ARTIFICIAL INSEMINATION of DAIRY CATTLE

H. A. HERMAN and A. C. RAGSDALE

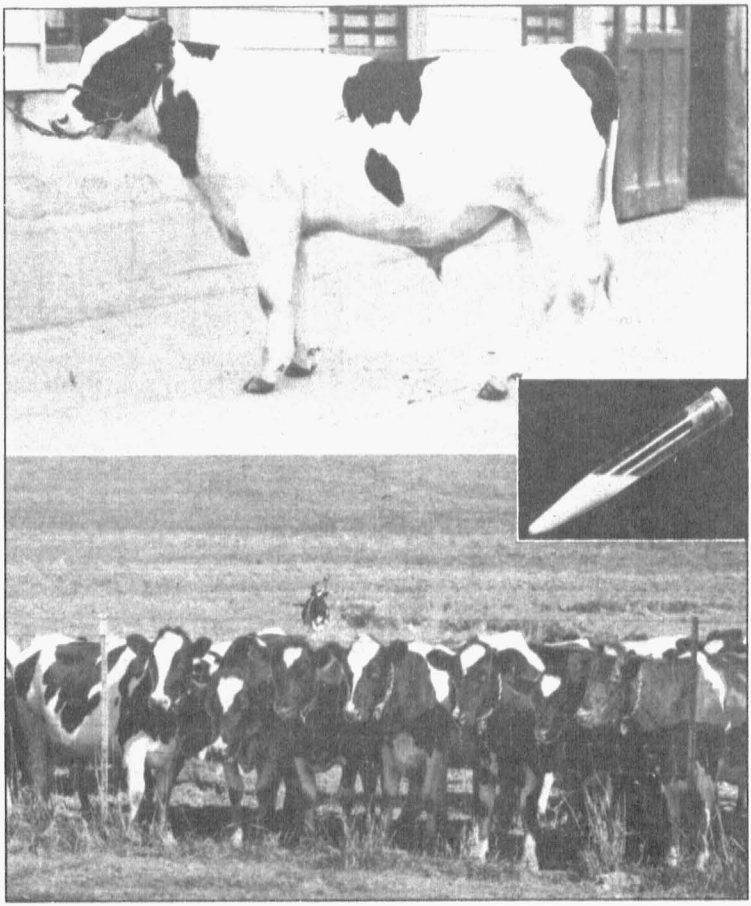

Fig. 1.—The first calf resulting from artificial insemination in the University of Missouri dairy herd. Dropped April 18, 1938. Photograph taken at 10 months of age.

Fig. 2.—Holstein, Jersey, and Guernsey heifers in the University of Missouri herd produced by artificial insemination. Photographed November 1, 1945.

Bulletin 494 *January 1946*

University of Missouri College of Agriculture
Agricultural Experiment Station

E. A. TROWBRIDGE, Director

THE DEVELOPMENT OF ARTIFICIAL INSEMINATION

The first authentic account of the use of artificial insemination was by the Italian, Spallanzani, who successfully inseminated a bitch in 1780. There is, however, some evidence that the Arabs used artificial insemination as early as the Fourteenth Century. Much of the present stimulus for artificial breeding of dairy cattle traces to developments in Russia where as early as 1909 this method was used on mares. About 1928 the Russians began inseminating cattle and by 1938 they were thus breeding some 1,500,000 head. In 1937 a large cooperative association was organized in Denmark.

The first artificial breeding association in America was organized in May, 1938, at Clinton, New Jersey. Additional units were added until in 1945 six associations with 1700 members had 15,000 cows enrolled. A recent survey shows that there are now about 250 dairy cattle artificial breeding associations in the United States. These are operating in practically every state and are made up of over 30,000 members, owning about 300,000 cows which will be inseminated with semen from some 900 bulls.

The first association in Missouri was organized in Pettis County, June, 1938, when the Farm Security Administration at Hughesville, Missouri, and the Department of Dairy Husbandry, University of Missouri, set up a cooperative project whereby artificial breeding was provided for the cows on two large cooperative farms and approximately 30 individual farms located within a 10-mile radius of Hughesville. This project included about 500 cows and operated until 1944.

The Midwest Breeding Farms at Trenton (successor to Herndale Farms, Fayette, which began operations in April, 1939, with horses, adding dairy cattle in the spring of 1940) now have the oldest and largest artificial breeding organization in the state with 12 to 15 bulls providing service for 10,000 cows.

The third major association was organized at Carthage, Missouri, with the support of the Chamber of Commerce and private funds. It is now breeding more than 4,000 cows annually.

In October, 1945, the Missouri Farmers' Association, through its Producers Creamery and the M. F. A. Milling Company at Springfield, launched an artificial breeding project to service the cows of its 5500 patrons. This project calls for the use of 25 to 30 dairy sires and is expected to service 15 to 25 thousand cows annually with operations beginning in January, 1946.

Several smaller associations operating in Southwest Missouri during the past few years have been taken over by the Missouri Farmers' Association project.

Artificial Insemination Of Dairy Cows

H. A. HERMAN and A. C. RAGSDALE

Artificial insemination was first used in the University of Missouri dairy herd July 15, 1937. The calf dropped to this service, U-Mo Aaggie Sultan Valiant, 772584, born April 18, 1938, is shown on the cover page. Since that time this breeding method has become widespread in Missouri with about 20,000 cows in the state so bred in 1945. It now seems likely that 40,000 to 50,000 cows will be serviced by this method in 1946. Artificial insemination is defined as the mechanical introduction of semen from the male into the genital tract of the female in contrast to the natural introduction by the male.

Breeders cooperating with the Department of Dairy Husbandry have supplied records showing over 5,000 cows settled in calf with an average service rate of 1.8, which compares very favorably with the results from natural breeding. Studies of the breeding efficiency of the Missouri Experiment Station dairy herd covering a 30-year period and involving the use of over 40 sires show an average service ratio of 1.6 services per calf. Beginning in February, 1938, for a two-year period the herd was divided into two groups, one group bred by means of artificial insemination and the other by means of natural service. During this period, in spite of many aged proved sires in use, 246 cows bred artificially required 1.81 services per conception, while some 300 cows bred by means of natural mating required 1.76 services per conception.

