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EXPERIMENT STATION, Stillwater, Oklahoma.

ARTIFICIAL INSEMINATION

L. L. LEWIS.

In March, 1906, a circular was issued which dealt mainly with the question of the use of the artificial impregnator in horse breeding. The edition of this circular has been exhausted. It dealt mainly with the practical use of the impregnator, and it is thought advisable to make use of some portions of it in this bulletin with such changes as our later experiments have demonstrated to be advisable. The use of the impregnator, or other artificial means of introducing the semen, in horse breeding has passed beyond the experimental stage, and its practical use has been thoroughly demonstrated in many breeding establishments as a very satisfactory means of breeding mares that have been difficult or even impossible to breed by direct service; also as a means of breeding several mares at one service of the stallion, making it possible to breed the stallion three or four times a week. This will give semen of greater fertilizing power than when the stallion is used for service daily.

While many are interested in the scientific questions relating to breeding and would like to understand the underlying principles and laws governing these matters, more are interested in the practical application of the laws and appreciate to the fullest extent information that will enable them to apply whatever knowledge may be gained by investigations of this character. There is generally a dread on the part of most people in undertaking anything new. But since there is nothing about the anatomy of the reproductive organs of the mare or of the stallion that is difficult to understand, and the necessary instruments to be used in artificial insemination are few and simple, there seems to be no reason, so far as the operation is concerned, why every breeder should not practice this method of horse breeding, and at times with others of the domestic animals, especially cattle. Further, the vitality of the semen is such that it may be transferred from one mare to

another without any apparent loss of fertilizing power. Many stallion owners are making extensive use of the method of artificial insemination and are succeeding in getting a high per cent of foals. Since there is nothing about the method but what may be known by every breeder, there is no reason why one man should meet with success and another with failure if the same care is exercised.

There are various forms of instruments on the market, but those shown in figures one and two are the ones in common use. The preference is generally for the metal instrument as illustrated in figure one.



Figure 1—A curved metal syringe for transferring semen to mares that are to be bred.

Some of the instruments are straight and have no glass barrel while others are curved and may be had either with or without the glass barrel. In many ways the instruments with glass at the tip are preferable as it enables one to see if the syringe is filled with the semen. Where the semen is collected from the vagina the curved instrument is easier handled and is easier to introduce into the neck of the womb than is the straight syringe. A specu-

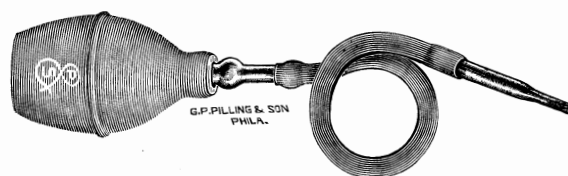


Figure 2. A rubber bulb syringe for the same purpose as the one shown in Figure 1.

lum should be kept in every breeding barn as they are necessary if an examination is to be made to determine the condition of the organs.

However, it is not necessary to use the speculum to secure the semen from the vagina or to introduce the instrument into an-

other animal for the purpose of impregnating them. The use of the breeding bag is recommended in all cases when it is possible to use it, as the semen may then be collected in a clean bag and unmixed with any other fluids or secretions, while if the breeding bag is not used the stallion will frequently place the semen in the womb where it is not altogether safe for the amateur to gather it with an instrument on account of the liability of causing injury. Cervical plugs such as illustrated in figure three are frequently used in order to prevent the semen from entering the womb direct from the service. These plugs are usually well protected on one side by a soft rubber ring. They are inserted into the neck of the womb without the use of vaseline or other lubricating material. When the plug is used semen is found in the vagina where it may be readily collected with the instrument. By using the stallion or jack for only three or four services a week the semen secured will have a much greater vitality and a greater number of sperm cells than when the animal is used once or twice every day.

