eight Holstein-Friesian bull calves six to twelve weeks old in the standing position. Each calf was effectively anesthetized by local infiltration of approximately

50 cc. of 2 per cent procaine hydrochloride solution into the skin, subcutaneous tissue, muscle, and peritonium. The calves were restrained in a working chute.

Peacock et al. (1964) described a technique for the splenectomy of calves, using local anesthesia, with the animal in the standing position. Section and hemostasis of the splenic vessels were effected by means of an ecraseur. Surgical interference was much less than in previously described methods. The operation was reported to be safe, with the minimum of distress and discomfort to both animal and surgeon. It took an average time of twenty minutes to perform.

Markowitz et al. (1964) recommended gentle squeezing of the spleen during surgery from time to time in order to restore as much blood as possible to the general circulation. Shrinking of the spleen may be caused by injecting 1 to 2 ml. of a 1:10,000 solution of adrenaline.

Witzel and Mullenax (1964) reported a new technique for splenectomy of the horse involving partial resection of the sixteenth, seventeenth, and eighteenth ribs.

Splenectomy in veterinary medicine, although primarily useful in studying anaplasmosis, has several other purposes. Anderson (1954) found it to be specific for healing splenodynia and splenic tumor in the dog. Foote et al. (1957) employed splenectomized calves for studies with eperythrozoonosis. Dimopoullos et al. (1962) found splenectomized calves useful not only in anaplasmosis studies, but also in studying chemical and physical changes in the plasma and erythrocytes. Splenectomized rats, according to Weinman (1938) served well in studies with

Bartonella muris infection while splenectomized donkeys were used by Jansen (1953) in studying therapeutic measures against Babesia equi infections.

IV. VASECTOMY IN BULLS AND RAMS

Different techniques have been employed by workers in performing vasectomy in bulls, buffaloes, and rams.

Warwick (1924), using general anesthesia, restrained the bull in the dorsal position. He also employed separate sutures for the tunica vaginalis and the skin. Following surgery the animal was injected with a prophylactic dose of tetanus anti-toxin.

Tharp (1955) preferred to cast the bull on its left side, with both rear legs pulled alongside the abdomen. The scrotum was drawn back between the hind legs to expose its anterior surface. This site was selected for the operation because it allowed the minimum amount of interference from the external cremaster muscle. Johari et al. (1961) also preferred this site. Two per cent procaine hydrochloride with 200 turbidity units of hyaluronidase per 100 ml. of solution was used as a local anesthetic. The author preferred to use young and unused bulls from a known healthy herd, in order to avoid transmission of genital diseases.

Working with rams, Anderson (1955) reported that a one-year-old ram was the best subject for surgery. He preferred winter months for the operation and thus avoided fly menace. Johari et al. (1961) employed epidural anesthesia combined with a tranquilizer on vicious bulls and buffaloes.

V. MUSCLE BIOPSY

Several workers have described the muscle biopsy technique and its versatility in studies of physical, chemical, and morphologic changes in muscle.

Bray (1953) described a technique for biopsy of semimembranosus muscle in cattle. A dehorning chute was used to restrain the animal in standing position. Adequate epidural anesthesia was provided by injecting 10 to 20 cc. of 2.5 per cent procaine solution to desensitize the rear quarters. An incision was made directly over the intramuscular septum (between the semitendinosus and semimenbranosus muscles), two to three inches ventral to the tuber ischii. With blunt dissection, the semimembranosus was separated medially, and a sample of muscle two inches in diameter and four inches long was removed. The bottom of the incision was leveled to permit adequate drainage. The wound was dusted with sulfonamide-urea powder and the ends of the skin brought in apposition by mattress sutures.

Merkel et al. (1954) developed a technique to obtain muscle samples from longissimus dorsi of hogs to study the intramuscular fat deposition. Hogs were anesthetized by an intraperitoneal injection of a solution of 5 per cent magnesium sulphate and 5 per cent chloral hydrate. In heavier hogs the anesthetic was administered via the posterior auricular vein or its branches. The animals were restrained in lateral recumbency on a table. A 1-1/2 to 3 inch incision was begun just posterior to the last rib and parallel to and one to two inches lateral

from the vertebral column. A section of muscle about 1-1/2 inch long by three-fourth inch wide and three-eighth inch thick was removed from the longissimus dorsi. Hemorrhage was controlled with gauze tampons and ligation. Healing was normal and the sutures were removed in ten days. A similar technique was reported by Wilson et al. (1955) for biopsy in steers.

Spurlock et al. (1962) employed identical techniques for removing samples from longissimus dorsi and semimembranosus muscles of lamb. The animal was required to stand in a holding crate for biopsy of longissimus dorsi muscle and was cast in lateral recumbency with the leg pulled forward for semimembranosus biopsy. The line of incision was infiltrated intradermally with 2 per cent procaine solution. The incised wound was dusted with aureomycin surgical powder and the cut edges of the would were closed with would clips.

Bowden and Gutmann (1945) described a technique commonly employed in human medicine for diagnostic purposes. A piece of muscle 1 cm. long, 0.5 cm. wide, and 0.5 cm. thick was removed from the affected muscle under local or general anesthesia. The specimen was placed at once on a strip of cardboard to prevent distortion and fixed immediately in 10 per cent formal saline. If a larger specimen was made, half was used for frozen section and the remainder embedded in paraffin.