Artificial insemination has proved helpful in increasing the use of outstanding sires, reduced the number of sires kept, and assisted in controlling breeding troubles in over fifty of the largest dairy herds in Missouri. In these herds the technical work is all performed by experienced herdsmen. In some cases long distance matings of exceptional animals have been made and the entire breeding program of such herds materially improved.

The service charge for artificial inseminations is usually $5.00 to $7.00 and provides a maximum of 3 services. Where cows require additional services proportionate increases in costs are made.

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 *epididymis*, which extends down the outside of the testicle to its base. The epididymes provides 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. 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.

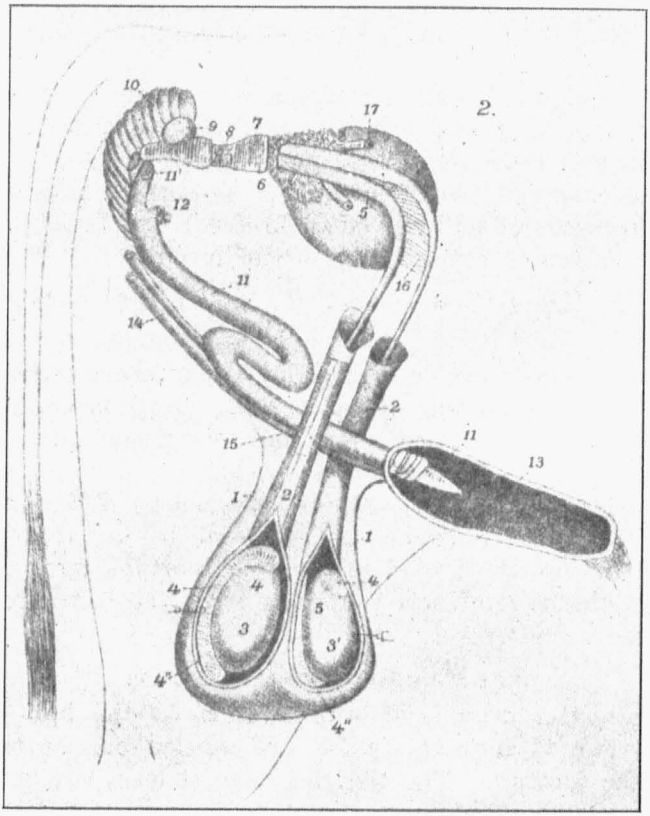

Fig. 3.—Reproductive and urinary organs of the bull. 1, scrotum; 2, spermatic cord; 3, testicle; 4, epididymis; 5, vas deferens; 6, vesicula seminals; 7, membranous portion of urethral canal covered by Wilson's muscle; 8, part of prostate gland covered by Wilson's muscle; 9, Cowper's gland; 10, accelerator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

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.

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.

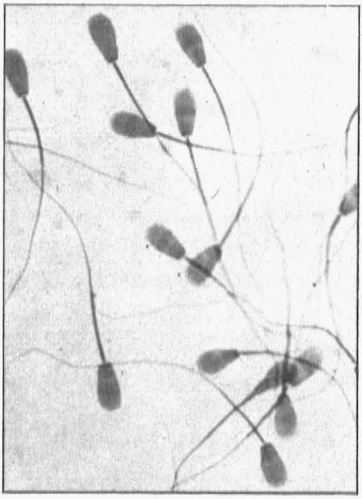

Fig. 4.—Spermatozoa of the bull (highly magnified).

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 centi-

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

meters. 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.

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. 5.

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.

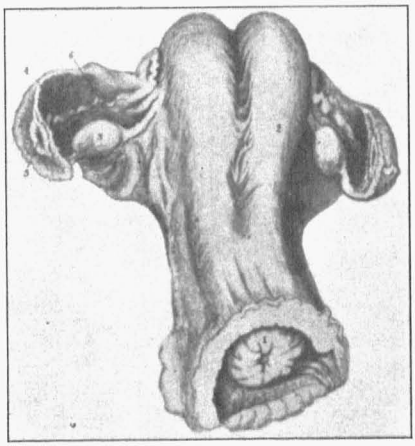

Fig. 5.—Reproductive Organs of the Cow. 1, os uteri; 2, right horn of uterus; 3, ovary; 4, ovarian ventricles; 5, fimbriated end of Fallopian tube; 6, Fallopian tube (from "Diseases of the Genital Organs of Domestic Animals", Williams).

The 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.

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.

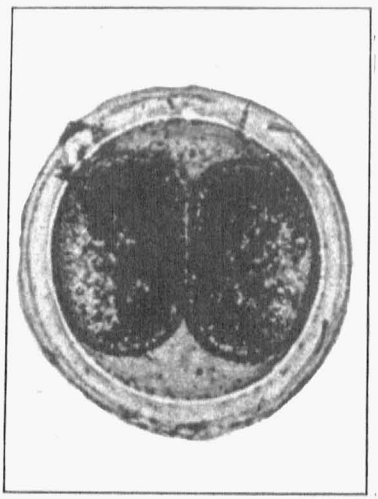

Fig. 6.—Two-cell stage of cow's ovum (from Hartman, Lewis, Miller and Swett, Anat. Rec., pub. Wistar Institute).

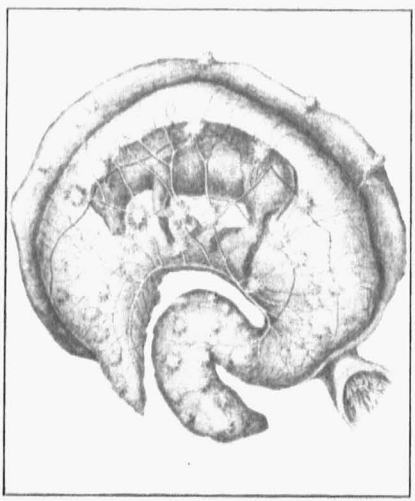

Fig. 7.—Fetal calf within its membranes (from U. S. D. A. Diseases of Cattle).

