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ARTIFICIAL INSEMINATION OF DAIRY COWS

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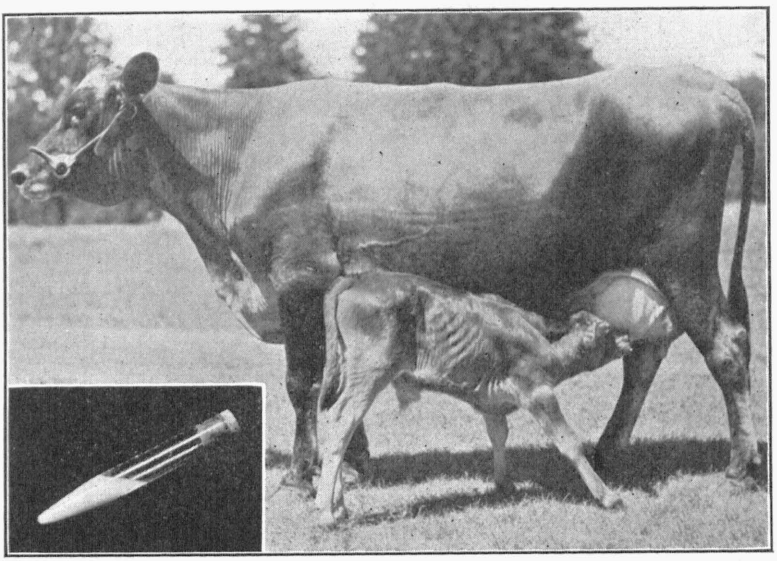

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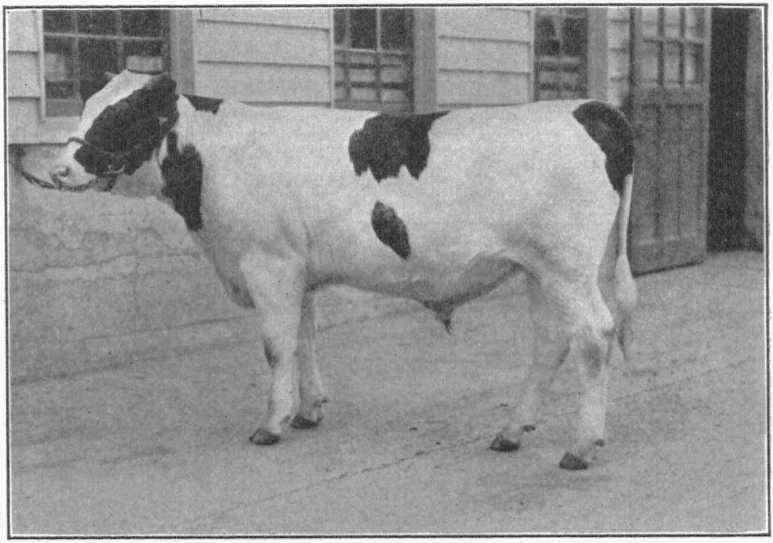

Fig 1.—The first calf resulting from artificial insemination in the University of Missouri dairy herd. Photograph taken at 10 months of age.

Artificial Insemination Of Dairy Cows

H. A. HERMAN and A. C. RAGSDALE

Artificial insemination has been used in the dairy herd of the University of Missouri since the first of July, 1937. During the past 18 months this method of impregnating dairy cows has given such encouraging results that the practice has been adopted by approximately 100 dairy farmers in Missouri. Breeders cooperating with the Department of Dairy Husbandry have supplied records showing nearly 500 cows settled in calf with an average service rate comparing favorably with natural service.

Studies of the breeding efficiency of the Missouri Station herd covering some 25 years and involving the use of 21 sires show an average of 1.53 services per calf for natural breeding. Since February, 1938, when this herd was divided into two equal groups, one bred naturally and the other by artificial insemination, with a total of nearly 100 cows settled in calf, the average number of services required per conception has been 1.56 for natural breeding and 1.40 for artificial insemination. In addition several "shy breeders" have been settled by artificial breeding using double inseminations.

The idea of impregnating animals artificially is not new. Spallanzani (1780) is reported as using artificial breeding on the dog, and records further indicate that the Arabs, centuries ago, were using artificial insemination in their horse breeding practices. It is only since the World War, however, that improved techniques in the collection (artificial vagina), storage, transportation, and utilization of bull semen have made this method of breeding dairy cows practical for wide use.

Much of the present stimulus for artificial breeding of dairy cattle traces to developments in Russia where as early as 1909 the Tzarist government began using the method on its stud farms. Beginning about 1923 the use of artificial insemination became more widespread. Artificial insemination of cattle came into use in 1930 when 19,970 cows were impregnated. In 1931 the number of cows reported to have been inseminated with successful pregnancies following had grown to 185,000 and in 1932 it had increased to 385,000. In 1937 the number of cows artificially bred had reached 1,000,000 and in 1938 1,500,000 were reported, approximately 5 per cent of all cattle in Russia.

One bull, Molodetz, of the Yaroslav breed, according to published reports, was used in the insemination of 530 cows; and the bull

Martika, of the Tagil breed, was used in the insemination of 1450 cows.

Breeding societies, operating in relatively small areas, have been organized and are now operating successfully in Denmark, Holland and several other European countries, in America, and elsewhere. In Denmark one society, the "Elite Breeding Society of Sams," in 1936-37 successfully bred over 1200 cows artificially. Two bulls were used and it is reported that one sire was used in the artificial breeding of approximately 900 cows. Semen was obtained by means of an artificial vagina. The average number of inseminations per conception was reported to be 1.68, a lower rate than was required in natural service on surrounding farms.

Various American dairy cattle breeders and experiment stations have carefully followed the progress of artificial insemination on a practical basis in Europe and have applied the technique to their own herds. The Department of Dairy Husbandry, University of Missouri, first used artificial breeding in the spring of 1937 when a valuable proved sire, Campus Aaggie Segis Sultan 586515, sustained a thigh injury which prevented his serving a cow. Semen was obtained by massage and several cows inseminated. The first calf obtained by this method (U-Mo Aaggie Sultan Valiant 772584, born April 18, 1938) is shown in Fig. 1.

County and community organizations sponsoring breeding projects for the artificial insemination of dairy cattle have been set up in several localities in America and others are rapidly being formed as the practicability of the method for large scale breeding is demonstrated.

The first artificial breeding society or unit in America was organized in May, 1938, through the cooperation of the New Jersey State College of Agriculture and the New Jersey Holstein-Friesian Association, with 102 dairymen entering 1050 Holstein cows. A well qualified veterinarian was employed to do the work. An outstanding proved sire was leased by the society for breeding purposes and as the demand for services increased, other young bulls of excellent breeding were introduced. Two additional breeding units were organized in New Jersey during November, 1938, adding some 1800 cows of the Holstein and Guernsey breeds.

In June, 1938, the Farm Security Administration at Hughesville, Missouri, and the Department of Dairy Husbandry, University of Missouri, set up a cooperative project whereby the cows on two cooperative farms and approximately 30 individual farms located within a 10 mile radius of Hughesville, would be artificially bred. This

project embraces approximately 300 Jersey cows at present, but the number is gradually being increased and will probably reach 500 to 600 in the near future. Three bulls, one a proved sire and the other two younger bulls of meritorious breeding, are being used. The bulls are all kept on one farm and each cooperator notifies the operator in charge by telephone when the cows are in heat. The semen is collected by means of the artificial vagina and is transported by automobile to the respective farms where the cows are to be bred. The service fee ($2.50 at the present time) is considered sufficiently large to cover (1) the cost of feed and care of the bull; (2) equipment; (3) travel and salary of operator; and (4) other miscellaneous expenses. This fee will be increased or decreased as experience warrants and as the project develops. The results of artificial insemination of dairy cows on this project will be discussed later.

