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Artificial Insemination of Dairy Cattle

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The great increase in the use of artificial insemination for dairy breeds of cattle during recent years has been fully justified by the satisfactory results achieved through thorough study of, and careful attention to, proper methods for collection of semen and its transfer to females. Considerable time can now be saved in proving young bulls, and the number of progeny from outstanding herd sires can be increased. The dairymen of this Country can give great help during the present emergency and the rehabilitation to follow by producing more and better dairy cattle through artificial breeding.

Successful artificial breeding of cattle requires training. Those undertaking this work must be familiar with the anatomy and physiology of the reproductive organs of cattle and have a sound understanding of correct sanitary precautions and the numerous opportunities that exist for doing irreparable damage.

The technique of artificial insemination of dairy cattle described in this bulletin has been tested at the Ohio Agricultural Experiment Station and found reliable.

COLLECTION OF SEMEN

EQUIPMENT

The equipment used in collecting semen from a bull is shown in figure 1.

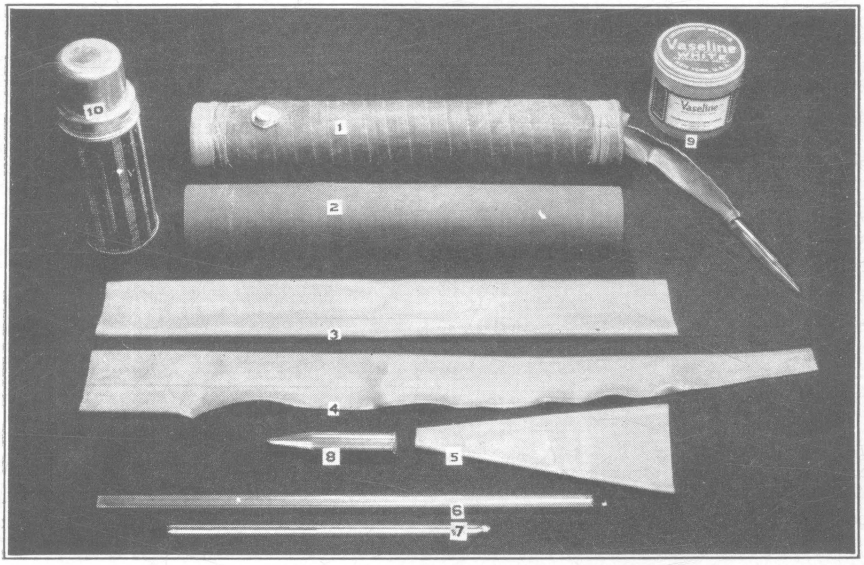

Fig. 1.—Equipment for collecting semen

1. Artificial vagina assembled with long rubber funnel
2. Outside casing, can be made from a radiator hose, size 2¼ to 2½ inches by 12 to 18 inches, with ½-inch water inlet through casing 4 to 5 inches from one end
3. Rubber inner liner of the water jacket. Size, 3 inches flat width by 18 to 22 inches long, depending on the length of the outside casing. This tube is placed inside the heavy hose or casing with each end folded over the corresponding end of the casing to form the water jacket. Rubber bands placed around each end of the water jacket hold the rubber tube in place.
4. Rubber funnel. Long type is placed inside the water jacket. The large end of the funnel is folded over one end of the water jacket. A small glass tube or vial (8, fig. 1) is then fastened in the small end of the funnel for semen collection.
5. Rubber funnel. Short type, can be used instead of long type. With short type, the wide end of the funnel is slipped over one end of the water jacket.
   Construction of the long funnel.—A piece of rubber band tubing material 3 inches wide (flat) by 20 to 26 inches long is cut in the shape of a funnel beginning 4 inches from one end (4, fig. 1). The small end of the funnel should be ⅝ inch flat width when completed. The cut edges are vulcanized together. About 8 inches from the small end a small slit is made through the rubber to allow air to escape during ejaculation. This rubber band tubing material can be purchased in desired lengths 3 inches wide (flat) from the B. F. Goodrich Co., Akron, Ohio, when production of such rubber items is possible. Advantages of the long funnel over the short (5, fig. 1) are: simplified cleaning; reduced expense of equipment, as a used funnel is easily exchanged for a sterilized dry one without a change of water jackets; protection of rubber equipment; allowance for increase or decrease of artificial vagina resistance for individual bulls without alteration of construction of the water jacket; and prevention of loss of semen resulting when an end of the water jacket is accidentally forced open or broken at the time of ejaculation.
6. Glass rod, size ⅜ or ½ inch by 20 inches, used to apply white vaseline or petroleum jelly to the inside surface of the vagina for lubrication
7. Thermometer, long type
8. Small pyrex glass tube or vial, capacity about 15 cubic centimeters, the semen collector
9. White vaseline or petroleum jelly
10. Thermos bottle for keeping semen at constant temperature and away from sunlight during transportation. It should have an open-center stopper in which the tube of semen is firmly held.