Figures 6 and 7 show the first division of the fertilized ovum and the subsequent development of the placenta.

Collection of Semen from the Bull

There are at least three recognized methods of 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.

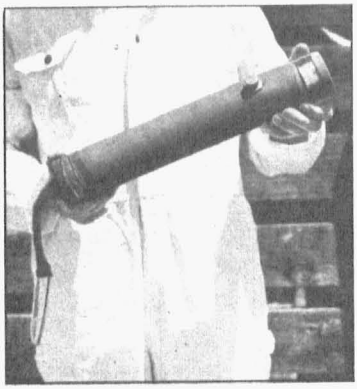

Fig. 8.—The artificial vagina assembled and ready for use. Note the collecting tube.

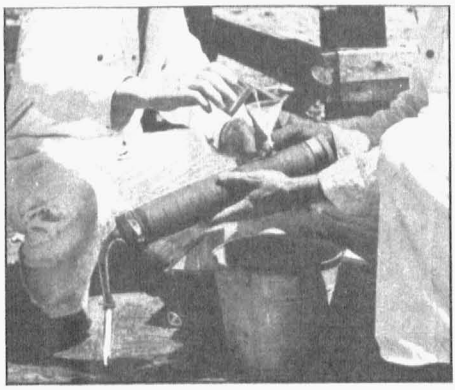

Fig. 9.—Filling the chamber between the rubber liner and the outer sheath of the artificial vagina with warm water.

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.

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, light mineral oil,

or a special lubricant made up of 3 grams gum tragacanth, 5 cc. glycerine, and 50 cc. distilled water and allowed to jell) may be smeared lightly over the lining of the upper one-half of the artificial vagina by means of a large sterile glass rod before the collection is made. Both mineral oil and pure vaseline seem to be non-injurious to sperm, but some lubricants seem to be definitely harmful. Used in small amounts the gum tragacanth mixture gives good results, but should never be used heavily nor placed into the lower half of the vagina. Some operators use no lubricant at all, but some bulls will not serve into a dry artificial vagina.

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". Even a fairly quiet cow not in heat may be used. A "dummy cow" which is constructed by covering a strong steel frame with padding and canvas to resemble a cow may be also used in collecting semen from bulls which have been trained to its use. Some bulls will not mount a dummy. A breeding rack or stocks will facilitate the handling of the bull and collection of the semen where 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 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.

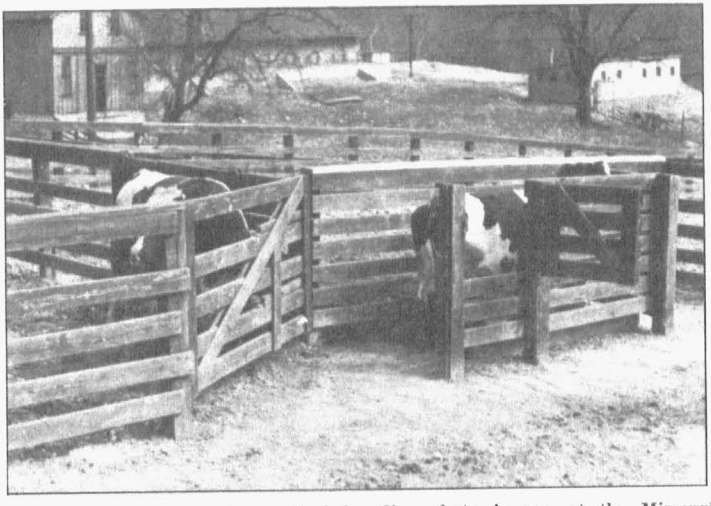

Fig. 10.—A convenient, practical breeding chute in use at the Missouri Station showing the bull and cow in place preparatory of the collection of semen. This chute simplifies the problem of handling old or mean bulls and reduces chances of injury to the operator.

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

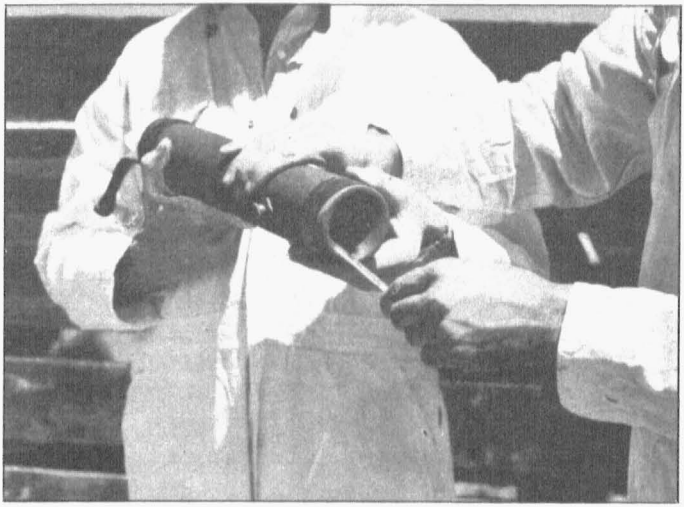

Fig. 11.—Taking temperature of the artificial vagina, which should be 41-43° C. (105-110° Fah.) at the time of use.