All instruments should be kept scrupulously clean. This is an extremely important matter. A corroded syringe, a greasy leather plunger or dirt or grease of any kind may very materially interfere with the work. No soap, cleaning powders or disinfectants should be used in cleaning the instruments. They should be cleaned with hot water and allowed to thoroughly dry in the sunlight. The sperm cells are very sensitive to the presence of chemicals even when these are present in very small amounts, consequently it is necessary to handle the instruments in such a manner as to prevent material of this character from coming into contact with the semen. If the breeding bag is used the semen should be removed within a short time as contact with rubber for any length of time is harmful. This probably would not be such as to necessitate immediate removal, but when the semen is left in the rubber bag for any great length of time it loses its vitality much more quickly than when it is left in clean glass vessels.

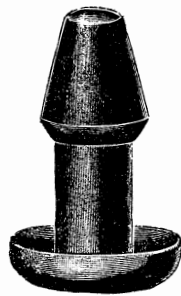


Figure 3. Rubber plug for placing in the opening of the womb.

While the sperm cells will live for some time under artificial

conditions the transfer of the semen from the breeding bag to mares that are to be bred should be made as quickly as possible. No cold instruments should be allowed to come in contact with the semen and no water should be allowed to mix with it. The instruments should be kept in water warmed from 90° to 100° F. When the semen is ready for injection the hand should be inserted and the opening into the womb located, after which the point of the instrument is guided into the neck of the womb and inserted for one or two inches, after which the semen is injected. Some make a practice of inserting the instrument into the womb for a distance of four or five inches. This is not necessary as the body of the womb is small at this time. Breeding capsules of gelatine are used by some in this work. Experiments with these breeding capsules made under laboratory conditions seem to indicate that the gelatine is not harmful to any great extent, if at all, to the vitality of the sperm cells. However, there seems to be no good reason for using the capsules. It requires some time for the heat of the body to soften the gelatine sufficiently for the semen to escape into the womb, and it appears to be unnecessary to have this condition existing when the semen may be placed at once into the womb with the instrument and under such conditions as to permit of the immediate fertilization of the egg cell, provided it is present at that time.

The majority of breeders who have used the breeding bag think that it will injure the stallion. From observations along this line

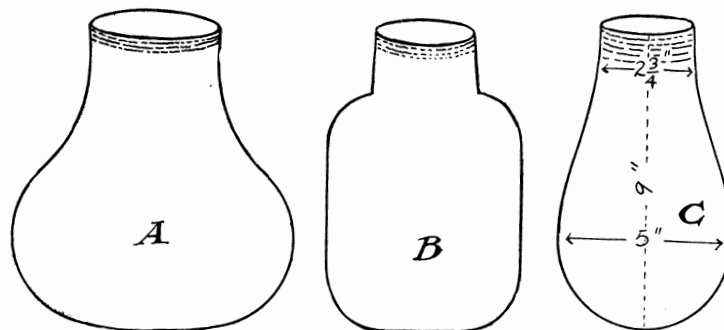


Figure 4. Various forms of breeding bags used in experiments. The form represented by C is the most desirable.

for two years we do not think that this is true in most cases. The stallion may refuse to serve with the breeding bag on, but will

serve readily with it off. Most young horses may be used from the beginning with the breeding bag at almost all services and will continue to serve with the bag, while an old stallion may refuse entirely to serve in this manner. The individual peculiarities of the horse or jack undoubtedly have a great deal to do with the successful use of the breeding bag. Some breeders prefer to take the semen from the vagina and by doing so do not have to bother with warm water to prevent the semen from cooling. While the breeding bag is not necessary for the work, its use is very desirable on account of always being able to get the semen for use and to secure it free from urine and secretions of the vagina.