Equipment which cannot be secured locally can usually be purchased through local veterinarians.

PREPARATION OF ARTIFICIAL VAGINA

Assemble the artificial vagina and reinforce all connections with extra rubber bands. Cover the glass tube (8, fig. 1) with rubber, cloth, or paper to prevent possible breakage. Fill the water jacket with hot water, 55° C. (132° F.), or hot enough that the inside temperature of the vagina is 41 to 44° C. (105-110° F.) at the time of service. Apply a light coating of vaseline over the inside of the vagina with the glass rod.

Remove about half the hot water from the vagina just before using.

PREPARATION OF BULL

Clip all long hair from the sheath. If the sheath is dirty, wash it with warm water (no soap) and allow it to dry before collecting the semen. Although washing the sheath is generally unnecessary when the bull's stall is adequately bedded, it is essential that it be free of dust and dirt in order that satisfactory semen can be obtained. Every care must be taken to keep semen clean and free from bacterial contamination.

METHOD OF COLLECTION

Allow the bull to mount a cow or another bull, and place the artificial vagina in an inclined position (with the open end lowest) close to the animal's rump. By grasping the sheath properly, the bull's penis can be directed into the artificial vagina. Immediately after ejaculation, the small vial is grasped with the free hand, while the vagina is held in a vertical position with the other. This position allows the semen to drain into the glass tube, where it is protected by the hand from sudden temperature changes and an excessive amount of daylight.

Using a mechanical or dummy cow (fig. 2) for holding the vagina is usually satisfactory.

Construction of a dummy cow and the ideas involved are easily discernible from figures 2, 3, and 4.

The framework (fig. 3) was made from 1¼- and ½-inch pipe and welded together at the joints. Top or back construction is made from wooden pieces.

The framework was covered with several layers of rug felting material before a cowhide was put in place as shown in figure 2.

The front end of the dummy rests on two 8-inch wheels which allow forward motion when the dummy is forced by a bull or moved from one place to another. This end should be chained to a solid object so that it cannot be raised off the ground or floor when a bull dismounts.

The rear end is supported by a low frame and a crossarm which is staked or fastened to the ground. This frame hinges on the rear body permitting forward motion of the dummy when excessive pressure is applied by a bull. A diagonal connecting iron rod and coiled spring arrangement between the front and rear ends keeps the dummy in a rigid position. Building a dummy cow with this hock action feature is not necessary, however.

Figure 4 shows that the support for the vagina, thermos bottle, and glass tube is hinged to the rear frame so that it can be lowered immediately after ejaculation and extra semen can drain into the glass tube. A heavy rubber apron which holds the open end of the artificial vagina in place can be raised or lowered as desired. This apron should be cleaned thoroughly after collection of semen.

Information on other techniques for collecting semen can be found in Lambert and McKenzie's circular (3), "Artificial Insemination in Livestock Breeding."

CARE OF COLLECTED SEMEN

The tube or vial of semen is quickly stoppered with a sterile cork and placed in a thermos bottle which contains enough cool water (28-30° C., 82-85° F.) that the tube will be in contact with the water. Protecting fresh semen in this way eliminates exposure to sunlight and extreme temperature changes,