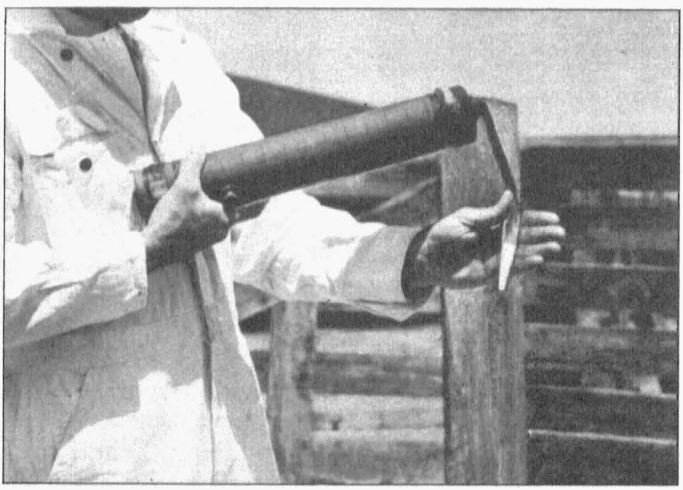

Fig. 12.—A sample of semen, collected, and ready for immediate use or storage.

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.

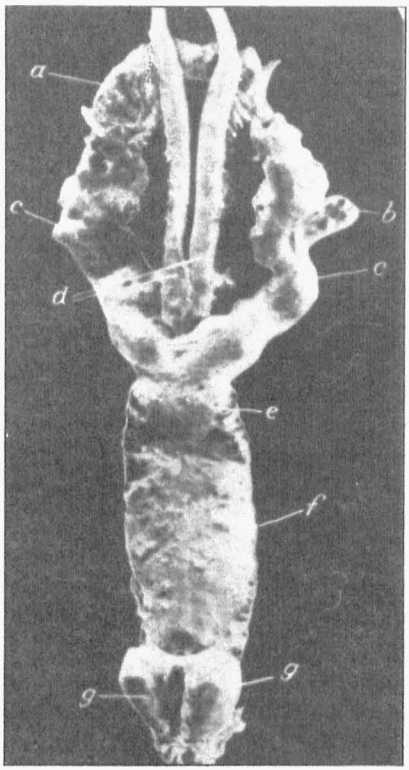

Fig. 13.—Dorsal view of the reproductive organs of the bull—a, bladder; b, ureter; c, seminal vesicles; d, ampullae; e, body of prostate; f, pelvic urethra; g, bulbo-urethral (Cowper's) glands. (From Miller and Evans, Jr. Agr. Res., vol. 48, 1934).

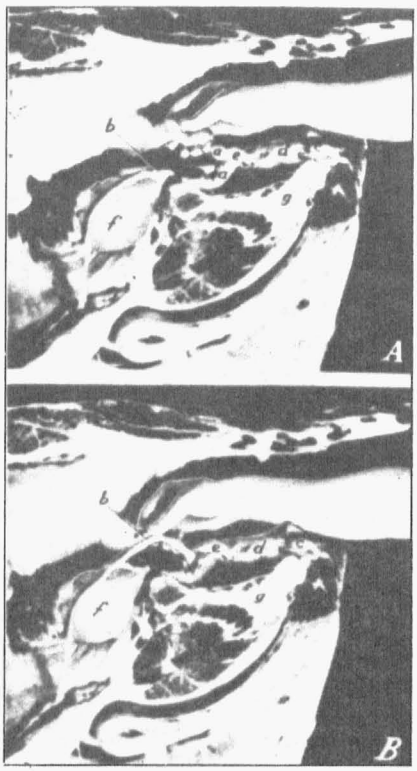

Fig. 14.—Position of the genital organs of the bull and method of manipulating them. A. Massaging the seminal vesicles. B. Massaging the ampullae of the ductus deferens. (From Miller and Evans, Jr. Agr. Res., vol. 48, 1934).

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 inter-

*See Missouri Research Bulletin 326 for a detailed discussion of semen characteristics.

vals 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. One of the most valuable indices, found in studies with dairy bulls has been the length of time semen maintains a high percentage of motile sperm under storage conditions at 35 to 40° F. Last, but not least is the sire's breeding record, or settling rate, which should be carefully followed, irrespective of the physical and chemical tests made on the semen.

1. *Where inseminations are to be made within a few minutes to one-half hour 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 held at room temperature until used.

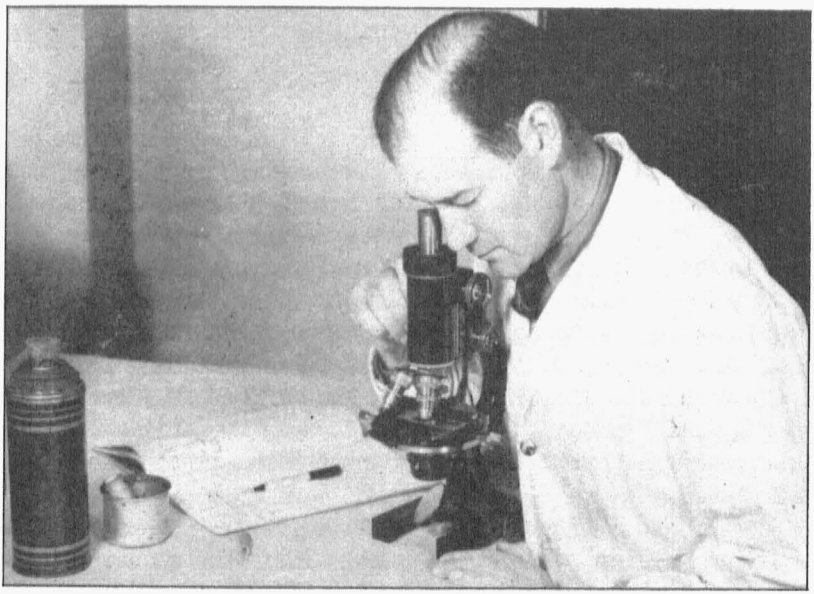

Fig. 15.—Examining a sample of semen for motility and sperm concentration.

2. *Where inseminations are to be made ½ to 48 hours after collection, every precaution should be taken to keep the semen in a high state of viability.* Sudden temperature changes should be avoided and the semen protected from the air. Lowered holding temperatures result in a lowered sperm activity, and is 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 35 to 40° F. If the semen is to be diluted, the diluter fluids should be added as quickly as possible after collection. 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 35 to 40° 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 need not be raised before insemination.