VI. SEMEN COLLECTION TECHNIQUE (ELECTRO-EJACULATION)

The early work of Batelli (1922) and of Moore and Gallagher (1930) with the guinea pig showed that it was possible to obtain semen by means

of an electric shock applied to the base of the brain. These workers employed two electrodes, one in the mouth and the other under the skin of the dorsal surface of the neck. An alternating current of about thirty volts and repeated low grade stimuli resulted in ejaculation. This method was modified for sheep by Gunn (1936) who inserted one electrode in the rectum and a needle electrode into the longissimus dorsi muscle. This avoided the hazard of brain stimulation. A stimulus of thirty volts of fifty-cycle alternating current resulted in a current of about 160 to 190 milliamps. Further, Gunn et al. (1942) noticed the reaction on parts of the animal was very severe and complete restraint was necessary.

Dalziel and Phillips (1948) made further studies of electro-ejaculation with guinea pigs and chinchillas. They found the muscular contractions and distress symptoms decreased with increasing current frequency and the optimum frequency for ejaculation was between 500 and 1,500 cycles in sine-wave current.

Bonadonna (1938) modified the method of Gunn (1936) and applied the extra-rectal electrode to the skin of the back, above the muscle. The area was soaked in saline prior to ejaculation to insure electrical contact.

Laplaud and Cassou (1948) introduced a bipolar electrode which was inserted in the rectum of the bull or stallion for ejaculation. This technique was further developed by Thibault et al. (1948) and Laplaud et al. (1948). The bipolar electrode consisted of thirty brass rings separated from each other by ebonite. Each alternate ring was

connected to one terminal of a potentiometer which delivered thirty volts alternating current. Stimulation was effected by varying the current from zero to maximum and back to zero, the cycle taking three to five seconds. Using this type of electrode the authors described two phases in the ejaculation: (1) the phase of copious secretion of high pH containing few or no spermatozoa, (2) phase of ejaculation in which there is a secretion of high sperm density. The first phase of secretion was produced as a result of stimuli of about twelve volts. In the second phase, the stimuli were increased progressively up to thirty volts.

Further modifications in the rectal type electrode were reported by Marden (1954) and Hill et al. (1956). The longitudinal electrode devised by these authors considerably reduced the external reaction of bull by lowering the excitation of lumbar and sciatic nerves. Blackshaw (1954) used an improved bipolar electrode in conjunction with a battery-operated portable stimulator.

An electroejaculator with finger electrodes was designed and constructed by Rowson et al. (1954). These electrodes consisted of two copper rings soldered to a thin flexible wire extension cord and shaped to fit the finger tips. The cord was attached to a control unit containing a step-down transformer, voltmeter, fuses, and a variable rheostat that operated on ordinary 110 volt house current. The copper ring electrodes were placed on the first and third fingers of the rubber gloved hand and held in position in the rectum. These authors state they have located a center of ejaculation existing on or near the ampulla of the vas deferns.

Swanson (1956) reported that undesirable stimulation of the hindquarters could be avoided almost entirely by use of ring-like electrodes.

Nilsson et al. (1959) successfully employed finger-electrode equipment for routine collection of semen from bulls and also found this method useful with bulls which repeatedly failed to ejaculate when stimulated by the rectal probe.

The effect of variation in the cycle of frequency of the current has been examined by several workers. The results of Dziuk et al. (1954), who obtained excellent results with frequency changes ranging from 15 to 900 cycles per second, do not agree with those of Marden (1954) who found the optimum frequency to be within the range of twenty-five to thirty cycles per second. Walton (1955) supported the reduced frequency wave of twenty-five to thirty cycles per second on the grounds that a sine wave of this frequency strongly stimulates the nerves of the autonomic nerve supply but barely excites the central nervous system. This would reduce undesirable side effects.

The controversial opinions among the workers are attributed to the results reported in different species. From the available literature, it is evident that satisfactory semen collections can be made with a wide range of frequency of stimuli. The cycle frequency of twenty-five to thirty cycles is reported to be normal physiological range; however, fifty cycles per second has been found entirely satisfactory.

CHAPTER III

MATERIALS AND METHODS

I. RECTAL PALPATION FOR DETERMINATION OF PREGNANCY IN THE COW

The method employed at the Tennessee Experiment Station for determination of pregnancy in beef cows by rectal palpation was used in this study and the procedure is reported in the following paragraphs.

Good equipment, consisting of one or more holding pens, a lead-in chute, and a working chute is essential to safe, rapid handling of the cows. A record book, rubber or plastic sleeves, liquid soap, buckets, a scrub brush, towels, and appropriate personal clothing are the only needed materials.

Examinations are generally made 60 to 120 days following the removal of the bull. Occasionally examinations are made the day the bull has been removed from the herd. In these latter cases there has usually been a sixty-day breeding season and the results are, for the "not pregnant" diagnoses, largely presumptive. Each cow to be examined is placed in the working chute and the head secured. A gate or other barrier is used to keep the next cow from crowing the operator. Usually the examinations are made while the operator stands directly behind the cow, but they are also made at times with the operator standing outside the chute and reaching through an opening in the chute wall. Usually the tail is held securely by the operator's free hand. The examining

hand is lubricated with soap and inserted into the rectum as the thumb and fingers form a cone. With the fingers immediately inside the pelvis, a thorough search is made for the non-gravid uterus or for the cervix. When the uterus is in the pelvis and non-gravid or in early pregnancy, the uterus, uterine tubules, and ovaries are examined. When the uterus does lie anterior to the brim of the pelvis, it is located most often by allowing the hand to follow the cervix. The uterus is then carefully palpated for size of uterine horns, presence of cotyledons, presence and size of fetus, and other diagnostic features. In cows pregnant approximately 150 days, it is sometimes difficult to make detailed palpations of the uterus. In these cases a search is made on either side of the pelvic cavity for the middle uterine arteries and these vessels are examined for size and nature of pulse. It is important that the person responsible for keeping records note the results immediately after they are given by the examiner. Errors have occurred when this precaution was not taken.