ADVANTAGES OF ARTIFICIAL INSEMINATION

1. The usefulness of superior sires may be increased many-fold. A proved sire with ability to transmit characters for high milk and butterfat production may be used to breed several hundred cows annually. Where cooperative breeding organizations are formed the use of such bulls may be extended to a large number of dairy herds.

2. Small breeders who would of necessity have to buy a bull (often of mediocre breeding) within their means may dispense with the keeping of a bull and through the use of artificial insemination participate in the use of a bull of outstanding merit at a lower cost.

3. The transmitting ability of a bull may be determined quickly and effectively. Young, unproved bulls should be used sparingly until their transmitting ability has been demonstrated. Where sires are used cooperatively this is easily accomplished and at best no one dairyman will have more than a few daughters of such a bull in his herd.

4. The danger of spreading genital diseases (such as trichomonads) is materially reduced. This is of much importance even though such diseases are relatively few in number.

5. Valuable sires which because of injury are unable to serve cows may be continued in service.

6. Yearling heifers and small cows may be bred to large, heavy bulls without danger of injury.

7. Because of the regular examination of the semen, infertile bulls are likely to be detected earlier than with natural breeding. Likewise, abnormalities of the cow's genital tract which may lead to shy breeding, may be discovered earlier.

8. In most cases better breeding and calving records will probably be kept. This is particularly true in organized breeding units where one man is responsible.

9. Line-breeding and the development of certain large families of superior dairy cattle within a community is possible.

10. The mating of outstanding individuals, though located hundreds of miles apart, is possible.

11. The participation in a breeding program and the study of breeding problems by a large number of cooperating dairy farmers should bring forth the best community spirit for the advancement of the dairy cattle industry.

DISADVANTAGES OF ARTIFICIAL INSEMINATION

There are certain disadvantages connected with artificial insemination in dairy cattle which should be recognized by any person planning the use of this method.

1. Artificial insemination requires a well trained operator and special equipment.

2. It requires somewhat more time than natural service which tends to limit its use to large herds or well organized cooperative projects.

3. All equipment and instruments used must be clean or infection may be spread. Proper facilities for cleaning instruments must be available.

4. The extended use of sires by this method may result in fewer purchases of bulls by small breeders and breeding establishments may suffer consequent loss of income.

THE TECHNIQUE OF ARTIFICIAL INSEMINATION IN DAIRY COWS

The technique of artificial breeding is concerned with the collection of a suitable sample of semen, its preservation under favorable conditions until used, and the insemination of the female.

A knowledge of the anatomy and physiology of the male and female reproductive organs is essential in the technique of artificial insemination, and the more important facts concerning these organs and their functions are given in the following discussion.

The Male Reproductive Organs

The essential organs of reproduction in the bull are the *testicles,* two in number, which are carried outside the body wall in the scrotum.

The testicles have at least two functions; (a) the production of spermatozoa (male germ cells), and (b) the production of endocrine substances which markedly affect the development and behavior of the male. Attached to each testicle is a convoluted tube, the

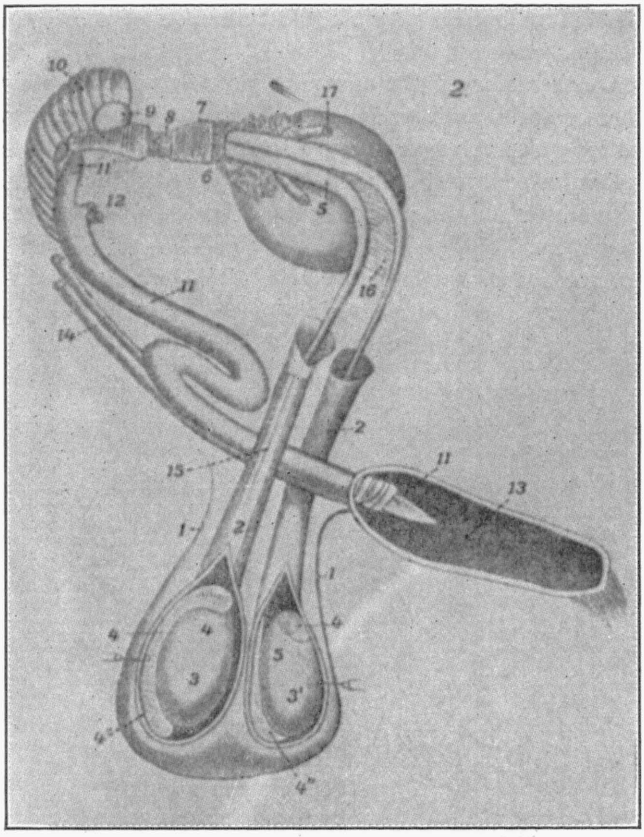

Fig. 2.—Reproductive and urinary organs of the bull. 1, scrotum; 2, spermatic cord; 3, testicle; 4, epididymis; 5, vas deferens; 6, vesicula seminalis; 7, membranous portion of urethral canal covered by Wilson's muscle; 8, part of prostate gland covered by Wilson's muscle; 9, Cowper's gland; 10, accelerator urinæ muscle; 11, penis; 12, cut suspensory ligaments of penis; 13, sheath, laid open; 17, ureters. (*From U. S. D. A. Diseases of Cattle.*)

epididymis, which extends down the outside of the testicle to its base. The epididymes provide storage space for spermatozoa and in addition have a secretory function. The spermatozoa, when formed by the testes, pass to the epididymes where they undergo a maturing process.

Leading upward from the epididymes are the *vasa deferentia,* which are slender tubes connecting with the *urethra,* which passes through

the penis and provides an opening to the exterior for ejaculation of the semen. Near their termination the vasa deferentia enlarge to form the *ampullae.* The ampullae are located just above the anterior part of the pubis, where they join and progress forward as the urethra. Sperm are stored in the ampullae and massage of these organs, by way of the rectum, makes possible the collection of semen as will be discussed later.

Lying on either side of the ampullae are the *seminal vesicles.* In the bull these glands, usually considered accessory organs, are about 2 to 3 inches long and approximately 1 inch wide. They secrete a thick alkaline, globulin-containing fluid which is added to the ejaculate and serves as a carrier for the spermatozoa. The seminal vesicles empty into the *ampullae.*

The spermatozoa of the bull are somewhat tadpole-like in appearance and consist of head piece, middle piece, and tail. They are minute in size, being 9 to 10 micra in length.

Fig. 3.—Spermatozoa of the bull (highly magnified).

Additional glands, accessory in nature, are (a) the prostate, located near the neck of the bladder and surrounding the urethra, whose function is the secretion of alkaline fluids apparently of value in neutralizing any acid condition of the urethra and also the vagina; and (b) Cowper's glands, two in number, located on either side of the urethra. The function of the secretions of these glands is not fully known and besides serving as a carrier for the sperm, may be similar in action to those for the prostate.

The *penis* has the function of draining the bladder and is also an organ of erection and ejaculation which serves to introduce spermatozoa into the vagina of the cow.

Semen is the normal discharge of the male at mating time. It is a whitish, somewhat thick fluid, and consists of the spermatozoa, milk-like fluid from the testicles and epididymes, and the secretions of the seminal vesicles, prostate and Cowper's glands. The amount of semen ejaculated by a bull at a single service normally varies from approximately 2 to 6 cubic centimeters. The number of sperm per cubic centimeter of bull semen usually ranges from a few hundred million to three billion.

Typical ejaculations, as to volume and sperm concentration, of samples collected from dairy bulls in the Missouri Experiment Station dairy herd are shown in Table 1.

TABLE 1. SPERM PRODUCTION—DAIRY BULLS.