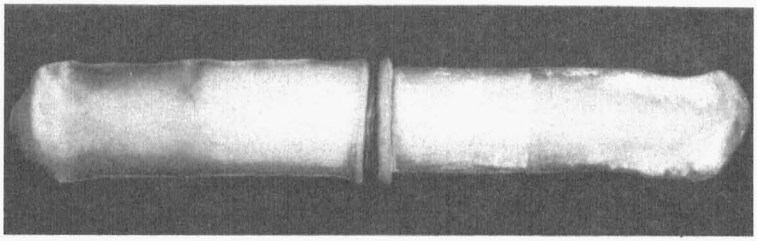

Fig. 16.—A vial of semen wrapped for storage. Two thicknesses of paper are placed about the tube, then a rubber finger stall is placed over either end, drawn together in the middle and held in place by a rubber band.

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 use 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 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.

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 35 to 40° 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.

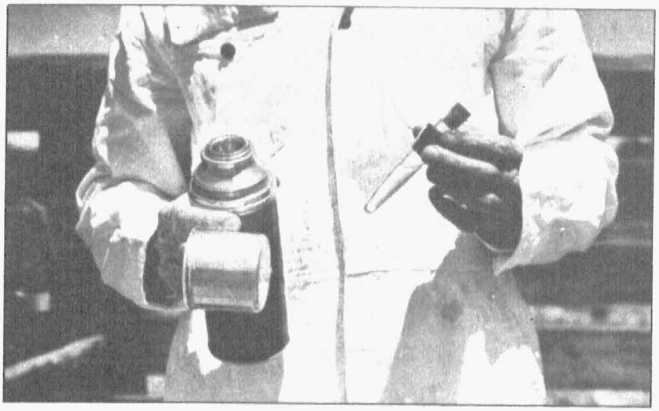

Fig. 17.—A thermos bottle fitted with a bored cork for holding semen vials during insemination.

Diluting Semen

Where only 3 to 6 cows are to be bred immediately from a single ejaculate, it is seldom necessary to dilute the semen. As a rule 1 cc. of undiluted semen is placed in the cervix for insemination. 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. Many diluters have been proposed, but the two now most widely used and with equally satisfactory results are the *egg yolk-phosphate diluter* and the *egg yolk-citrate diluter*. These are prepared as follows:

Egg Yolk-Phosphate Diluter.—To 100 cc. of boiling distilled water add 0.2 gram KH_2PO_4 and 2.0 grams $Na_2HPO_4 \cdot 12H_2O$. Both these chemicals must be of high grade commonly designated as "chemically pure". The solution thus made is termed the "phosphate buffer". To the phosphate buffer add an equal volume of fresh egg yolk and mix thoroughly. The diluter is now ready for use.

Egg Yolk-Citrate Diluter.—An M/15 sterile solution of sodium citrate ($2\ Na_3C_6H_5O_7 \cdot 11\ H_2O$) (47.615 grams per 1000 cc. of distilled water) is made up. A equal volume of the sodium citrate solution thus made up is mixed with an equal volume of fresh egg yolk for use.

The chief advantage of the citrate diluter over the phosphate seems to be that the citrate disperses the fat globules in the egg yolk so that microscopic examination is made much easier. It also seems to maintain a slightly higher motility level in semen stored for long intervals.

Water used in making up diluters should preferably be double distilled over glass. The complete diluter either phosphate or citrate should be made up with fresh egg yolk for every new collection of semen to be diluted. The buffer solutions (citrate of phosphate) may be prepared in fairly large volume and kept for several weeks in a refrigerator, provided no contamination takes place.

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

Rate of Dilution.—Semen may be diluted at the rate of 1:4 or even 1:10, 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 100 to 250 million for each insemination. Some experiments indicate a satisfactory settling rate with even higher dilution rates where only 20 to 50 million sperm are present in the usual 1 to 1.5 cc. of diluted sperm used.

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.

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 1¼ inches in diameter or a trivalve speculum) and a headlight are required.

Before insemination, the vulva and adjacent parts of the cow must be thoroughly cleansed with water. Soap should be used

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.

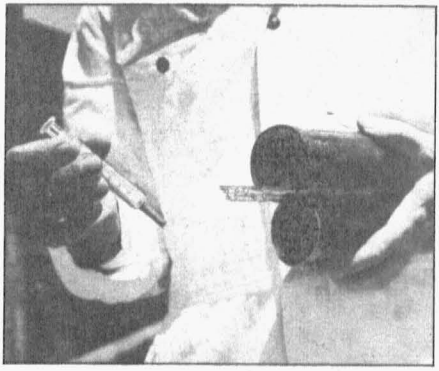

Fig. 20.—A handy metal tube, or cannister, for carrying sterile inseminating tubes.

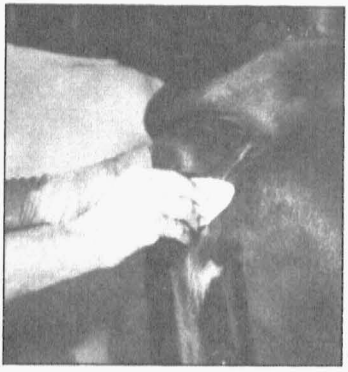

Fig. 21.—Cleaning the vulva of the cow prior to insemination.

sparingly and all of it washed off before inserting the speculum. The speculum is carefully inserted in the vagina. The cervix is then located. Next, about 1 cc. of the semen is drawn into the glass tube by means of the syringe. 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.

Insemination Without Use of Speculum.—Another widely used 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 or even into the horn of the uterus 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 by actual data.