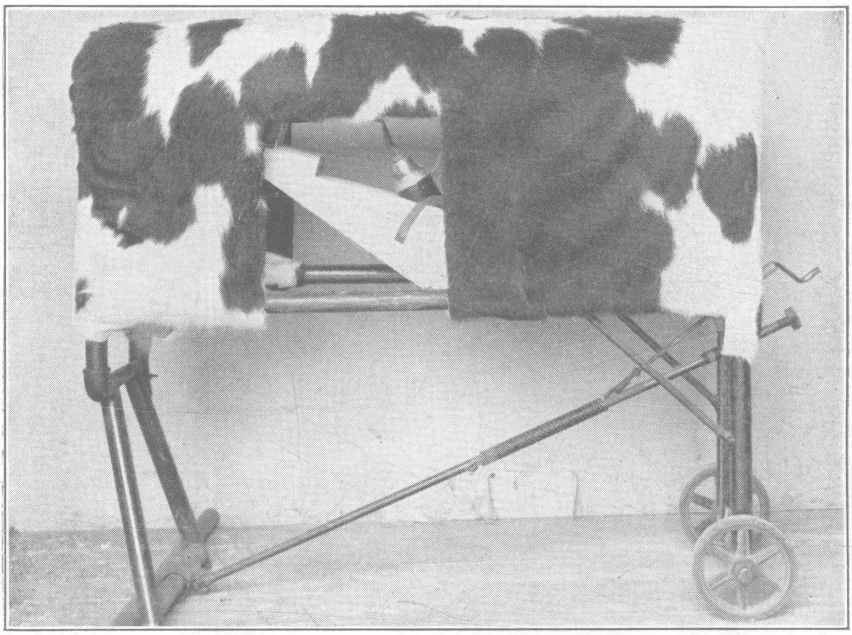

Fig. 2.—Dummy or mechanical cow

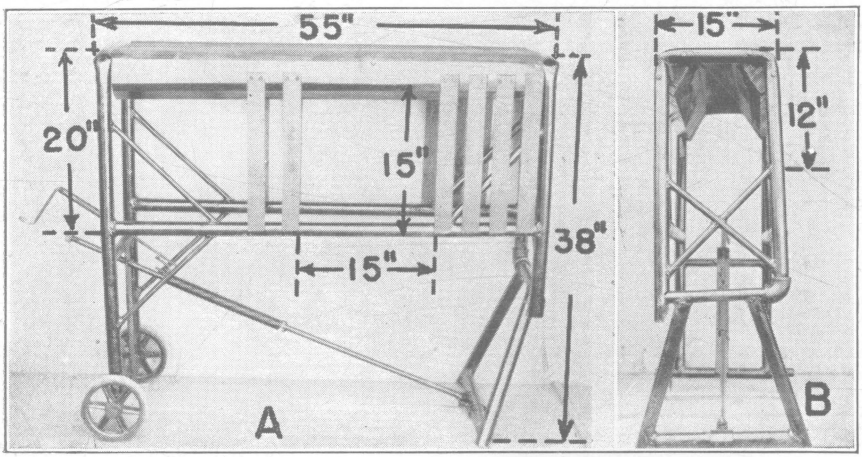

Fig. 3.—Skeleton of the dummy cow. A, side view; B, rear view

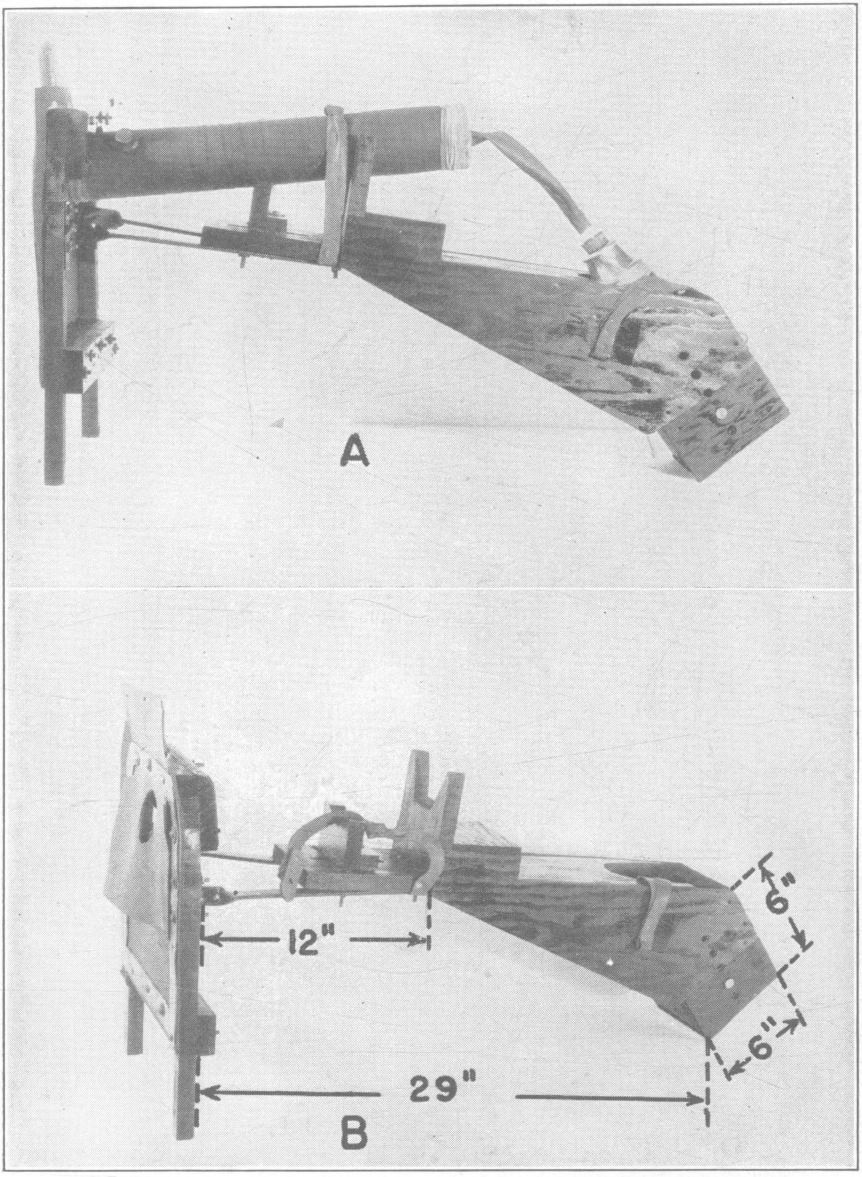

Fig. 4.—Dummy cow support for the artificial vagina and thermos bottle. A, complete; B, support construction

either of which kills sperm cells or shortens their life in storage. Normal semen can be held under these conditions for 1 to 2 hours before it is used for breeding purposes. Holding it for 24 to 48 hours for breeding purposes is possible if certain precautions are taken in storing. These precautions require that it be slowly cooled to 5° C. (41° F.) and held at that temperature until it is used. A simple method of cooling semen slowly is to place the tube or tubes containing the semen upright in a closed pint mason jar that contains ½ inch of cotton and then place the jar and its contents in a mechanical refrigerator which is operating at 5° C. (41° F.).

DILUTION AND STORAGE OF SEMEN

Considerable experimental work has been done on diluents, or diluting mixtures, for semen. It is now possible through their use to breed many cows with one collection and to retain semen in storage in a satisfactory condition for several days. This practice avoids too frequent use of a bull, one cause of sterility.

The best diluents at the present time contain egg yolk, buffer salts, and other materials in water solutions. Directions for using them should be followed closely for desirable results. Diluters do not improve poor-quality semen, and semen of this type is seldom satisfactory for storage (see "Examination of Semen for Quality").

The following suggestions will prove helpful in prolonging a high degree of sperm motility in stored semen.