Bull No.	Breed	Age (years)	Number of Ejaculates	Average Volume (cc.)	Sperm per cc. (millions)
1	Holstein	2½	14	2.3	910
2	Holstein	3	18	5.4	650
3	Holstein	3	21	4.1	1510
4	Holstein	8	9	5.6	695
5	Holstein	3½	12	5.1	360
6	Holstein	1½	4	2.4	720
7	Guernsey	3½	9	6.1	1450
8	Jersey	3	15	2.1	1950
9	Jersey	10	31	4.3	412
10	Jersey	13	16	3.7	760
11	Jersey	6	8	3.2	990
12	Jersey	7½	6	4.6	876

The Female Reproductive Organs

The essential organs of reproduction in the cow are the *ovaries, Fallopian tubes, uterus, cervix, vagina,* and *vulva,* as shown in Fig. 4.

The two ovaries of the cow produce the reproductive cells (ova or eggs). Normally one or more ova are shed by the sexually mature bovine each 18 to 24 days, preceded by estrus or heat. In addition to their production of eggs, the ovaries produce hormones which are concerned with reproduction and growth of the mammary gland.

The Fallopian tubes, suspended in the broad ligaments, open at the fimbriated end near the ovaries and serve to conduct the egg from the ovary to the uterus. The Fallopian tubes, or oviducts, end in the body of the uterus, which is a tubular, very glandular, muscular organ with two branches or horns. The two horns of the uterus, right and left, are really continuations of the Fallopian tubes but are much larger and thicker walled. The horns join to form the main body of

the uterus. The uterus, in pregnancy, contains the developing fetus and because of its muscular elasticity is capable of changing its size with the growth of the young.

The uterus opens posteriorly in the cervix or *os uteri.* The cervix of the cow is about 3 inches long and ¾ to 1 inch in width. It is quite thick-walled and the cervical canal has many folds which makes the introduction of any instrument somewhat difficult. During heat the cervix relaxes slightly but at parturition it relaxes a great deal to permit expulsion of the fetus. During pregnancy it is sealed with a mucus plug, thus guarding the developing embryo and uterus from germ invasion.

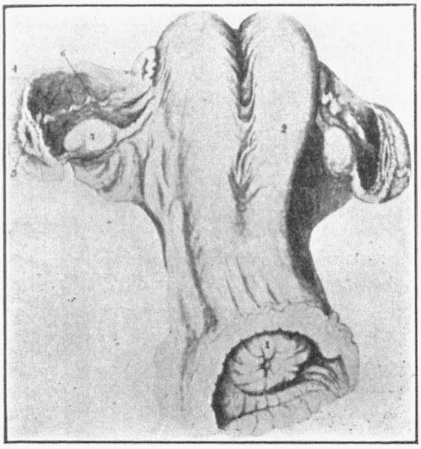

Fig. 4.—Reproductive Organs of the Cow. 1, os uteri; 2, right horn of uterus; 3, ovary; 4, ovarian ventricles; 5, fimbriated end of Fallopian tube; 6, Fallopian tube (from "Diseases of the Genital Organs of Domestic Animals", Williams).

The cervix opens posteriorly into the vagina. The vagina is strictly an organ of copulation and extends from the vulva to the cervix. It is from 10 to 14 inches long in the cow. In copulation the semen is ejaculated into the vagina. A small depression just in front of the cervix apparently aids in collection of some of the semen near the opening of this organ. The vagina is somewhat restricted from the vulva or external opening of the reproductive tract. The vulva is an organ common to both the reproductive and urinary tract and is of greater diameter than the cervix.

Cows, unlike some animals in their domesticated state, are not seasonal breeders, and may normally be expected to come into heat about every 18 to 24 days until settled in calf. Occasionally irregular

heat periods due to abnormal conditions of the ovaries are experienced, and such conditions usually call for treatment by a skilled veterinarian.

The cow's *ovum* or egg is fertilized by a single sperm from the male and growth begins at once by a series of divisions. The single fertilized cell divides to make two, then four, eight, etc. cells.

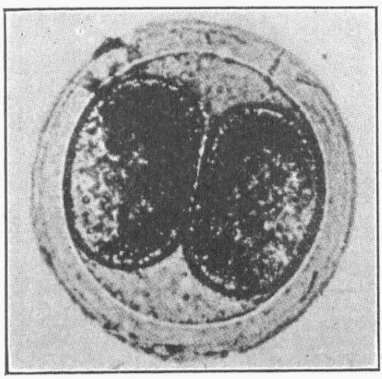

Fig. 5.—Two-cell stage of cow's ovum (from Hartman, Lewis, Miller and Sweet, Anat. Rec., pub. Wistar Institute).

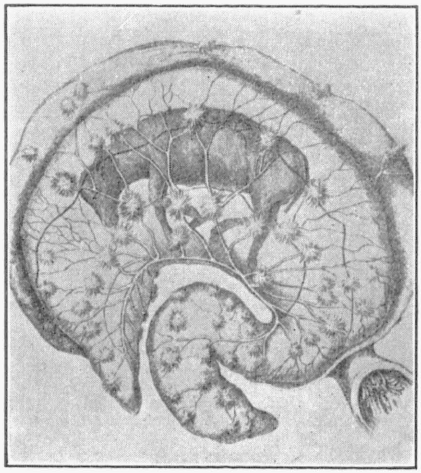

Fig. 6.—Fetal calf within its membranes (from U. S. D. A. Diseases of Cattle).

Figures 5 and 6 show the first division of the fertilized ovum and the subsequent development of the placenta.

Collection of Semen from the Bull

Three are at least three recognized methods for obtaining semen from a bull—(1) the use of a breeding sheath or artificial vagina; (2) the massaging of the accessory genital organs (ampullae); and (3) the recovery of semen from the vagina of a cow that has just been bred by natural mating.

1. **The Artificial Vagina.**—This method seems to be the most practical and satisfactory. Where conditions are properly regulated, sires are active and mount readily, semen is quickly and easily collected. The normal ejaculate is 2 to 6 cc., with an average of about 3.5 to 4.0 cc.

The artificial vagina or sheath used consists of a heavy rubber cylinder 16½ inches long and 2¾ inches in diameter with a thin rubber lining, and may be fitted with a valve or a hole in the outer cover to permit filling with warm water to provide proper pressure. The lining is a thin rubber tube which is folded over each end of the

cylinder to hold water. The amount of water used between the inner and outer linings of the vagina must be regulated according to the size and age of the bull. If too much water is used, ejaculation is interfered with or the semen collecting receptacle is blown off the end by the bull's thrust.

Fig. 7.—The artificial vagina assembled and ready for use. Note the collecting tube.

Fig. 8.—Filling the chamber between the rubber liner and the outer sheath of the artificial vagina with warm water.

The proper pressure is usually obtained by filling the outer jacket about one-half to two-thirds full of water at a temperature of 115 to 140° F. (depending on environmental temperature). After the warm water has been added the valve is closed, or if the artificial vagina is not fitted with a valve, the hole is covered by a fold of the end of the rubber lining. A thin coat of lubricant such as sterile white vaseline, mineral oil, or a mixture made up of 3 grams gum tragacanth, 5 cc. glycerine and 50 cc. distilled water and allowed to jell, is smeared over the whole surface of the inner lining by means of a large sterile glass rod. Both mineral oil and vaseline are very injurious to rubber and are difficult to wash off. The lubricant containing tragacanth and glycerine is much preferred. This mixture may be kept for several days if held in an ice box, but must be made up fresh at frequent intervals. A slender thermometer should be in readiness for checking the temperature inside the vagina. *It is very important that the temperature on the inside of the artificial vagina at the actual time of collection be 105 to 110° F.* Higher temperatures may kill the sperm, and lower temperatures may retard ejaculation.

The large end of a funnel-shaped piece of rubber is slipped over the end of the artificial vagina. Into its smaller end by means of rubber bands is fitted a sterile glass tube (preferably a 12 or 15 cc.

graduated centrifuge tube) for collection of the semen. The cow used for collection may be in heat, or a "chronic buller" or a fairly

Fig. 9.—A convenient, practical breeding chute in use at the Missouri Station. This chute simplifies the problem of handling old or mean bulls and reduces chances of injury to the operator.