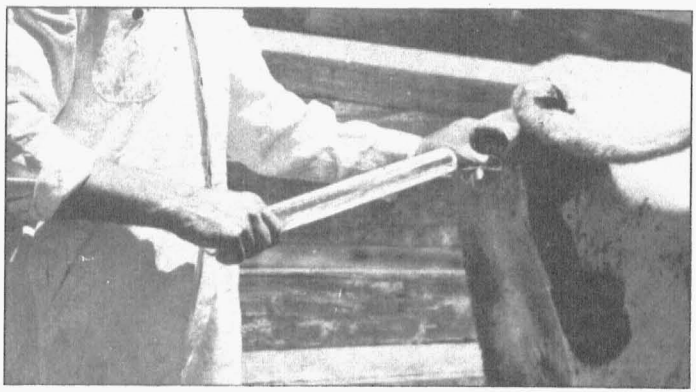

Fig. 22.—Inserting the speculum into the vagina.

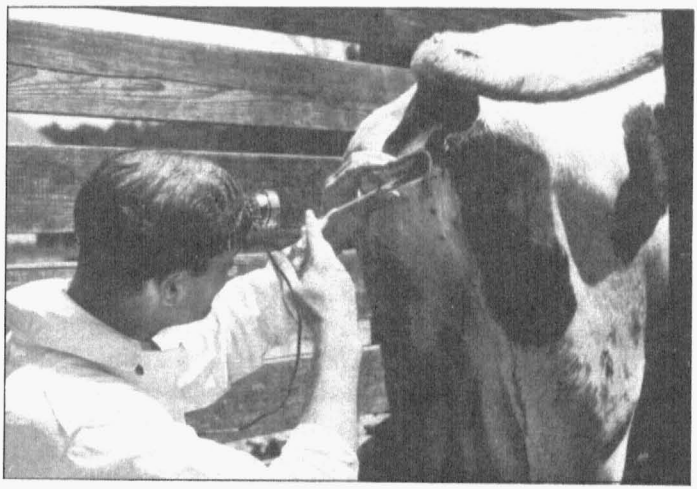

Fig. 23.—Inseminating the cow.

Amount of Semen Used.—In work done thus far by the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, 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. Most American inseminators use 1 to 1.5 cc. of diluted or undiluted semen and there seems to be little occasion for using lesser amounts with the rate of dilution possible.

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

Fig. 24.—Inseminating the cow. The left hand is inserted into the rectum and the cervix grasped so as to permit the introduction of the catheter into the cervix by way of the vagina.

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.

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 while in heat and again 4 to 6 hours after she is out of heat. If but one insemination is used it should usually be

about 6 to 10 hours after the beginning of the heat period or when the cow is in full estrus.

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 and the temperature at which held.

Long distance shipping or holding of semen requires cooling and storing at temperatures between 35 and 50° F. For quick transportation semen may best be shipped *air express*. Suggested directions for shipping semen are:

(A) **Short-time Shipping (up to 12 to 24 Hours).**—Where semen is shipped intra-state as often occurs in organized artificial breeding association work, or between herds several hundred miles apart, a strong shipping box made of corrugated paper or wood may be utilized. Such a box is usually about 6x6x12 inches and is of sufficient size to hold the refrigerant material and the semen containers with the necessary insulation. Such shipping containers have been found satisfactory in Missouri for organized breeding associations. The directions for shipping semen by this plan are:

1. After the semen has been tested for quality, diluted, and cooled to 35-40° F., the amount to be shipped is placed in a properly labeled, sterile vial and sealed with paraffin.
2. The vial is then wrapped in several thicknesses of paper for insulation purposes and placed along side a toy balloon, or small can filled with solidly frozen ice.
3. The vial and ice container are then wrapped together tightly in heavy insulating paper commonly referred to as "Jiffy Wrapper".

4. The wrapped package is inserted into the shipping container, and sufficient corrugated paper, packing, etc., used to hold it firmly in place. The container is then sealed, addressed, and mailed.

(B) **Long-time Shipments (60 to 80 Hours).**—The requirements of long distance shipping are:

1. Adequate refrigeration of the package.
2. Protection of the semen from the intense cold of the refrigerant.
3. Insulation of the refrigerant against the external temperature.

The University of Missouri, Department of Dairy Husbandry has met these requirements by using the small sized, jacketed, insulated shippers employed in shipping ice cream.

The refrigerant used is mainly crushed ice with a can of frozen brine used for extra hot weather. A small vacuum bottle filled with water at 40° F. was used to contain the vials of semen; and commercially available small insulated shipping packages such as are commonly used for ice cream were used as external insulation. These were combined as diagrammed in Figure 25.

A PACKED SEMEN SHIPPER

The cooled or partially cooled semen in an insulated, rubber-wrapped vial is placed in a vacuum bottle of water at 40° F. A few strands of excelsior in the bottle prevent undue bobbing of the vials during transit. The vacuum bottle, with supporting wires to position it at the center, is put into a metal can and chipped ice is packed around it as shown in figure 25. The can is sealed and placed in the small ice cream shipper. For extremely hot weather a pad of frozen brine may be placed on top of the can containing the cracked ice and thermos bottle in which the semen vial is carried.

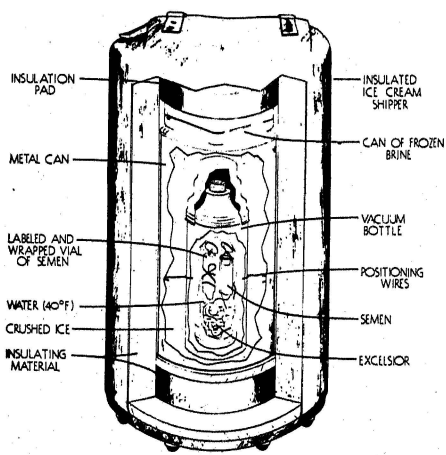

Fig. 25.—A cut-away diagram of the packed semen shipper.

Twenty-four shipments of semen have been made by this method in the course of twelve months' investigations. When

the proper precautions as outlined above have been observed, the shipments have been successful in delivering semen with motility as good or better than that obtained from the same semen stored at 40° F. in a refrigerator. This shipper has maintained temperatures of below 50° F. for 84 hours at midsummer temperatures and in one study involving shipments to the New Jersey Agricultural Experiment Station, 20 calves from 42 routine inseminations have resulted.