Preparing the diluents before collecting the semen is advisable in order to avoid unnecessary delay in getting the semen down to storage temperatures.

Egg yolk which is to be added to a diluting material should be freshly prepared from healthy fresh eggs, preferably not over 3 days old. For best results, the eggs should be washed with water, rinsed with alcohol, and allowed to dry before the shell is broken. The egg white is completely removed from around the yolk by holding the yolk in one half of the shell. The yolk membrane is then punctured with a sterile glass rod and the yolk allowed to flow into a sterile container. A mixture of not less than three yolks is best.

The semen is collected and transported in the thermos bottle from the place of collection to the laboratory for dilution.

The temperature of the diluent is adjusted to that of the undiluted semen.

Semen is then added, usually in the proportion of one part semen to three to five parts diluent, and mixed well by gentle whirling.

The diluted semen is slowly cooled as previously explained for undiluted semen (see "Care of Semen").

Suitable containers for storing semen are those which allow portions of the semen to be removed for breeding purposes without contamination of the stored supply. Either one of the following methods will prove satisfactory for this purpose:

1. Divide the supply into small vials so that each one will contain enough semen to inseminate one cow.
2. Put the total amount of diluted semen into one or two large vials (capacity 8 to 16 cubic centimeters) and stopper with thin-centered rubber stoppers. A 3-inch, 16-gauge hypodermic needle attached to a 2-cubic centimeter hypodermic syringe (4, fig. 7) can be forced through the stopper to withdraw diluted semen without contaminating what remains. Wetting the surface of the rubber stopper with alcohol before using the needle is advisable.