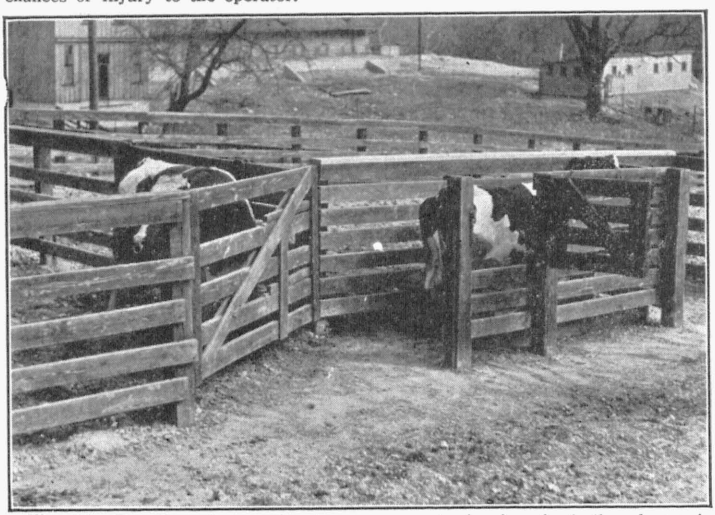

Fig. 10.—Breeding chute and gate arrangement, showing the bull and cow in place preparatory to the collection of semen.

quiet cow not in heat may be used. A breeding rack or stocks will facilitate the handling of the bull and collection of the semen artificial insemination is to be practiced.

As the bull starts to serve the cow, the artificial vagina at the proper temperature and properly lubricated, is placed in position so that

as the bull makes his thrust he ejaculates into the artificial vagina, or more exactly, into the rubber funnel and the receptacle attached to its farthest end.

During collection the artificial vagina is held at the cow's side with the open end tilted downward at an angle of about 45 degrees. In

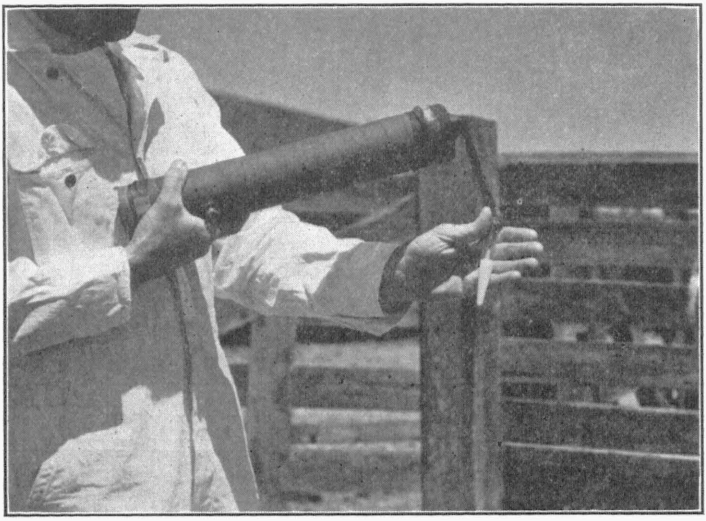

Fig. 11.—Taking temperature of the artificial vagina, which should be 41-43° C. (105-110° Fah.) at the time of use.

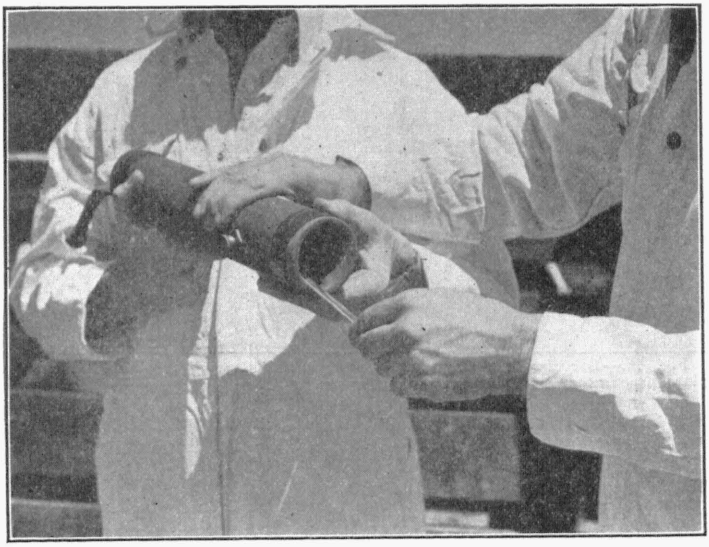

Fig. 12.—A sample of semen, collected, and ready for immediate use or storage.

addition to being in the most favorable position for collection of semen, the tilting of the artificial vagina results in the water collecting in the lower part of the jacket surrounding the thin rubber lining and creates the proper pressure for stimulation of the ejaculatory nerves of the bull which are located near the end of the penis.

As the bull starts to mount the cow, the penis is quickly guided into the artificial vagina by means of a hand *placed on the sheath.* The penis itself should not be touched as this frequently causes the bull to dismount without ejaculating. After the bull ejaculates, the vagina is held upright and the semen allowed to run down into the collecting tube.

2. **Collection of Semen by Massaging the Ampullae.**—This method has the disadvantage of requiring special training and familiarity

Fig. 13.—Dorsal view of the reproductive organs of the bull—a, bladder; b, ureter; c, seminal vesicles; c, ampullae; e, body of prostate; f, pelvic urethra; g, bulbo-urethral (Cowper's) glands. (From Miller and Evans, Jr. Agr. Res., vol. 48, 1934).

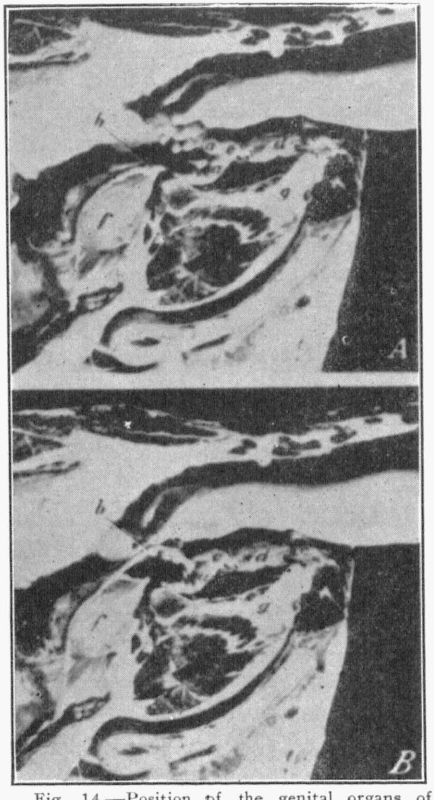

Fig. 14.—Position of the genital organs of the bull and method of manipulating them. A. Massaging the seminal vesicles. B. Massaging the ampullae of the ductus deferens. (From Miller and Evans, Jr. Agr. Res., vol. 48, 1934).

with bovine anatomy, and in addition the semen is often contaminated with dirt and urine during the collecting process. However, this method is of much value where bulls are incapacitated and unable to serve. The semen obtained by this plan seems to be just as fertile as that obtained by means of the artificial vagina. Where it is necessary and the operator is sufficiently versed in bovine anatomy, semen may be obtained from the bull by massaging the ampullae. Before anyone attempts this method of collection he should first obtain training from some person who is experienced. Some bulls respond very poorly to the massage method of collection and the semen obtained is often more contaminated than that obtained by the use of the artificial vagina. For lame bulls which cannot serve naturally or into the artificial vagina, this method may be used.

Figures 13 and 14 show the reproductive organs of the bull and the general technique employed in obtaining semen by this method.

3. **Collection of Semen from the Vagina.**—A third method of collection of semen and one of the oldest, is to recover the semen from the vagina of a cow that has been served by the bull. The semen may be easily collected from the vagina by means of a pipette. This method is not recommended because of the danger of spreading diseases of the genital organs of the cow and bull. Furthermore, the semen is usually contaminated with mucus, urine, etc.