For transportation by car, or short distances by bus or rail, semen may also be shipped by placing the insulated vials in a thermos bottle filled with cracked ice. The thermos bottle should be well packed in a large container to prevent breakage. The ordinary thermos bottle, however, will not stand the rigors of shipping and one of the above methods is best if routine shipping is practiced.

Semen has been kept alive at a temperature of 40 to 45° F. for over 20 days, but all is not yet known regarding the fertility of stored semen. English workers report successful impregnation with semen 96 hours old. The writers have successfully settled many cows using semen over 48 hours old. In one case a 198 hour old sample produced a calf.

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. Bulls vary greatly in the ability of their semen to survive long shipment and storage. Some bulls cannot be successfully used to produce semen for this purpose.*

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 no chemical changes when in contact with body fluids, and is easily cleaned and sterilized. The equipment normally used is shown in Figure 26.

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.

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

*See Missouri Agricultural Experiment Station Research Bulletin, No. 326.

glass speculums 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.

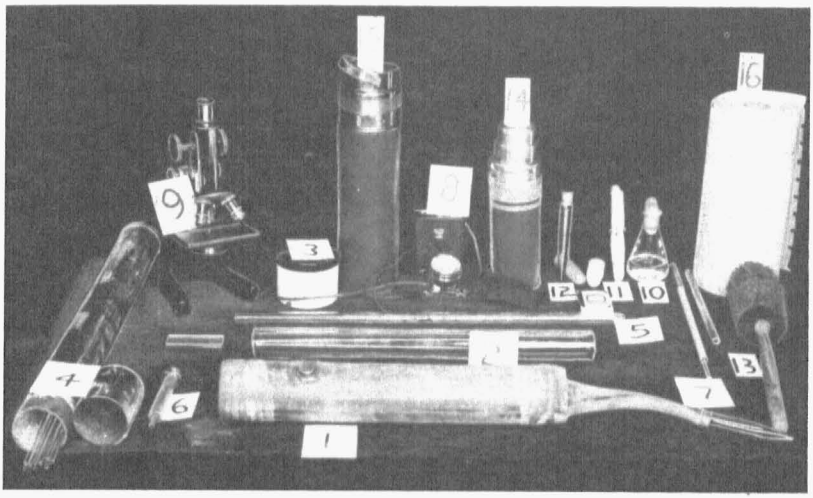

Fig. 26.—Equipment used for artificial insemination of dairy cattle: (1) assembled artificial vagina and collecting vial; (2) heavy walled glass speculum; (3) lubricating material; (4) metal tube for carrying sterile inseminating tubes or pipettes, 3/16" diameter, 1/8" bore, about 20-22" long, for removing 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 speculum; (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.

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. A summary of over 5000 breeding records from

Missouri dairy herds depending on artificial insemination indicate that 1.8 to 1.9 services are required.

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.

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. 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.

DAIRY CATTLE BREEDING ORGANIZATIONS

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 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 $125.00 per year to maintain a bull and this, plus the purchase price, results in a rather high service fee. Experiences with artificial breeding indicates the cost per service may be expected to range within the approximate limits of $5.00 to $7.50 per cow.

There are two common types of artificial breeding organizations. These are (a) *local artificial breeding organization* and (b) *central or federated breeding organizations.*

The local artificial breeding organization is most commonly a cooperative association although the local organization may be privately owned and operated. Creameries, feed companies, or other organizations may sponsor or assist in the financial backing. *To successfully operate any artificial breeding association* there are certain problems of organization, operation and management which must be met satisfactorily. The most important of these with special reference to local organizations are:

1. A survey and careful analysis should be made of the area from the standpoint of available cattle, interest in herd improvement, and general adaptability to dairy farming. Hard surface roads and adequate telephone facilities are necessary to the efficient operation of a breeding association.

2. The territory should have 1000 to 1200 cows preferably of one and not more than two breeds available for artificial service. These should be located in an area with a radius of not more than 15 to 20 miles.

3. A broad educational program should precede organization and beginning of operation. The advantages and shortcomings of artificial breeding should be fully explained.

4. The association, if cooperative, should be properly organized and incorporated with a president, or chairman, secretary, treasurer, and board of directors, all of whom are cooperators.

5. The financial set-up must be sound:

a. There should be a sign-up of all cooperators with a membership fee of $5.00 to $10.00 payable in advance so as to purchase necessary equipment and provide a working fund.

b. The service fee, usually $5.00 to $7.00 should be set sufficiently high to cover all costs of service.

c. 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 insemination.

d. Complete and accurate records, kept on standard forms approved by the association should be kept.

6. Only trained inseminators or technicians with an intelligent understanding of work, and sympathetic toward the problems involved, should be employed.

7. Three or more good sires will be needed. Include at least one proved sire. These bulls should all be kept at one centrally located place. All sires should meet rigid standards from the standpoint of production and type and should preferably be approved by the State Agricultural College before purchase.

8. All sires should be free from T. B., Bang's Disease, Trichomoniasis, and produce viable semen capable of withstanding storage.

9. Provision for adequate housing, feeding, and handling of sires.

10. Establishment of proper laboratory facilities for the evaluation, dilution, storage, and shipment of semen, and the sterilization of equipment.

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

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

13. 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.

14. Do not use make-shift methods nor practice short-cuts in organization and management.

The central or federated organization is one operating in an area of several to a dozen or more counties. It may be *cooperative* or *privately* fostered. In this type of organization all of the activities diverge from one central location or headquarters. Such an organization may keep from 10 to 30 or more sires and serve from a few counties to one entire state if transportation facilities for semen are adequate and other factors favorable. This type of organization is operating successfully in many states including New York, Wisconsin, and Missouri. The central or federated organization is often the result of *merging several local associations*, with a resultant improved financial condition. Only one set of buildings for housing sires, one laboratory, one office and accounting set-up are required.