EXAMINATION OF SEMEN FOR QUALITY

The artificial breeding of healthy cows with normal bull semen will produce favorable results. Both bull and semen should be pronounced healthy before use in any artificial breeding work. The examination of bull semen, usually conducted under field conditions, includes observation of its volume and physical properties and the motility, concentration, and morphology of the spermatozoa.

APPEARANCE

Physical observations which should be made on every collection of semen are as follows:

1. Volume. A normal ejaculation may range from 1.0 to 10.0 cubic centimeters.

2. Physical properties. Semen should be light creamy to creamy (opaque) in appearance.

Watery semen, as well as that obtained from the first ejaculation of a bull not in service for 12 days or more, should be discarded. Yellowish-colored semen need not be condemned if the yellow color is not due to pus in the semen, because this yellowish semen is normal for some bulls. Pink-colored semen indicates the presence of blood, and it is questionable whether it should be used for breeding purposes. Dirty semen may be dangerous to use for breeding purposes and should be discarded. Allowing a bull to ejaculate more than once into the same artificial vagina will result in contaminated semen.

MOTILITY

Sperm motility is a percentage estimation of the number of active spermatozoa in semen. The initial sperm motility of undiluted fresh semen is determined by covering a small drop with a glass cover slip and examining it under a microscope (×300 to 400) after warming to body temperature. An initial sperm motility of 85 to 95 per cent is considered normal for healthy bull semen. Semen with a sperm motility of 50 to 60 per cent should not be diluted. However, it can usually be used satisfactorily in a fresh undiluted condition.

A more exact sperm motility count in semen can be obtained after the semen has been diluted 20 to 22 times with a clear diluent like that recommended by Milovanov (4), which contains 3.4 grams of sodium sulfate, 3.0 grams of glucose (dextrose), 1.25 grams of Witte's peptone, and 250 cubic centimeters of distilled water. Omitting the peptone from this diluent can be done without harm to the active cells. After the diluted sample is warmed to 39° C. (102° F.), the percentage of living cells can be determined under the microscope (×300 to 400) by counting the number of active cells present in counts of 10 cells each. An average of about five such counts taken from different fields of the microscope will give a fairly accurate motility count.

The ability of sperm to remain active in storage has a definite relationship to its ability to settle cows with calf, even though variations exist between bulls. A satisfactory degree of spermatozoa motility requires that they be vigorously active and swimming more or less in a straight line. Semen of excellent quality usually retains a sperm motility count of 80 to 90

per cent for 4 days or longer under such handling and storing conditions as described. When the sperm motility count of stored diluted semen declines from 90 to approximately 50 per cent after 2 or 3 days in storage, the semen can be classified as poor in quality. Whenever semen of poor quality is obtained, it does not necessarily follow that a bull should be disposed of, but a rather thorough review of management and environment should be made. The production of poor-quality semen is characteristic for some bulls, and others produce semen of poor quality at certain seasons of the year, or under poor management and feeding conditions.

The ability of normal healthy spermatozoa to remain active in storage is demonstrated in table 1. These data include 43 different collections of semen from 3 Holstein and 3 Jersey bulls over a 10-month period from November 1941 to October 1942. This table also shows that the egg yolk-gelatin-buffer diluent (2) maintains a higher degree of sperm motility longer than an egg yolk-buffer diluent (6).

An egg yolk-sodium citrate diluent (8) has recently been developed which disperses the egg yolk fat globules and thereby makes the sperm cells more visible. Adding gelatin to this mixture may make a more desirable diluent for preserving active bull spermatozoa in storage than the egg yolk-gelatin-buffer diluent.

TABLE 1.—Motility of bull spermatozoa after dilution and storage at 5° C. (41° F.) for 14 days

In storage	Semen diluted four times with—			
	Egg yolk-buffer diluent (a)		Egg yolk-gelatin-buffer diluent (b)	
	Average	Range	Average	Range
Days	*Per cent*	*Per cent*	*Per cent*	*Per cent*
2	84	64-90	86	75-90
4	73	42 90	83	66-90
6	62	34-78	77	40-90
8	55	20-80	75	30-90
10	53*	14-74	70*	36-88
12	42	16-70	70	44-85
14	36	6-60	66	42-80

Diluents contained—	(a)	(b)
Fresh egg yolk, cc.	50	50
Distilled water, cc.	50	50
Gelatin (Knox), gm.	—	1.07
Dibasic sodium phosphate (Na_2HPO_4), gm.	.39	.45
Monobasic potassium phosphate (KH_2PO_4), gm.	. 1	. 1

*Many samples were removed because of low motility count resulting from poor-quality semen at time of collection, spoilage, improper handling before or after dilution, etc.