Handling and Transporting Bull Semen

Where a number of cows are to be bred in one day with the semen from one ejaculation, as is usually the case in a large herd or an organized cooperative artificial breeding association, several methods, depending upon the circumstances, are recommended for handling the semen.

Quality of Semen.—Semen varies greatly in its density of sperm and every precaution should be taken to obtain a good sample of highly viable semen for artificial breeding. The semen from bulls should be examined microscopically at frequent intervals for the concentration and motility of sperm. Occasional sperm counts and examination for abnormal morphology are also useful in evaluating the semen, particularly where there is some question concerning a bull's fertility. Other characteristics indicative of fertility in the male are the quantity, consistency, and color of the semen.

1. *Where inseminations are to be made within one to two hours after collection* it is sufficient to place the semen in a small vial which should be stoppered and wrapped in several thicknesses of paper or cloth, and set in a room at 80 to 85° F. until used. Where insem-

inations are to be made immediately after the semen is drawn, the tube containing the semen should be wrapped to prevent breakage and too rapid temperature changes and the cows inseminated immediately.

Fig. 15.—Examining a sample of semen for motility and sperm concentration.

2. *Where inseminations are to be made 2 to 10 hours after collection,* every precaution should be taken to keep the semen in a high state of viability. Sudden temperature changes must be avoided and the semen protected from the air. Lowered holding temperatures and cutting off the air supply results in a lowered sperm activity, two factors of vital importance in semen storage. The semen should be placed in a small sterile vial immediately after collection, then wrapped in two or three thicknesses of paper and set in a refrigerator at 45° F. Another means of holding, particularly where a thermos bottle is used to maintain the lowered temperature, is to cover the paper wrapped vial with two rubber thumb stalls, after which

it may be dropped into a vacuum bottle containing water at 45 to 50° F. This treatment results in gradual cooling of the semen. A layer of neutral mineral oil poured over the top of the semen in the vial is helpful in excluding air. Upon removal from the refrigerator or thermos bottle, the temperature of the semen should be raised gradually before insemination. The rubber-paper insulated vials should be placed in a room at 80 to 85° F. with the wrapping intact for some 20 to 30 minutes before use so that the semen may warm up gradually. Another scheme for gradual warming is to transfer the vials to thermos bottles at 65, 75 and 90° F., allowing the vials to stay in each thermos bottle 10 to 15 minutes.

The properly insulated vial for semen storage is shown in Fig. 16. If all of the semen is not to be used at one time it is best to divide it into several vials and warm the individual vials as they are needed. Frequent warming or cooling of semen, or rapid changes in temperature may cause "temperature shock" which sometimes results in irreversible immotility of the sperm.

Clean paraffin treated corks should be used to stopper the semen vials. In our work a 12 or 15 cc. graduated centrifuge tube has been found to be the most satisfactory for semen collection.

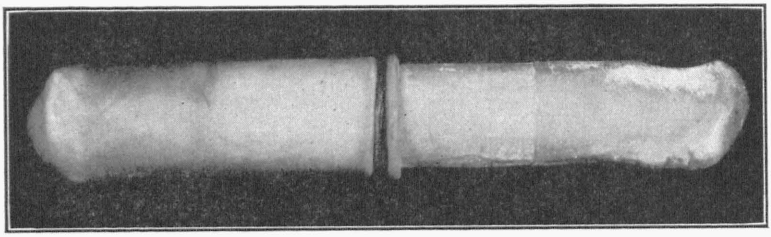

Fig. 16.—A vial of semen wrapped for storage. Two thicknesses of paper are placed about the tube, then a rubber finger stall is placed over either end, drawn together in the middle and held in place by a rubber band.

3. *For field use,* a thermos bottle with a hole bored in the stopper to accommodate the vials containing the semen may be helpful in holding the vials as well as maintaining the proper temperature. In the technique employed by some operators a pint thermos bottle is filled about two-thirds full of water at 45 to 50° F. and the stoppered vial slipped into place in the hole in the cork. Where semen is to be used at once this plan seems to be satisfactory, although it does perhaps subject the semen to more drastic temperature changes than the plan described under 1 and 2, and which has been used most extensively in our field work at this Station.

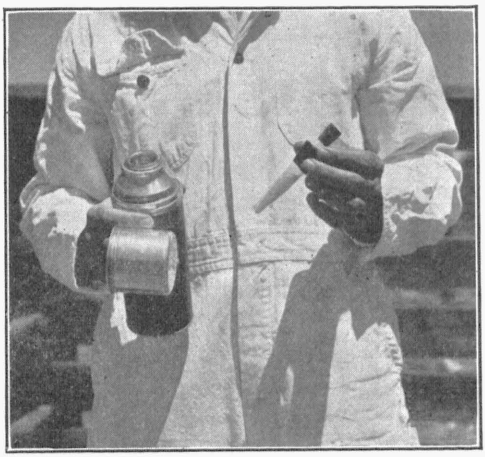

Fig. 17.—A thermos bottle fitted with a bored cork for holding semen vials during insemination.

Diluting Semen

Where only 3 to 6 cows are to be bred from a single ejaculate, it is seldom necessary to dilute the semen. As a rule about 1 cc. of undiluted semen is placed in the cervix for insemination. However, where a large number of cows (8 to 20) are to be bred from a single collection, it is necessary to dilute the semen. The purpose of a dilutor is to increase the volume so that more cows may be bred from a single ejaculate. The dilutors are also credited with providing energy, buffering, and preserving the sperm. This is not always the case, however, as preliminary studies on storage of semen at this Station show that undiluted semen may live as long or longer than diluted. Dilutors may be satisfactorily used where semen is used the same day drawn. The dilutor should preferably be added just before the work of insemination for the day is begun.

A number of solutions have been successfully used as dilutors, including normal physiological salt solution, and a $\frac{3}{5}$ per cent glucose solution. The most satisfactory dilutor found by the Russian workers is known as the Milovanow Formula SGC-2, which is quite generally used in this country. The formula for this solution is:

Na_2SO_4 .....13.6 g. per 1000 ml. distilled water.
Dextrose .....12.0 g. per 1000 ml. distilled water.
Peptone ..... 5.0 g. per 1000 ml. distilled water.

This solution must be carefully prepared, using only high quality, pure materials, put into clean glassware, and sterilized at fairly low temperatures for at least two hours. For field use the dilutor solu-

tion may be put up in 5 or 10 cc. vials. Storage of the solution in an ice box to retard the growth of organisms is also recommended.

Semen may be diluted at the rate of 1:2, 1:4, or even 1:8, depending upon the number of cows to be bred. One point to keep in mind is that semen of low sperm concentration should not be diluted as much as that which is highly concentrated. As a rule the number of sperm in a sample of diluted or undiluted semen should be about 200 to 250 million for each insemination.

In adding the diluting material to the semen, care should be taken to make sure that both the semen and the diluting fluid are at similar temperatures.

Insemination of the Cow

Cows should be inseminated while in heat or up to a few hours after the end of heat. Russian experiments indicate greater success in settling cows where they were inseminated in the later stages of the heat period (a cow usually remains in heat 12-36 hours). As a rule cows may be successfully inseminated up to six hours after the heat period.

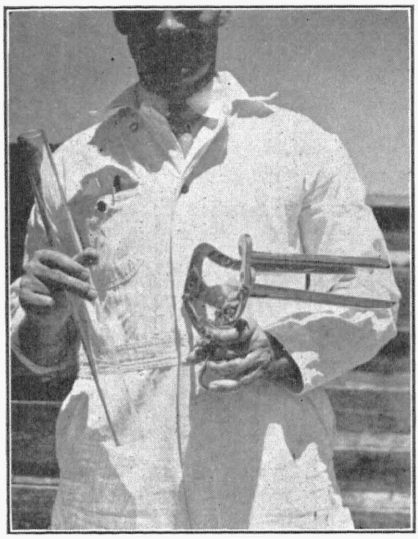

Fig. 18.—Showing the types of speculae employed in artificial insemination. The glass tube shown at the left is preferred.