II. RUMEN FISTULA IN CATTLE

Several surgical techniques for rumen fistulation were mentioned in the literature. Most of the methods were based on the work of Schalk and Amadon (1928). Each successive worker made attempts to develop an improved technique which was simple and less injurious to the animal. However, no single technique found in the literature was clained as superb or best.

The following technique was used in this study and has been employed, with various modifications, at the Tennessee Experiment Station:

The materials needed for the operation are listed in Table I.

Preparation of the surgical site and of the animal for fistulation was in keeping with techniques of abdominal surgery in cattle. Normal feed was not withheld. Six to twelve hours prior to surgery, the animal was treated intramuscularly with approximately one mg. of the tranquilizing agent, ethyl isobutrazine hydrochloride (Diquel)[1] per pound of body weight. Shortly before surgery, the left side and flank were clipped and scrubbed. The surgical site was the left para-lumbar fossa as described by Sission and Grossman (1953). This area was shaved, scrubbed, dried, and covered with tincture of iodine. A squeeze chute was used for restraint of the animal in standing position. Anesthesia of the surgical area was accomplished by the injection of approximately 100 ml. of a 2 per cent solution of procaine hydrochloride into the skin, subcutaneous tissue, and muscle. Openings five inches in diameter had been requested for each fistula. A sterilized plastic ring of that size was used for a pattern and the circular line of incision marked with a sterile knife blade.

The initial incision carried through the skin. The disc of skin was grasped at the edge, dissected free, and removed. A corresponding

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[1]Diquel, an animal tranquilizer, manufactured by Jensen-Salsbery Laboratories, Kansas City, Mo.

TABLE I

MATERIALS NEEDED FOR RUMEN FISTULATION OF STEERS

Name of the Materials	Number Required
Diquel (ethyl isobutrazine hydrochloride)	one hundred ml.
Clipper, electric or hand	one
Bucket and brush	one
Surgical trays	two
Syringe, 20 ml. capacity	one
Syringe, 10 ml. capacity	two
Hypodermic needles, 18 gauge x 1"	six
Tincture of iodine	
Razor blade holder	one
Razor blades	four
Liquid soap	
Quatenary ammonia solution	
Procaine hydrochloride solution (2%)	
Cannula for marking incision	one
Bard-Parker handle No. 4	two
Bard-Parker knife blades No. 22	six
Scissors, curved, medium	two
Hemostats	one dozen
Sterile gauze pads	three dozen
Suturing needle, half-curved (2" size)	six
Suturing needle, full-curved (2" size)	six
Needle holder	one
Chromic catgut suture, No. 4	
Chromic catgut suture, No. 0	
Umbilical tape, standard size	one tube
Keraspray	one tube
Co-ral	
Gauze bandages	
Paper towels	one bundle
Rubber gloves (sterile)	four pairs

NOTE: All the surgical equipment and rubber gloves were sterilized in an autoclave for thirty minutes under fifteen pounds pressure.

incision was carried through the external and internal oblique muscles and the peritoneum. Hemorrhage was controlled by hemostats as the incision progressed. Continuous sutures of chromic catgut, size four, were used to unite the cut edges of the muscle and peritoneum and to secure them to the inner surface of the skin at the rim of the incision. The rumen was not incised. The rumen was secured to the cut surface of the skin with interrupted sutures of size four catgut placed at one-fourth inch intervals. These sutures were placed in the skin and in the serous coat of the rumen. However, when one suture accidentally penetrated the entire rumen wall, there were no ill effects. This completed the first phase of the surgery.

The wound, with the rumen intact but secured to the cut skin surface, was covered with an antiseptic powder and a layer of gauze. It was nearly impossible to keep adhesive plaster on the skin of these animals, and the gauze was held in place primarily by the tissue exudates. One animal became febrile on the second post-operative day and was treated with intramuscular penicillin and streptomycin. No further post-operative illness was noted and there was no treatment of the other animals between the two phases of surgery.

The second phase of surgery, removal of the disc of the rumen wall, was accomplished nine to twelve days following initial surgery. No anesthesia was required. Restraint was by means of a head gate and chute. The disc of rumen wall within the area to be removed was not atrophied. In fact the blood supply appeared to have been increased. The rumen wall was incised and the disc removed. Bleeding was profuse

and controlled with difficulty by means of hemostats and pressure. The pneumatic plugs were inserted at once. There were no post-operative complications and the animals were released in three to five days for nutritional experimentation.

III. SPLENECTOMY IN CATTLE

Several methods for performing splenectomy have been cited in the literature. Cattle of all ages have been successfully splenectomized. Young calves are preferred by many workers for the best results.

The technique used in this study and described below is identical to that used in the Tennessee Experiment Station with the exception that the use of a young calf made it necessary to restrain the animal in lateral recumbency. The surgical equipment used for the operation is listed in Table II.

A Jersey male calf weighing fifty-one pounds was splenectomized under the influence of a tranquilizing agent and local anesthesia. The calf was not fasted before surgery. An area approximately one foot square on the left flank was shaved, then scrubbed with soap and water, and, after drying, painted with tincture of iodine. The animal was placed on a surgical table in the right recumbent position. The calf was kept in that position with the feet secured to the table. The calf was then injected intravenously with 0.4 cc. (about 20 mg.) of ethyl isobutrazine hydrochloride (Diquel),[2] an animal tranquilizer employed to

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[2]Diquel, an animal tranquilizer manufactured by Jensen-Salsbery Laboratories, Kansas City, Mo.