The semen used in all local areas is produced at the central bull barn or stud and mailed, sent by bus, or other suitable means to the technician in the local association who in turn inseminates the cows in his area. This type of organization is particularly suited to the expansion of an artificial breeding program. As new areas sign-up 300 to 600 cows it is often possible to align them with the central organization using a technician working part time while gradually building up the number of cows to 1000 or more when a full time technician will be needed.

BARN BREEDING RECORD

A barn breeding record form has been developed by the Department of Dairy Husbandry of the Missouri College of Agriculture for use in herds practicing artificial insemination. It should be posted in the barn and used to record every heat date, insemination date, and the other information indicated for each cow. Where a herd is experiencing breeding troubles it is easy to tell at a glance whether the cows are coming in heat regularly (every 18-24 days) and also when cows may be expected in heat. This latter point is very important as many patrons fail to observe cows in heat and report same promptly to the technician. This form may be obtained from the Department of Dairy Husbandry, University of Missouri, Columbia, Missouri.

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 breed association and follow the advice of the association in reporting such matings.

In the case of regularly organized dairy cattle breeding associations, where the organization and its technicians are approved by the College of Agriculture, Director of Agricultural Extension or his representative, a special triplicate form is used for recording the insemination. One copy goes to the herd owner, who files it with the breed association when he makes application for registry of the resulting calf, one copy goes to the breeding association secretary and the other copy is retained by the inseminators. 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 owning or leasing bulls, the procedure in registration is now no different than where natural breeding is followed.

(name and address of breed association)

CERTIFICATE OF COLLECTION OF SEMEN

This is to certify that _____ took semen

from the bull _____ Herd-Book No. _____

owned by _____ on _____
(date)

The semen was placed in a container which was sealed and labeled as follows: _____

_____

and shipped or delivered to _____
(name and address)

Signed _____
(by owner of bull or in owner's name by authorized agent)

Address _____

CERTIFICATE OF ARTIFICIAL INSEMINATION

This is to certify that _____ inseminated the

cow _____ Herd-Book No. _____

owned by _____ on _____
(date)

with semen from container marked as follows: _____

and certified to be from the sire (name and no.) _____

Signed _____
(by owner of cow or in owner's name by authorized agent)

Address _____

Signature of witness of insemination _____

IMPORTANT—This form must be forwarded to the breed association office within 7 days after the cow is inseminated if the resulting calf is to be registered. (This form is not required where the owner of the bull is also the owner of the inseminated cow, but is required wherever the owner of the bull is not the recorded owner of the cow.)

SAMPLE BUDGET - LOCAL ARTIFICIAL BREEDING ORGANIZATION

Capital investments include such items as - cost of bulls, equipment, a laboratory, buildings, paddocks, land, etc. The necessary funds are usually provided by membership fees, assessments per cow, and interested individuals, companies, and organizations.

Operating costs include such items as - salaries, labor, travel expense, feed, bedding, laboratory supplies, semen shipping costs, office, telephone, insurance, reserve for bull replacements, depreciation of the physical plant. Experience has shown that rigid rules should be established relative to the payment and collection of all fees.

Sample Budget for the first year

	Minimum		Maximum	
Capital Investment:				
100 memberships at $5.00 to $10.00	$ 500.00		$1000.00	
Assessment 1000 to 1200 cows at $1.00	1000.00		1200.00	
Operating Income:				
Breeding fees for 1000 to 1200 cows at $6.00	6000.00	$7500.00	7200.00	$9400.00
Capital Costs:				
Three sires	$1050.00		$1800.00	
One young sire for reserve	--------		300.00	
Laboratory and equipment	400.00		600.00	
Buildings and land purchased or leased	--------	$1450.00	--------	$2700.00
Operating Costs:				
Salary of technician	$2000.00		$2400.00	
Travel expense of technician	800.00		900.00	
Managerial expense, travel, etc.	200.00		200.00	
Labor, feed, bedding, veterinary, laboratory, office, telephone, water, light, heat, laundry, miscellaneous supplies, insurance, etc.	1500.00		1800.00	
Depreciation on bulls (33 1/3 %)	350.00		600.00	
Reserve fund	350.00		400.00	
Depreciation or rental on buildings and land	--------	$5200.00	--------	$6300.00
TOTALS		$6650.00		$9000.00

After the first year capital expenses will be less but the income from membership fees and assessments will decrease. The above budget is suggestive only.

BREEDING RECORD--DAIRY CATTLE

(Artificial Insemination)

Department of Dairy Husbandry - Missouri College of Agriculture

Herd Owner _____ Address _____

Post this card on Barn wall

Cow's Name & Registry No.	Ear Tag & Tattoo	Date in Heat	Date Insemi-nated	Due to Calve	Sire Used	Condition of Vagina & Cervix	Remarks & Insemi-nated by
		1					
		2					
		3					
		1					
		3					
		1					
		2					
		3					
		1					
		2					
		3					

Note to Herd Owners: (a) List each heat date for all animals whether they are inseminated or not.

Note to Technicians: (a) List every insemination and proper sire for each cow — identify purebred cows.
(b) Record observations on condition of vagina and cervix also note abnormal heat periods.

The Requirements for Recognition as an Approved Artificial Breeding Organization*

Records must include: Identification of the cow, when bred, by comparison with color markings or tattoo number shown on registration certificate.

Proper labeling of the semen used must provide: 1. Recording of name and number of sire and date when semen was taken. 2. Accurate labeling of tube containing semen at time of collection and preparation for use.

The original, or white sheet, of the insemination certificate shall be filled out in complete form in indelible pencil and left with the owner of the cow at the time of each insemination.

Permanent herd breeding records must be kept up to date on the association uniform breeding report blanks by the technician and he must have this report when on official duty and keep up to date records of all inseminations.

Bulls must be registered and ownership recorded in the name of the artificial breeding association or properly on lease to the association. Bulls purchased or leased by association, when in and out of service, must be reported to the designated college representative within ten days, stating the time acquired or taken from headquarters.

The records and procedure shall be open at all times for inspection by the designated college or breed association representatives.

*(Adopted by the American Dairy Science Association for cooperative associations June 24, 1943)