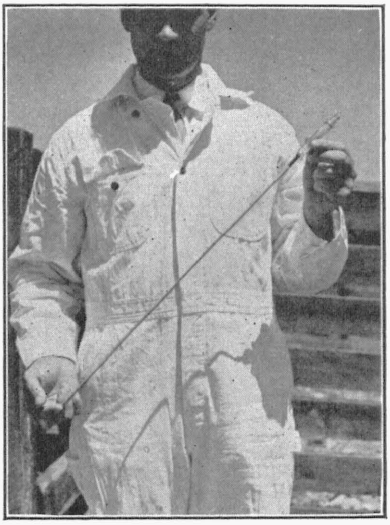

Fig. 19.—Showing the glass syringe, rubber connector, and inseminating tube in place.

The equipment used for insemination consists of a small glass syringe (5 cc.) attached, by means of a piece of fairly stiff rubber tubing about 1 to 1½ inches long, to a piece of 3/16 inch glass tubing about 20 inches long. The tips of the glass tubing should be rounded

by heating in a flame. The pipettes must be sterile and free from water, soap, and disinfectants, all of which are harmful to sperm. In addition, a speculum (either a heavy walled glass tube $1\frac{1}{4}$ inches in diameter or a trivalve speculum) and a headlight are required.

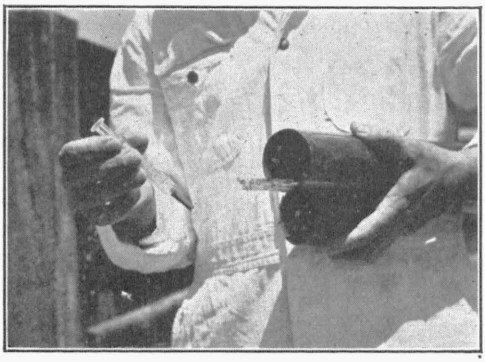

Fig. 20.—A handy metal tube, or cannister, for carrying sterile inseminating tubes.

Before insemination, the vulva and adjacent parts of the cow must be thoroughly cleansed with water. Soap should be used sparingly and all of it washed off before inserting the speculum. The speculum is lubricated and carefully inserted in the vagina. The cervix is then

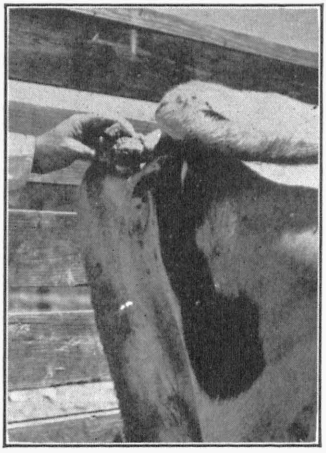

Fig. 21.—Washing the vulva and adjacent parts of the cow preparatory to insemination.

located. Next, about 1 cc. of the semen is drawn into the glass tube by means of the syringe. The pipette should be at a temperature similar to that of the semen used in order to avoid temperature shocks.

The free end of the glass tube is placed in the cervix to the depth of about 1 inch (not difficult when cows are in heat and the *os uteri* is open) and the semen is slowly expelled by pushing in on the plunger of the attached syringe. The tube and speculum are then withdrawn.

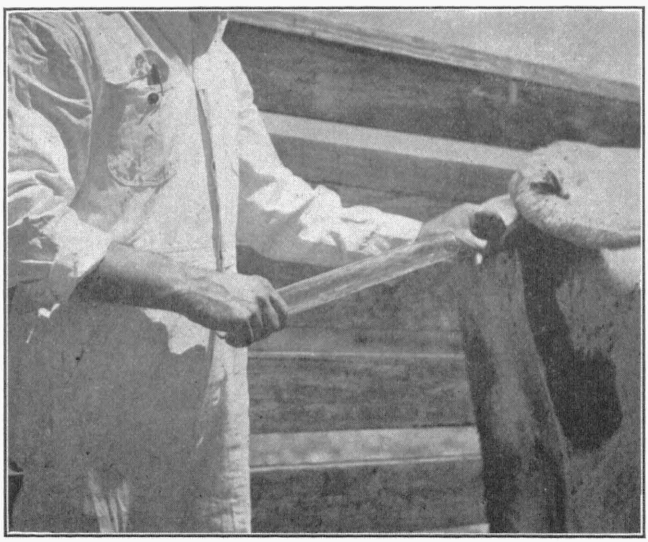

Fig. 22.—Inserting the speculum into the vagina.

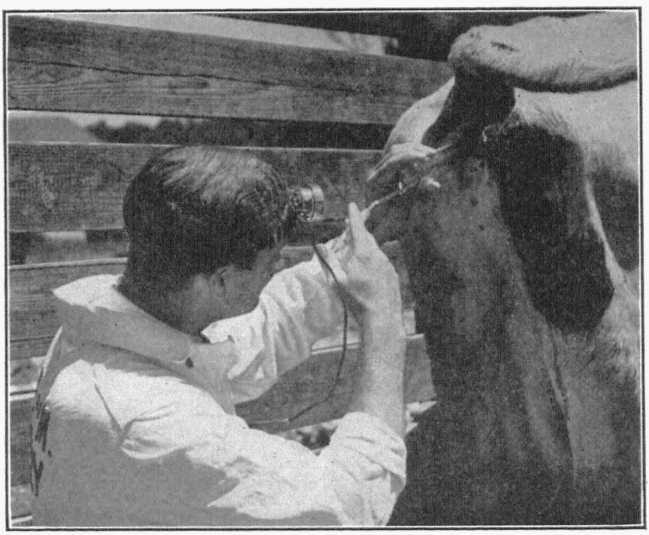

Fig. 23.—Inseminating the cow.

Amount of Semen Used.—In work done thus far by the Department of Dairy Husbandry, Missouri Agricultural Experiment Sta-

tion 1 cc. and in some cases 0.5 cc. of semen has been used successfully. Russian workers report considerable success using 0.2 cc. and 0.5 cc. With smaller amounts more animals can be inseminated without diluting the semen. More work needs to be done on this phase of the problem.

General evidence shows the greatest number of pregnancies where the semen is placed directly in the cervix. Some Russian workers report considerable success where the semen is simply placed in the vagina. American investigators, however, do not report this plan as effective in settling cows as when the semen is placed directly in the cervix.

Insemination Without Use of Speculum.—Another method of insemination is to place one hand in the rectum of the cow and grasp the cervix. With the other hand, the inseminating tube (a special heavy-walled, shatter-proof tube or catheter is required) is gently guided through the vagina and deep into the cervix, 1 to 2 inches, before expelling the semen. By this method it is possible to implant the semen fairly deep into the cervix and little or none of it can run back into the vagina. The necessity of using a speculum and disinfecting this piece of apparatus is thus obviated. Whether this method of insemination is more effective than that of placing the semen in the mouth of the cervix is yet to be demonstrated.

Double Insemination May be Helpful.—Where a breeder is having difficulty in securing a high percentage of conceptions it may be advisable to inseminate each cow twice during the estrus period. This plan does not lend itself very well to field work where organized breeding associations are operating, but it does seem to have a distinct place where cows are hard to settle. Russian workers have found this plan very encouraging and recently several cows in the Missouri Station dairy herd which had repeatedly failed to settle, either by natural or artificial breeding, were successfully impregnated by double insemination.

Where two inseminations are used, the cow should first be inseminated shortly after the beginning of heat and again 12 to 20 hours later. If but one insemination is used it should usually be about 10 to 15 hours after the beginning of the heat period.