TABLE II

MATERIALS REQUIRED FOR SPLENECTOMY IN CATTLE

Name of Article	Number Required
Clipper, electric or hand	one
Bucket and brush	one
Razor blades	two
Razor blade holder	one
Liquid soap	
Tincture of iodine	
Syringe, 20 ml. capacity	one
Syringe, 10 ml. capacity	two
Hypodermic needles, 18 gauge x 1"	six
Diquel (ethyl isobutrazine hydrochloride)	one hundred ml.
Procaine hydrochloride solution (2%)	
Cloth shroud (sterile)	one
Algicide	
Bard-Parker handle No. 4	two
Bard-Parker knife blade No. 22	six
Scissors, curved medium size	two
Hemostats	six
Surgical trays	two
Sterile gauze pads	three dozen
Rubber gloves (sterile)	three pairs
Chromic catgut suture No. 0	
Chromic catgut suture No. 4	
Umbilical tape, standard size	one tube
Suturing needles, full curved, 2" size	three
Suturing needles, half curved, 2" size	three
Keraspray	one tube
Co-ral	one tube
Paper towels	one bundle

NOTE: All the surgical instruments and rubber gloves were sterilized in an autoclave for thirty minutes under fifteen pounds pressure prior to surgery.

reduce excitement and struggling. In addition, adequate local anesthesia was provided by injecting about 30 ml. of procaine hydrochloride solution, into the skin, subcutaneous tissue, muscle, and peritoneum of the area. A sterile shroud with an opening for the proposed incision was spread over the entire surgical area.

In cattle, the dorsal end of the spleen lies under the dorsal ends of the last two ribs and extends back as far as the first lumbar transverse process. The ventral end lies opposite the ventral ends of the eighth and ninth rib as per Sission and Grossman (1953).

A five inch incision was made one-half inch posterior to and parallel to the last rib through skin, muscle, and peritoneum. The left hand of the operator was passed through the incision until the parietal surface of spleen was felt. By forcing the hand between the spleen and the rumen the operator loosened the spleen of all attachments but the splenic vessels and withdraw the organ through the incision. The splenic vessels and nerves were clamped by means of two forceps. The vessels were ligated with size zero chromic catgut at two points about an inch from the hilus. The vessels were severed very close to the ligatures and the spleen was removed. The peritonium and muscle were brought in apposition and closed with continuous sutures of number four chromic gut. The skin was closed by means of mattress sutures of umbilical tape. The closed wound was dusted with Co-ral[3] to prevent fly infestations.

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[3]Co-ral, livestock duster, manufactured by Chemagro Corporation, Kansas City, Mo.

The splenectomized calf recovered from effects of the tranquilizer approximately eight hours from the time of surgery. It was placed on a straw bedding and was fed the next morning. The wound healed by first intention and post-operative recovery was non-eventful.

IV. VASECTOMY IN BULLS AND RAMS

Two yearling Hereford bulls and two yearling Hampshire rams were vasectomized, using identical surgical procedures as discussed in the following paragraphs.

Instruments and supplies needed for the operation are listed in Table III. Bulls, in standing position, were restrained in the squeeze chute while the rams were held in the standing position on a low table. The animals were fed normally prior to surgery. The surgical areas were located on the posterior surface of the scrotum about a half-inch lateral to the median raphe, and extending from the scrotal attachment ventrally for about three inches. The surgical site was clipped, cleaned with soap and water, and painted with tincture of iodine. Approximately 20 ml. of 2 per cent procaine hydrochloride solution were infiltrated into the skin and subcutaneous tissues of the surgical areas. The scrotum and the testes were grasped by the left hand of the operator, with enough pull to cause stretching of the skin and the spermatic cord to facilitate incising the skin and tunica vaginalis. A vertical incision, one and a half inch in length, was made below the external inguinal ring. Upon penetration of the fascia and the subcutaneous tissue the spermatic cord was exposed. The vas deferens, which can be palpated in the spermatic

TABLE III

LIST OF MATERIALS REQUIRED FOR VASECTOMY IN BULLS AND RAMS

Name of the Article	Number Required
Clipper, electric or hand	one
Bucket and brush	one
Algicide	
Soap (liquid)	
Razor blade holder	one
Razor blades	two
Tincture of iodine	
Extension cord with spare bulb	one set
Procaine hydrochloride solution (2%)	
Surgical trays	two
Syringe, 20 ml. capacity	one
Syringe, 10 ml. capacity	two
Hypodermic needles, 18 gauge x 1"	six
Rubber gloves (sterile)	three pairs
Bard-Parker handle No. 4	two
Bard-Parker knife blade No. 22	six
Hemostats	six
Sterile gauze pads	two dozen
Chromic catgut suture, No. 0	
Chromic catgut suture, No. 4	
Microscope	one
Glass slides	one dozen
Cover slips	six
Scissors, curved medium size	two
Suturing needle, half-curved, 2" size	three
Suturing needle, full-curved, 2" size	three
Umbilical tape, standard size	one tube
Co-ral	
Paper towels	one bundle

NOTE: All the surgical instruments and rubber gloves were sterilized in an autoclave for thirty minutes under fifteen pounds pressure prior to surgery.

cord posterior and medial to the other cord structures, is enclosed by a special fold of the tunica vaginalis propria. The spermatic sheath was incised and the deferential fold containing the vas deferens was exposed and secured at two places with forceps. The vas deferens, identified as a greyish-white, glistening tube, was ligated at two places with number zero chromic catgut. The vas deferens was severed at two points between the two ligatures and the isolated part, approximately one inch long, was removed. To confirm the identity of the vas deferens, a drop of lumen content was placed on a glass slide and examined under a microscope for detectable spermatozoa. The incision was closed by means of mattress sutures using number four chromic gut suture. Co-ral,[4] an insecticide, was dusted externally to protect against insects. The opposite vas deferens was approached similarly from a separate incision on the opposite side and the subsequent surgery was identical.