DENSITY

Density or concentration refers to the number of spermatozoa present in semen per unit of volume. The determination is made by diluting 1 part of well-mixed semen 200 times with a 3 to 5 per cent solution of sodium chloride in a Trenner diluting pipette and, after mixing, placing a drop of it on a Levy-Hausser counting chamber and estimating the number of cells according to the general procedure used in making blood cell counts. Such counts are usually stated in terms of the number of cells per cubic millimeter of undiluted semen.

The higher the concentration of cells, the greater will be the opacity of the semen. This characteristic has made it possible to devise rapid methods for estimating the number of spermatozoa present in ram (1), stallion (5), and bull (7) semen. Salisbury and coworkers (7) report that their method of comparing diluted semen samples with opacity standards is slightly less accurate than the direct counts previously explained.

The concentration of normal semen generally varies between 350,000 and 1,500,000 spermatozoa per cubic millimeter. It is not advisable to dilute semen for breeding purposes when it contains less than 300,000 spermatozoa per cubic millimeter. Such semen can be used undiluted if the initial sperm motility is not less than 80 per cent.

MORPHOLOGY

Sperm morphology refers to the physical shape of the spermatozoa as observed under the microscope. In making a preparation for morphological study, a thin smear of semen is made on a clean glass slide. This is accomplished by placing a large drop on a slide and quickly spreading it with an air blast from a rubber bulb fitted with a small outlet. Slow spreading will result in a thick smear, which is undesirable. Spreading semen with another glass slide is also objectionable, because it may damage spermatozoa.

Under the microscope (×400 to 900) the observer should look for tailless and irregularly shaped cells. By simple calculation, the number of abnormal cells per hundred is determined. Unsatisfactory breeding results can be expected with semen containing 25 per cent or more of abnormal cells.

Fixing the smear can be accomplished by air-drying or by immersion in a fixing solution of some kind. A fixing solution is preferable for accurate results. A simple one is methyl alcohol containing a small amount of sodium sulfate and 5 per cent of glacial acetic acid. After ½ to 2 hours, the slide is removed and placed in 95 per cent alcohol for several days, with frequent changing until the acid has been removed. Staining can be done as desired.

Either of the following methods for staining spermatozoa can be used successfully:

1. Staining in chloral hematoxylin 2 to 5 minutes, according to the age of the solution used. After staining, wash well with water and counterstain in a ½ per cent aqueous eosin solution for ½ to 2 minutes. Wash away the excess stain with distilled water and allow to dry.

2. Staining with "fast green" for approximately 2 minutes, rinsing in water, and drying.

Stain formulas:

(a) Chloral hematoxylin:

Potassium alum	8	grams
Distilled water	250	cubic centimeters
Hematoxylin	.2	gram

Boil 5 to 10 minutes in agate or porcelain dish; cool; add 6 grams of chloral hydrate; place in open bottle for 2 weeks before using.

Old chloral hematoxylin solutions generally need filtering.

(b) Eosin, ½ per cent:

Eosin (tetrabromfluorescein-sodium)	.5	gram
Distilled water	.100	cubic centimeters

Place in bottle.

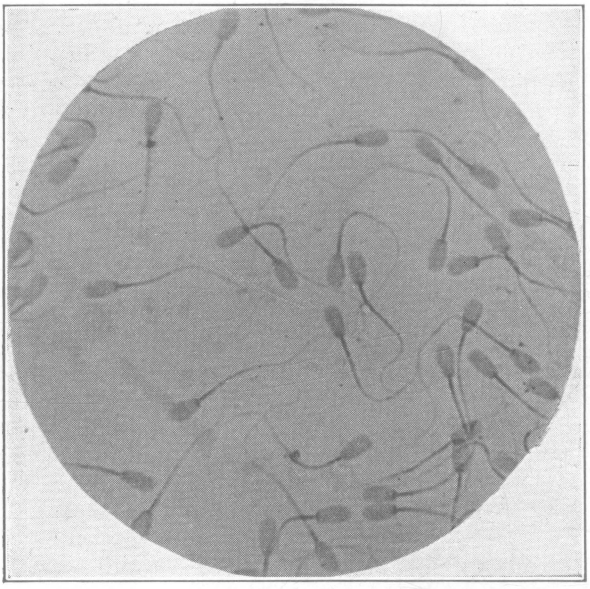

Fig. 5.—Photomicrograph of normal bull spermatozoa (× 600)