SHIPPING SEMEN—LONG DISTANCE MATINGS

The long distance transportation of bull semen and the mating of widely separated animals has not been as well perfected as the technique for immediate insemination. This phase of the problem is being very extensively investigated and the future will no doubt see

many improvements in the handling and utilization of bull semen to be stored for long periods. The Bureau of Dairy Industry, U. S. Department of Agriculture, Washington, D. C. reports the successful impregnation of several cows in Buenos Aires, Argentina, with semen collected at Beltsville, Maryland, and shipped by plane to its point of use. In Europe bull semen has been shipped by plane from England to Holland. Eight of 26 cows inseminated conceived. The maximum age of the semen used was 57 hours. In general, good viable semen seems to decline in fertility at a rate somewhat proportional to the holding time. In studies conducted by the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, it was found that approximately one-third more services per conception were required when dairy bull semen was collected, cooled, transported 75 to 80 miles, and used some 4 to 5 hours after collection than where the cows were bred within one-half to two hours after the semen was collected and used without cooling or diluting.

Long distance shipping or holding of semen requires cooling and storing at temperatures between 40 and 50° F. For quick transportation semen may best be shipped *air express*. Suggested directions for shipping semen are:

1. Place the collected semen, undiluted, in a small sterile vial, 2 to 4 cc.
2. Fill any unoccupied space with pure, clean mineral oil to exclude air.
3. Wrap the vial in two or three thicknesses of paper and place a rubber finger cot over each end lap and fasten the ends in the middle by means of a rubber band (see Fig. 16).
4. Place the insulated vial in a thermos bottle containing ice water at a temperature of 45° F. The vial may be tied to the cork to secure it if necessary.
5. Wrap the thermos bottle in several thicknesses of paper and pack it in a small wooden or pasteboard box with plenty of cotton or waste packing. The ends of the bottle may be cushioned with rubber sponges. Then wrap and seal the entire package, label plainly, and ship by the fastest transportation.

Some alternative plans of handling semen for long distance shipping are (1) to dilute the semen with an equal volume of 4 to 5 per cent sterile glucose solution and (2) to cool the semen by placing the insulated vial in a thermos bottle at 50° F. for one hour, then into a thermos bottle of cracked ice for final shipping. It is not yet established that the alternative steps mentioned give better results than the outlined procedure. However, where semen will be in transit

two or three days, it is preferable to use cracked ice rather than cold water in the thermos bottle to maintain low temperatures.

The semen vials should be removed from the thermos containers, with the insulation intact, and allowed to gradually warm to room temperature before use. Another plan is to place the cool, insulated vials into successive containers of water at temperatures of 60, 75 and 90° F. so as to step up the temperatures gradually before use.

Semen has been kept alive at a temperature of 40 to 45° F. for over 20 days, but little is yet known regarding the fertility of stored semen. English workers report successful impregnation with semen 96 hours old and the writers have successfully settled two cows using semen 48 hours old.

Semen for shipping or storage must be of excellent quality, dense, good motility, and sperm of normal morphology. In almost every instance the most successful use of stored semen has been with the most dense, best quality material.

EQUIPMENT FOR ARTIFICIAL INSEMINATION

All containers and other apparatus which comes in direct contact with the semen should be made of glass, as glass undergoes little or

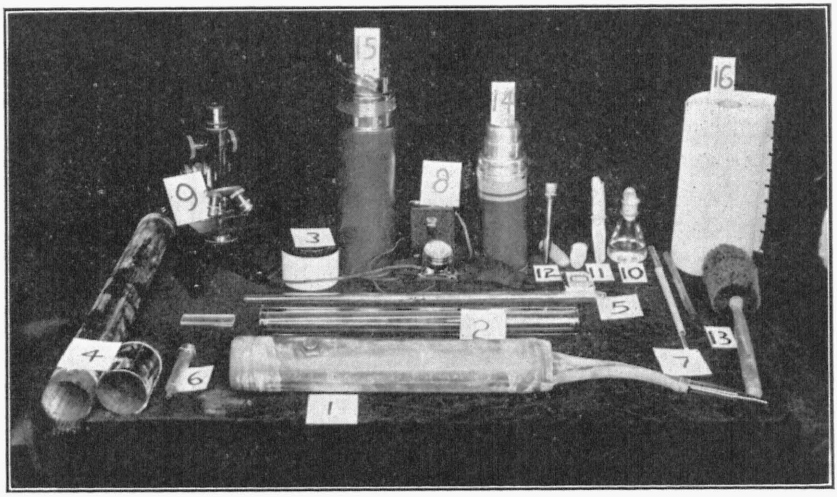

Fig. 24.—Equipment used for artificial insemination of dairy cattle: (1) assembled artificial vagina and collecting vial; (2) heavy walled glass speculum; (3) lubricating material; (4) metal tube for carrying sterile inseminating tubes or pipettes, 3/16" diameter, ⅛" bore, about 20-22" long, for removing semen from vial and introducing it into the cervix. This glass tube is connected at one end by means of a short rubber tube to a glass syringe (a sterile glass tube should be used for inseminating each cow); (5) heavy glass rod for applying lubricant to artificial vagina; (6) 5 cc. graduated glass syringe; (7) thermometer (calibrated from 0 to 200° F.); (8) headlight; (9) microscope and slides; (10) diluting fluid; (11) vial wrapped in paper and rubber for insulation; (12) collecting vial (15 cc. graduated centrifuge tube); (13) brush for cleaning artificial vagina and speculums; (14) pint thermos bottle; (15) thermos food jar; (16) paper towels; (17) bottle of 70% alcohol; (18) cotton; (19) soap and sponge; (20) box of gummed labels.

no chemical changes when in contact with body fluids, and is easily cleaned and sterilized. The equipment normally used is shown in Figures 24 and 25.

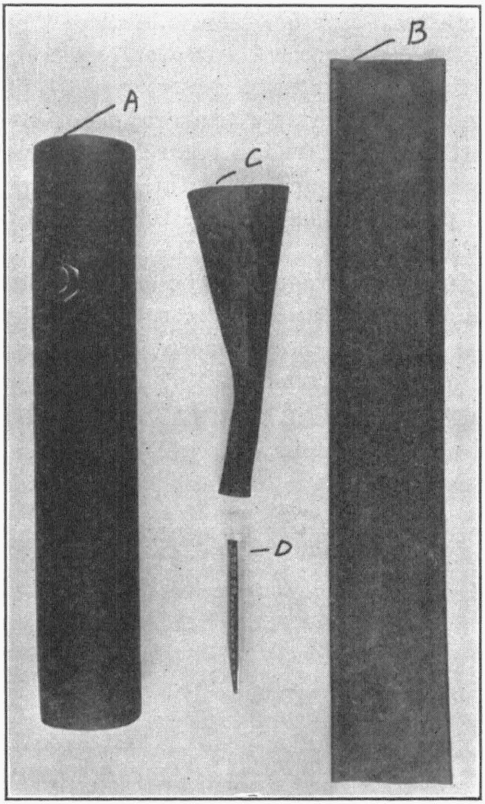

Fig. 25.—The artificial vagina, unassembled. (A) Outer casing; (B) rubber liner; (C) rubber funnel; (D) centrifuge tube for semen collection.

The equipment may be very satisfactorily transported in a large, inexpensive metal covered suitcase. Partitions may be inserted to insure each piece of equipment being snugly fitted into its proper place.

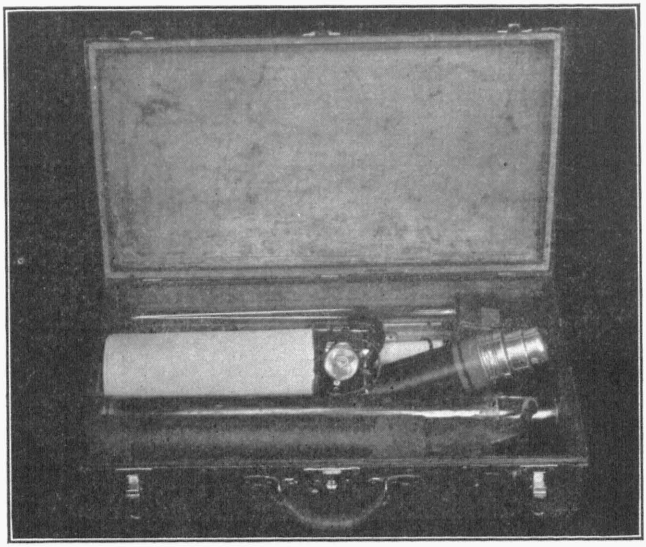

Fig. 26.—Equipment and carrying case.