V. MUSCLE BIOPSY

Descriptions of surgical techniques for biopsy of muscle have been published by Bray (1953) in steers, Merkel (1954) in hogs, Wilson et al. (1955) in cows, and Spurlock et al. (1962) in lambs.

The following muscle biopsy technique has been developed at the Tennessee Experiment Station and was used in a fifty-five pound Jersey calf for this study:

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[4]Co-ral, a livestock duster, manufactured by Chemagro Corporation, Kansas City, Mo.

The surgical instruments and equipment required for the operation are listed in Table IV.

The calf was fed normally and there was no restriction of feed prior to surgery. The operative area was shaved, scrubbed with soap and water, and, upon drying, painted with tincture of iodine. The animal was restrained in the right lateral recumbency on the operating table. Adequate local anesthesia was provided by injecting about 20 ml. of 2 per cent procaine hydrochloride solution subcutaneously over the entire surgical area.

An incision of approximately two inches in length and centered over the head of the twelfth rib was made parallel to and one and a half inches lateral from the vertebral column. A skin flap of one-half inch was made by extending a perpendicular incision medially from either end of the linear incision. The incisions were continued through the subcutis, the adipose, the connective tissues, and longissimus dorsi muscle. Final separation of the muscle was made by blunt dissection.

A rectangular piece of muscle nearly two inches long, one inch wide, and one-half inch thick was removed from the longissimus dorsi. Hemorrhage resulting from the excision was slight and was controlled by sterile gauze pads. A sterile gauze pad was plugged into the cavity of the wound. Keraspray[5] was dusted over the incised would. The cut edges

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[5]Keraspray, manufactured by S. E. Massengill and Co., Veterinary Division, Bristol, Tenn.

TABLE IV

LIST OF MATERIALS REQUIRED FOR MUSCLE BIOPSY IN YOUNG CALF

Name of the Article	Number Required
Clipper, electric or hand	one
Bucket and brush	one
Algicide	
Razor blade holder	one
Razor blades	two
Tincture of iodine	
Liquid soap	
Procaine hydrochloride solution (2%)	
Surgical trays	two
Syringe, 20 ml. capacity	one
Syringe, 10 ml. capacity	two
Hypodermic needles, 18 gauge x 1"	six
Cloth shroud (sterile)	one
Scissors, curved	two
Rubber gloves (sterile)	three pairs
Bard-Parker handle No. 4	two
Bard-Parker knife blade No. 22	six
Hemostats	one dozen
Sterile gauze pads	two dozen
Gauze bandage (sterile)	two rolls
Suturing needle, half-durved, 2" size	three
Suturing needle, full-curved, 2" size	three
Needle holder	one
Chromic catgut suture, No. 4	
Umbilical tape, standard size	two tubes
Keraspray	one tube
Co-ral	
Paper towels	one bundle

NOTE: All the surgical instruments and rubber gloves were sterilized in an autoclave for thirty minutes under fifteen pounds pressure prior to surgery.

of skin were brought in close apposition by mattress sutures of umbilical tape. Co-ral[6] was dusted routinely to ward off flies. The gauze pack was removed after forty-eight hours. Post-operative recovery was normal.

VI. SEMEN COLLECTION TECHNIQUE (ELECTRO-EJACULATION)

Twenty-seven Angus, ten Hereford, and eleven Polled Hereford bulls were used in this study.

The electro-ejaculation procedures routinely followed at the Main Experiment Station, University of Tennessee were used in this study and are described in the following paragraphs.

Materials used for the collection of semen are listed in Table V.

The electro-ejaculation apparatus consisted of an isolation transformer, to isolate the circuit from the 110 A. C. power source, a variable transformer, a voltmeter, an ammeter, fuse, pilot light, an on-off switch, and two copper ring electrodes.

The collecting apparatus consisted of a latex rubber cone with a collection tube attached and enclosed in a clear plastic jacket containing warm water (95 to 105° F.).

The bulls were confined in a specially-constructed collection chute. The "stand by" bulls were confined to a holding chute to take their respective turn in the chute for collection of semen.

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[6] Co-ral, a livestock duster, manufactured by Chemagro Corporation, Kansas City, Mo.

TABLE V

MATERIALS NEEDED FOR COLLECTION AND EVALUATION OF SEMEN FROM BULLS BY ELECTRO-EJACULATION

Name of the Article	Number Required
Electro-ejaculator with copper ring electrodes (complete set)	one
Extension cord	one
Liquid soap	
Semen collection tube, graduated	one dozen
Semen collection cones	six
Semen collector	one
K.Y. sterile lubricant jelly	one tube
Plastic protecting tubes	six
Cork, stopper	one dozen
Test tube	one dozen
Test tube rack	two
Funnel	two
Glass measuring jar, 250 ml. capacity	two
Physiological saline sol., 250 ml.	one bottle
Distilled water, 250 ml.	one bottle
Hot water bath	one
Scissors	one
Bucket and brush	one
Microscope	one
Glass slide	one dozen
Cover slip	one dozen
Gloves and sleeves	two pairs
Paper towels	one bundle
Detergent powder	
Glass rods	two
Grease pencil	one
Incubator	one

The preputial hair was clipped and the area of the preputial orifice cleansed with water and brush, so that a clean sample of semen would be obtained from the occasional bull which did not extend the penis from the sheath.