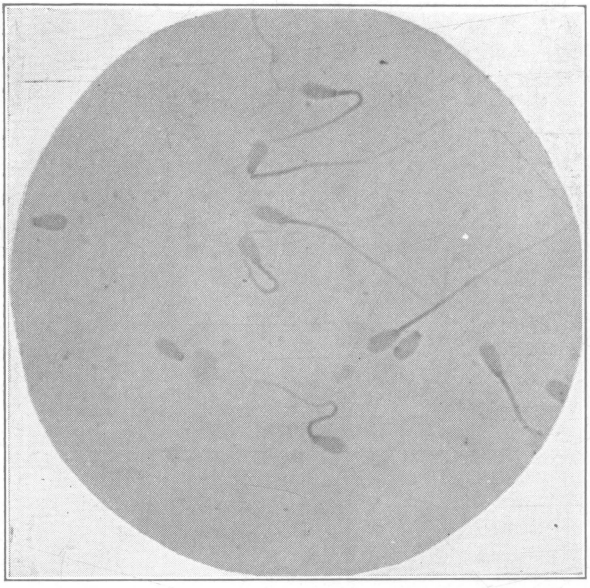

Fig. 6.—Photomicrograph of abnormal (tailless) bull spermatozoa (× 600)

(c) Fast green:

Stock solutions:

Aqueous phenol	5 per cent
Aqueous iron chloride	30 per cent
Fast green	1 per cent
Acid fuchsin	1 per cent

Prepare stain from stock solutions by using:

15 cubic centimeters aqueous phenol
2 cubic centimeters aqueous iron chloride
1 cubic centimeter fast green
1 cubic centimeter acid fuchsin

BACTERIOLOGY

Much variation exists in the bacteriological flora found in semen samples from different bulls. For instance, some contain great numbers of diphtheroids; others practically none. Whether these and other contaminating organisms affect the efficiency of the semen is not altogether clear, but certainly some kinds are of importance. *Pseudomonas pyocyaneus* has recently been found in the semen of bulls having histories of poor breeding efficiency. Consequently, it is advisable to have a careful bacteriological examination of semen when breeding problems appear and the cause cannot be otherwise determined.

It is well to keep in mind that there are numerous pathogenic bacteria which can invade the reproductive tract of cattle and that unlimited opportunity exists for dangerous organisms to gain entrance through carelessly handled equipment, semen, and diluents.

INSEMINATION

EQUIPMENT

Inseminating instruments are shown in figure 7.