CLEANING AND STERILIZING EQUIPMENT

During the day's work a separate, sterilized pipette, and a clean speculum must be used for inseminating each cow. The glass specula are very inexpensive and it is convenient to keep 6 to 12 on hand to eliminate the necessity of cleaning them in the field. At night the glassware should be thoroughly washed, then sterilized by boiling water.

After using the artificial vagina the liner should be washed at once with warm water. Washing soda may be used but it is very important that the rubber be rinsed well and then thoroughly disinfected with 70 per cent alcohol. The liner and rubber funnel should then be loosely wrapped in soft paper and allowed to dry.

PRACTICAL RESULTS AND SUGGESTIONS

Reports show that artificial insemination is as efficient and in some cases even more efficient than natural breeding. Results from Denmark indicate that in one organized society the number of inseminations per conception was 1.68 for 937 cows. Cows in the same district naturally bred required 1.8 to 2.0 services per conception. In the New Jersey Artificial Breeding Association it is reported that 762 cows bred required 1089 inseminations with 589 pronounced pregnant, or an average of 1.9 services per conception.

There are many factors affecting the number of services required for conception. Bulls are sometimes temporarily sterile or lowered in potency, the genital tract of the cow may be diseased, or an abnormal estrus cycle may result in lowered breeding efficiency. As a rule cows are far more responsible for breeding failures than any impotency of the sire. Irrespective of the method used, there is always a small percentage of cows that will not breed, because of some genital malformation or lack of coordination in the reproductive functions. These factors should be borne in mind at all times whether artificial or natural breeding is being practiced.

The typical results in a number of Missouri herds, artificially bred, are presented in Table 2.

TABLE 2.—RESULTS WITH ARTIFICIAL BREEDING—MISSOURI DAIRY HERDS.

Herd No.	No. of cows Inseminated	Services	Cows settled	Average services per conception
1	14	18	14	1.28
2	7	24	6	4.00
3	41	71	40	1.80
4	5	16	5	3.20
5	64	108	62	1.70
6	12	19	12	1.57
7	7	14	7	2.00
8	5	17	5	3.40
9	5	9	5	1.80
10	7	24	7	3.40
11	5	13	5	2.60
12	4	11	4	2.70
13	5	19	5	2.75
14	7	12	7	1.70
15	7	14	7	2.00
16	5	6	5	1.20
17	6	13	6	2.10
18	9	22	9	2.40
19	6	11	6	1.80
20	6	17	6	2.80
21	7	11	6	1.80
22	52	73	52	1.40
23	55	80	53	1.50
24	50	95	48	1.90
25 to 40 miscellaneous herds with 2 to 15 cows each	126	202	121	1.60
Total and average	517	919	503	1.80

It will be noted that in some 40 herds for which complete records are available, that 517 cows were inseminated and 503 became pregnant with an average service rate of 1.8 per conception. This rate would be materially lower had some of the "shy breeders" and those which failed to settle been eliminated from the averages. The circumstances under which artificial insemination was started in several of these

herds (Nos. 2, 4, 8, 10, 13, 20, 3 and 5) all a part of the Farm Security Administration project at Hughesville, Missouri, were none too favorable for establishing a very efficient record the first year. Practically all of these herds were assembled by random purchases just before the work of artificial insemination started. All of the cows were Bang's disease free, but in some cases had been open a long while, and in practically every instance the previous breeding history was lacking. With additional information accumulating in each herd a much better breeding record is being compiled for the year of 1939. Herds 22, 23, and 24 are all large breeding herds, free of disease, using mostly aged sires, but well managed and with very complete and accurate records kept on each cow. The greater breeding efficiency in these herds is quite apparent. Complete and accurate records regarding heat dates, breeding, calving, etc. are invaluable in an artificial breeding program.

COOPERATIVE DAIRY CATTLE BREEDING ASSOCIATIONS

Artificial breeding has its greatest possibilities where a large number of cattle are located in a small area. Community farm projects or organizations of small breeders offer the best opportunities. This method will not entirely replace the herd sires on the larger dairy farms, but it will extend their use. It may, however, reduce the expense of breeding in the small herd of approximately 6 to 10 cows. It normally costs approximately $75.00 per year to maintain a bull and this, plus the purchase price, results in a rather high service fee. Experience with artificial breeding indicates the cost per service may be expected to range within the approximate limits of $2.00 to $5.00 per cow.

Cooperative breeding associations, using artificial insemination as a means of furthering the use of outstanding dairy sires, as well as proving young bulls, are being formed in various parts of America. The best use of artificial insemination in dairy cattle will probably be realized by members of such organizations. To successfully operate an organized artificial breeding association, there must be sufficient members and cows included to insure the project receiving the income necessary to pay the cost of the sires used, hire and provide transportation for a well trained man, and provide for such equipment and laboratory facilities as are needed. There is a tendency for breeders to attempt the organization of breeding associations involving the use of artificial insemination, with too little regard for the hiring of a technically trained man, and the setting up of the organization on a sound business basis with sufficient cattle in a

given locality to be bred to insure the necessary finances for successful operation.

Some general recommendations concerning the organization and operation of an artificial breeding association for dairy cattle are:

1. Have at least 1000 cows, preferably of one but not more than two breeds, located within a radius of 15 to 20 miles, before starting the organization.

2. Three good sires will be needed. At least one and preferably two should be proved. These bulls should all be kept at one centrally located place.

3. Set up a well organized association with a president or chairman, secretary, treasurer, and board of directors, all of whom are cooperators.

4. Have an entry fee for all cooperators payable in advance so as to purchase the necessary equipment.

5. Set the service fee sufficiently high to cover all costs and leave a reasonable balance in the treasury.

6. The officers and board of directors should hire a well qualified, technically trained man, a veterinarian if possible, to collect the semen and inseminate the cows.

7. Adhere to a strict program of treating all cooperators alike. Do not allow special breeding privileges and use each bull in the scheduled rotation.

8. Collect for each cow inseminated as soon as the job is finished. Do not allow members to become delinquent. As a rule a cow may be inseminated three times, and if not then pregnant special charges must be made for additional inseminations.

9. Set up a constructive breeding program, perhaps involving some line-breeding, for the entire project.

10. Provide for proper feeding, handling, and management of the bulls.

11. Advise members concerning all matters of importance and acquaint them with the fact that they must not expect much better results than with natural breeding. Also stress the fact that both bulls and cows vary in their breeding efficiency and that all cows will not settle with the first insemination.

12. Do not use make-shift methods nor practice short-cuts in organization.

REGISTRATION OF PUREBREDS RESULTING FROM ARTIFICIAL INSEMINATION

The plan by which purebred dairy cattle breed associations accept signatures and proof as to the identity of the semen used and the cow inseminated, where animals not on the same farm are mated, has been tentatively worked out. Special forms, one to be filled out by the owner of the bull from which the semen is obtained and the other by the owner of the inseminated cow, have been devised. Every purebred breeder who uses semen obtained from bulls not owned by him and located on another farm, should obtain a supply of these forms from his respective breed association and follow the advice of the association in reporting such matings. Where cattle are artificially inseminated on the same farm where the bull is kept, even though he may be leased or cooperatively owned, and in purebred bull associations or cooperative breeding associations owning or leasing bulls, the procedure in registration is now no different than where natural breeding is followed.