Complete evacuation of feces from the rectum was done manually before electrical stimulation was begun. The operator's gloved hand was lubricated with soap and water. The ring-like electrodes were slipped on to the first and third fingers of the gloved hand, at levels which would avoid their coming in contact with each other. The operator, working with the electrodes and control unit, had his assistant hold the collecting apparatus and observe the nature of the ejaculation.

The ring electrodes with no current passing to them, were placed inside the rectum one on either side of the urethra near the prostate gland region. Current was then allowed to pass to the electrodes. After several intermittant surges of electricity, erection of the penis was usually obtained. The electrodes were then moved slightly forward and close to the ejaculatory duct. The voltage was slightly increased. A voltage of three to ten volts at three to four seconds was applied to each bull for production of rhythmical contractions of urethral muscles. No particular rhythm was used routinely, but each individual bull was observed by the operator and the most suitable rhythm used. Frequency and degree of stimulation was governed by physical and physiological response. Collection was generally made in two tubes. Both were used for assessment of the quality of the ejaculate. Collection time ranged from three to six minutes. Semen collection averaged 6 ml. in volume

with a range from 4 to 10 ml. The concentration of spermatozoa ranged from none to a high of over two billion sperm per ml.

CHAPTER IV

POST-OPERATIVE CARE AND COMPLICATIONS

I. RECTAL PALPATION FOR DETERMINATION OF PREGNANCY IN THE COW

In this study no post-operative care was necessary for the cows which have been examined per rectum.

Pregnancy diagnosis per rectum with reasonable care and skill is virtually without danger. Williams (1939) found that sometimes cows abort after digital palpation of the pregnant uterus, just as they abort without it. During the last ten years, at the Tennessee Experimental Station, Merriman (1963) found no cases of illness, abortion, or cow death reported due to the rectal palpation. However, Fosgate et al. (1954) and Ball et al. (1963) reported evidences of danger of inadvertently terminating pregnancy by injuring the embryo and fetal membranes at thirty-five to thirty-seven days of gestation.

In determinations of pregnancy made at 60 to 120 days post-conception, Salisbury and Van Demark (1961) found no evidences of abortion or fetal mortality and, in any event, the percentage of embryonic deaths appears to be very much lower than that recorded by Fosgate and Smith (1954).

Cattle examined for pregnancy in this study were 60 to 200 days pregnant at the time of palpation. There were no untoward effects due to the examinations. There may be slight risk of skin irritation for the examiner. Nestel et al. (1963) reported possible development of

pustular dermatitis among practitioners who do not use a rubber sleeve and gloves for the rectal examination. They also noticed sweat rash developing in the long time use of rubber gloves.

II. RUMEN FISTUAL IN CATTLE

Despite the apparent surgical success of the four fistulations in this study, the experimental use of the animals was disappointing and ended in death for two of the steers. After release from surgery, the cattle were placed in fields where they were to be used for sampling of pastures. They were employed for sampling on the fourteenth and twenty-eighth days after they were placed on pasture. In preparation for sampling, all rumen content was removed from each animal and placed in a tub. The rumen was washed and the washings removed. With the cannula plug replaced, the steers were allowed to graze for thirty to forty-five minutes. The freshly ingested material was collected from the rumen and original content was replaced. The pneumatic plugs fell from the fistulae or became punctured with the result that the fistulae were opened inadvertently at frequent intervals. The steers became cachectic. No evidence of endoparasitic ova was found in the feces except for coccidia. There was no response when animals were treated for coccidiosis. The hemotocrit levels reached 44 per cent or above. All were afebrile.

The cattle were placed in a barn thirty-seven days after going on pasture. Appetites were poor and weakness pronounced. There was no response to intravenously administered electrolytes and dextrose. Improved plastic cannulae were installed and massive ruminal inoculations

of rumen content from healthy cattle were given on the forty-second day after the start of the pasture experiment. One steer died that day and a second that night. The other two survived but remained unthrifty.

Post mortem examination of the dead steers revealed no significant numbers of gastro-intentional parasites. Both carcasses were emaciated. The abomasums and omasums were almost empty. There were no gross liver changes. There were no signs of speticemia. Urine collected after death of one steer had a pH 5.5.

Gross changes were confined to the rumens and were similar in the two steers. Each rumen was more than one-half full of fluid. Healing of the fistular wound was complete and normal. A large portion of the mucosa of the medial wall of each rumen was involved in deep, ulcerative, necrotic lesions. Death was thought to be due to loss of ruminal anaerobic conditions, ruminal ulceration, and ruminal and systemic acidosis.

III. SPLENECTOMY IN CATTLE

Withholding the animals' feed and water twenty-four hours immediately after splenectomy was recommended by Gates (1953). Dukes (1955) observed that when the spleen was extirpated from the body, other adjustments are made, so that little or no disturbance of health ordinarily resulted.