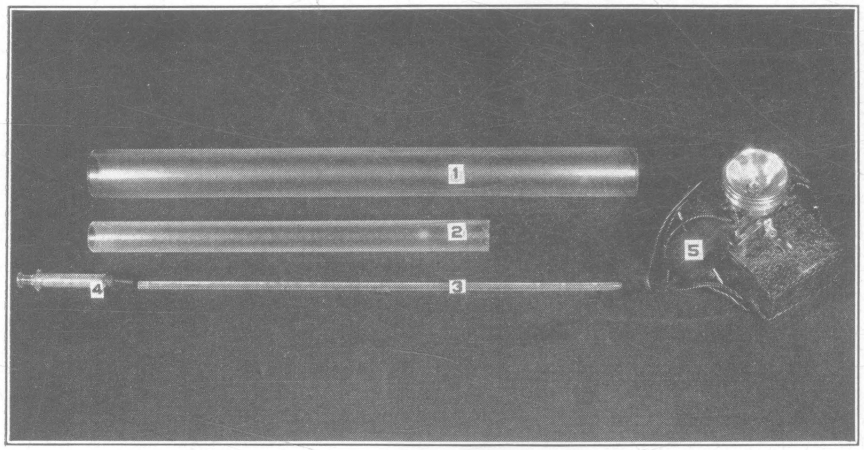

Fig. 7.—Inseminating equipment

1. Glass speculum for cows, size 1⅝ by 16 inches
2. Glass speculum for heifers, size 1 inch by 12 to 16 inches
3. Inseminating glass tube (thick walled) for transferring semen into the female reproductive organ. Diameter outside, ¼ inch (6 millimeters); inside diameter, 0.078 inch (2.0 millimeters); by 16 to 18 inches long
4. Glass hypodermic syringe, capacity 2 cubic centimeters. A small rubber connector is used between the syringe and the glass inseminating tube (3, fig. 7).
5. Electric light used for locating the os by way of the speculum. A long-sleeved rubber glove, not shown in figure 7, is also used by most technicians for making rectal examinations and for inseminating purposes.

Equipment which cannot be purchased locally can usually be purchased through a local veterinarian.

METHOD OF BREEDING

The insemination of cows can be accomplished either with or without a speculum. Most people prefer to use a speculum when they are not too familiar with the anatomy involved or where small heifers (Jersey breed) are to be bred. A speculum permits the operator to expose the os, through which the inseminating tube is passed into the cervix about ½ to 2 inches before the semen is deposited. Lubricating a speculum with vaseline or vaseline oil before using is advisable.

Insemination without a speculum is the method usually chosen by veterinarians and others trained in the technique. The operation is carried out by picking up the cervix through the rectal wall with one hand and gently passing the inseminating tube (3, fig. 7) through the vagina into the cervix with the other. This method permits deposit of the semen in almost any place in the tract. The third ring of the cervix is probably the location of choice in most instances. Deposition of semen far into the uterus, which is occasionally done, is somewhat too hazardous a practice for routine use. It may result in serious consequences due to pathogenic organisms' being carried in; an already pregnant animal may be caused to abort; and there is always the possibility of serious injury to the uterine wall.

The quantity of semen required for each insemination is 0.5 cubic centimeter when undiluted semen is used, 1.0 to 1.5 cubic centimeters when the diluted (1:3 dilution) material is used. More can be used with safety, but more is not required.

CLEANING AND STERILIZATION OF EQUIPMENT

The successful breeding of dairy cattle by artificial means requires that all equipment which comes in contact with bulls, cows, and semen be clean and sterilized.

RUBBER EQUIPMENT

1. Flush with warm or cold water immediately after using.

2. Immerse in or fill with hot water and scrub the inside surfaces with a round brush. Do not use soaps regularly, because they are detrimental to rubber. Traces of soap are also sufficient to kill spermatozoa and, consequently, must be carefully removed.

3. Rinse thoroughly with clean hot water, 85° C. (185° F.).

4. Rinse twice with distilled water.

5. Rinse with grain alcohol (not rubbing alcohol). This helps remove vaseline and water and kills bacteria.

6. Suspend in air and allow to dry before using again.

All newly constructed funnels or new rubber equipment which is to come in contact with semen should be scrubbed with hot soapy water and then rinsed with water from a faucet for 30 to 60 minutes in order to remove all traces of soap.

GLASS AND METAL EQUIPMENT

1. Flush with warm or cold water immediately after using.

2. Scrub and clean the outside portions with a good washing powder.

3. Flush the inside and outside several times with hot water, 85° C. (185° F.).

4. Rinse the inside with distilled water, grain alcohol, and finally distilled water.

5. Place in a covered pan or tray and heat until dry. When possible, sterilization of equipment can be done by boiling in water or steaming for 5 minutes or more. All equipment which comes in direct contact with undiluted semen must be dry. After sterilization, equipment must be kept covered until used. Glassware which remains cloudy after it has been cleaned as previously explained should be cleaned with some kind of cleaning solution. Forcing a hot lye solution or a hot 2 to 5 per cent solution of sodium phosphate through the equipment several times will usually be sufficient. When alkali solutions are insufficient for cleaning glass, a cleaning solution prepared by pouring 1 liter of concentrated sulfuric acid into 35 cubic centimeters of a saturated sodium dichromate solution can be used. This cleaning solution must be stored in crockery or pyrex glass and not allowed to come in contact with the skin or clothing. Extreme care should be used in removing any cleaning solution, because foreign materials of this kind kill spermatozoa.

VASELINE OR PETROLEUM JELLY

Lubricants should be sterilized from time to time by heating in an oven to about 100° C. (212° F.) for 15 minutes.

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