It is generally agreed that post-operative recovery of splenectomized animals is rapid and uneventful. No loss of animals due to surgical complications is reported in the literature except the loss of

four animals reported by Gates (1953); two of these died from the anesthesia and two died of hemorrhage. Davis (1952) reported no cases of shock or hemorrhage and found that the splenectomized animals were eating within a few minutes after the operation. Gates (1953) found the animals which had been given general anesthesia prior to splenectomy took a few hours to recover sufficiently to get on their feet. Normally, no treatment was given to the animals except daily application of fly repellents on the wound surface. At the end of the ten days, the superficial sutures were removed. Usually, the wounds heal by first intention. Occasionally, slight infection appears at the sites of the skin sutures as reported by Davis (1952). Such infections were quickly eliminated by the application of antiseptic powders. Gates (1953) recommends prompt removal of sutures and a daily check during hot weather to prevent fly larvae infestation in the splenectomized animal. Merriman (1963) reported severe but manageable post-operative shock in one of eight calves splenectomized. In this study, a splenectomized, three-week old Jersey male calf recovered without incident.

IV. VASECTOMY IN BULLS AND RAMS

There were no post-operative complications and recovery was normal in each of four cases in this study. However, post-operative complications such as abscess formation, atrophy of testicles, tetanus, and infections of the females with pus organisms have been reported by Warwick (1924). Merriman (1964) observed apparent failure of vasectomy in one bull and, in another, atrophy of one testicle several years

after surgery. It should also be borne in mind that vasectomized bulls which serve cows with genital infections may transmit the infections to other cows.

V. MUSCLE BIOPSY

Post-operative recovery was uneventful in the calf used for longissimus dorsi (loin muscle) biopsy in this study. Such uncomplicated recovery is generally seen. Bray (1953) found no noticeable permanent adverse effects on the health of animals. Merkel et al. (1954) penned the biopsied hogs separately and held them off feed for twelve hours before returning them to their respective lots. Fly repellants were applied routinely on the wound surface to ward off flies. In semimembranosus biopsy, Bray (1953) found it necessary to open the ventral portion of the incision to allow drainage. Merriman (1964) advocated removal of the sterile packings from the wound cavity about forty-eight hours from the time of operation. He observed a large hematoma in one of three cases. Sutures were removed seven to ten days after the operation. The biopsy wound was completely healed in three to five weeks. Wilson (1955) reported that failure to observe sterile technique resulted in tetanus in cattle.

VI. SEMEN COLLECTION TECHNIQUE (ELECTRO-EJACULATION)

No post-operative care was found necessary in this study. This agrees with published reports which also indicate that no mortality has resulted from electro-ejaculation of the bull. However, Marden (1954)

stated that the electrical stimulators designed by Gunn (1936), Bonadonna (1938), and Thibault et al. (1948) to obtain semen from animals caused severe stress, generalized muscular contractions, distress, and hazard of brain stimulation. The reported side effects have been considerably reduced by the development of refined techniques by Dziuk et al. (1954) and Rowson (1956). With the application of the latest commercial equipment, Hafez (1962) found that bulls respond favourably to the electro-ejaculation technique with no apparent ill effects, even after continuous routine collections over a period of a year or more. Some bulls which are subjected to electro-ejaculation often bellow during the operation, giving the impression that the technique is painful. The absence of other signs of distress indicate that bellowing is probably not due to pain.

CHAPTER V

DISCUSSION

I. RECTAL PALPATION FOR DETERMINATION OF PREGNANCY IN THE COW

Determination of pregnancy in cows by rectal palpation has distinct advantages over other methods of clinical diagnosis. It is simple, economical, reliable, and relatively rapid compared to other methods. Rectal palpation can be employed both in dairy and beef cattle practice under varied seasonal and environmental conditions. It is not a technique that could be employed by an average beef and dairy farmer. The accuracy of diagnosis mainly depends on the training, skill, and experience of the practitioner. The information given here about the accuracy of diagnosis attained by one operator under a particular set of conditions does not enable one to generalize about the accuracy of the method. There is good reason to believe that in more practiced hands the method may allow greater accuracy in prediction of parturition dates.

It has been reported that a clinical diagnosis of pregnancy can be made with a considerable degree of accuracy after the thirty-fifth day of gestation. It may be better, under conditions of normal beef cattle management, to make the examinations 60 to 120 days after the bull is removed from the herd.

II. RUMEN FISTULA IN CATTLE

Several techniques have been described in the literature for fistulation of the rumen in cattle and sheep. Satisfactory results have been claimed by the workers both from the one-stage and two-stage techniques. The surgical procedure which has been followed at the Tennessee Experiment Station for fistulation are identical to the conventional two-stage method. Post-operative recovery was normal in three cases and none of these received any special treatment. Delay in removing the ruminal disc from one steer resulted in localized necrosis and infection. The infection was readily controlled by administration of antibiotics.

Mendel (1961) reported no mortality in animals due to rumen fistulation. However, post-operative complications such as depraved appetite, dehydration, seepage of ingesta, peritonitis, loss of body condition, and cachexia have been reported by Schalk and Amadon (1928). Schnautz (1957) reported similar complications as a result of improper closure of the fistular openings. High (1964) reported that the use of pneumatic plugs has resulted in collapse of the plugs due to accidental punctures and abrasion. This resulted in improper closure and entrance of air into the rumen. In these cases, improper closure of the fistular opening and complete evacuation of rumen content resulted in severe cachexia. Subsequent cannulation of fistulae of these animals with plastic cannulae was not satisfactory. The illness and, in two cases, deaths of these animals is described in Chapter IV.

III. SPLENECTOMY IN CATTLE

Splenectomy has been found by many authors to be useful therapy in man and dog. It has been widely used, especially in cattle, in studying diseases caused by protozoa and protozoa-like organisms. Various satisfactory techniques of splenectomy for calves and mature cattle have been developed by Reese (1933), Gates (1953), Merriman (1963), and Peacock et al. (1964). The modification reported in this study has proved entirely satisfactory under conditions encountered at the Tennessee Experiment Station. Post-operative care and observation will usually ward off the few complications which occur.

IV. VASECTOMY IN BULLS AND RAMS

The technique of vasectomy described offers a simple and quick method for effective sterilization in rams and bulls. Results were satisfactory. In many respects the technique was less complex than others described in the literature. Pre-surgical fasting and casting were avoided. However, good restraint equipment was available. It is suggested that calves of less than 400 pounds in weight be cast for vasectomies. While working with bulls, it was found a larger number of sperm cells could be identified from the lumen contents of the second vas deferens than the first. This variation might be due to additional handling of the second testicle.

V. MUSCLE BIOPSY

Many descriptive methods are found in the literature concerning muscle biopsy for hogs, lambs, steers, and cows. To date no method has been found in the available references for biopsy in calves. The technique followed in this study for biopsy in the calf is more or less similar to conventional methods. No general anesthesia or tranquilizer was administered. Local anesthesia was sufficient for surgery. No considerable hemorrhage or struggling occurred as reported by other workers. Since the claf was subjected to splenectomy twelve days earlier to biopsy, it was restrained in the right lateral recumbency to keep the surgical interventions on one side of the body and allow the animal to rest comfortably on the other side. A sample of muscle was recovered from the incision made medial to the heads of the twelfth and thirteenth ribs.

VI. SEMEN COLLECTION TECHNIQUE (ELECTRO-EJACULATION)

Several techniques have been described in the literature for collection of semen by electrical stimulation. The method which has been practiced at the Tennessee Experiment Station is identical to the conventional methods which are routinely followed in beef bull management. The bulls under study are subjected to electro-ejaculation for routine collection of semen for evaluation studies.

The amount of voltage needed to cause erection was variable in each bull and the operator was guided by the response of the bull's

genital organs to the stimulation. In some bulls, the sigmoid flexure of the retracted penis was straightened out by the operator by manual downward and forward massage between the thighs. This caused the semi-erect penis to protrude from the sheath. Ejaculation followed a consistent pattern. After the stimulus had been applied for between one-half to one minute, accessory fluid commenced to leave the prepuce. After a second interval the sperm-bearing fraction was ejaculated. This later fluid was ejected with each stimulus over a well defined period and was followed by more accessory fluid.

CHAPTER VI

SUMMARY

I. RECTAL PALPATION FOR DETERMINATION OF PREGNANCY IN THE COW

Cows at the Tennessee Experiment Station are examined for pregnancy and prediction of parturition time when they are approximately 60 to 120 days pregnant. Accuracy of pregnancy determination under these conditions has been routinely close to 100 per cent, while the predictions of parturition dates have been less accurate. These methods for pregnancy determination by rectal palpation were used in this study and are described herein.

II. RUMEN FISTULA IN CATTLE

A survey of the literature indicates that fistulation of the rumen in cattle is accomplished generally by one of the following methods: (1) skin, muscle, peritoneum, and rumen wall are incised and secured in a single "one-step" operation, (2) the non-incised rumen wall is sutured to the margin of the abdominal incision and is not incised until seven to ten days following the initial stage of surgery. In this study four Hereford steers were fistulated by the second of these two methods. Recovery from surgery was uneventful in three cases and satisfactory in the fourth.

Subsequent difficulty with fistula closures and use of the fistulated animals for pasture sampling techniques resulted in apparent loss of anaerobic conditions in the rumens, death of two steers, and prolonged unthriftiness of the remaining two.

III. SPLENECTOMY IN CATTLE

Techniques generally reported in the literature and used, in slight modification, at the Tennessee Experiment Station for the splenectomy of cattle to be used in anaplasmosis research were employed in this study for the splenectomy of a fifty-one pound Jersey calf. The use of a tranquilizing agent, local anesthesia, restraint in lateral recumbency, and careful surgical technique were followed by uneventful surgery and recovery. A brief history of splenectomy and a review of post-operative complications are presented.

IV. VASECTOMY IN BULLS AND RAMS

Two bulls and two rams vasectomized in this study were restrained in the standing position for surgery and the vas deferens approached from the rear. Identification of each severed section of vas deferens was attempted by microscopic examination of the lumen content. Earlier experience with vasectomy at this station had revealed a possible surgical failure in one case, resulting in only partially-reduced fertility. Another case resulted, several years after surgery, in the atrophy of one testicle in a bull. Other cases were satisfactory.

The uses, techniques, and post-operative complications of vasectomy as reported in the literature are reviewed.

V. MUSCLE BIOPSY

Literature on the uses, development, and techniques of muscle biopsy in domestic animals is reviewed. In this study a relatively large section of longissimus dorsi was removed from a Jersey calf. Techniques similar to those used previously at the Tennessee Experiment Station were employed. Surgery and recovery were uneventful.

VI. SEMEN COLLECTION TECHNIQUE (ELECTRO-EJACULATION)

Electro-ejaculation was used in this study to secure semen samples from twenty-seven Angus and twenty-one Hereford bulls for fertility evaluations. The technique, routinely used at the Tennessee Experiment Station, was satisfactory and there were no post-operative complications. A review of the literature discussing the development and merits of early semen collection methods and of the electro-ejaculation technique is presented.

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