The shape and size of the breeding bag has a great deal to do with the convenience and success with which it may be used. The breeding bags that were obtainable four or five years ago were very unsatisfactory. They were not only too large, but were not of the right shape or made of the most suitable material. In our experiments we have made use of a considerable number of breeding bags of different shapes and made of different materials. Some were made of surgeon's silk, others of cloth of various qualities and some of rather heavy rubber. Very thin rubber is

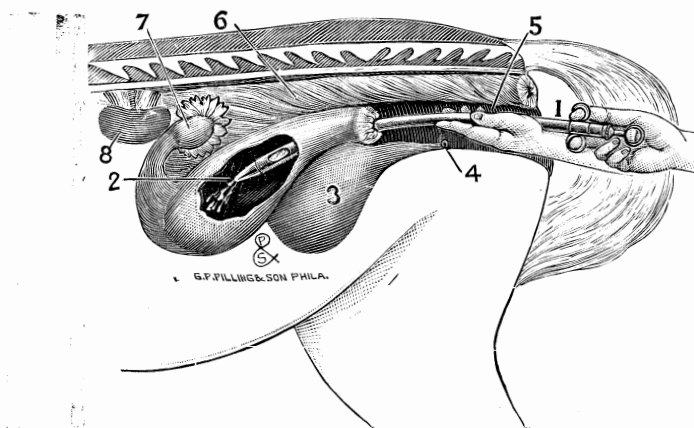


Figure 5. This figure shows the impregnator in use, also the various organs of the mare. 1. Impregnator in place. 2. End of instrument in womb. 3. Bladder. 4. Where bladder empties into vagina. 5. Interior of vagina. 6. Rectum. 7. Ovary. 8. Kidney.

the only satisfactory material that we have been able to obtain for this purpose. The principal objection to rubber is that the material is fairly easily torn and the bag rendered useless.

Figure four represents the most common forms of breeding bags used. The forms represented by A and B were among the earlier forms used and were not at all satisfactory. The form represented by C has given very satisfactory service. Drawings for this bag were furnished a manufacturer of rubber goods and the bags were used in the spring of 1909 and proved very satisfactory. Any rubber breeding bag should have a reinforced rubber band around the top in order to prevent tearing, as this is one of the weakest points in their construction. The bag represented by C had a half-inch reinforcing band around the top.

In using the artificial method of insemination in breeding it is not necessary to place a large amount of the semen into the womb. In most cases the sperm cells are very abundant and only one cell is needed or used in fertilizing the ovum. During the past few years a great many counts have been made of the sperm cells present in the semen from the horse and from the hog. The number present varies with the individual, and in the same individual under different conditions. Samples have been counted where the number of sperm cells in the semen from one service of the stallion was more than twenty billion. From this one may readily see that in as much as twenty drops of such semen there is likely to be as many as two hundred million sperm cells. As mentioned before, only one of these cells can take part in the fertilization of the ovum or egg. The semen should always be introduced into the womb and not merely into the vagina. In a great many cases the neck of the womb is found to project for two or three inches and is difficult to enter. In such cases it is not very easy to insert the syringe and extreme care should be taken to know that the syringe has passed into the womb before the semen is injected.

The semen from the horse is a whitish colored fluid having a peculiar odor, while that from the hog is more nearly white in color, contains a large amount of lumpy, albuminous material with a quantity of thin, watery fluid in which is found the sperm cells. The sperm cells of the horse are often found in clumps, especially where the services are infrequent. Under these conditions the vitality is much greater than in cases where the horse is frequently bred. Contact with the air seems to have little effect upon the vitality of the sperm cells. A number of tests were made where the semen was placed in closed receptacles and kept

from the air, but the vitality of the cells under these conditions was no greater than that of samples of the same material kept in open vessels. Direct sunlight is injurious and semen left in the sun soon loses its vitality. Undoubtedly there are conditions affecting the vitality of the semen or sperm cells with which we are not familiar. Under natural conditions the semen is never exposed to a temperature of less than 38° C., is not exposed to the light and only very slightly to the air. The semen is also surrounded and mixed with the natural secretion of the female organs while under laboratory tests the conditions are quite different. However, in the experiments that have been carried out during the past few years the results obtained seem to indicate that even under normal or natural conditions the life of the sperm cell is short, in fact but little longer than when the semen is kept under artificial conditions and at a temperature of from 15° to 20° C. When the semen has been confined in celluloid or parchment bags and inserted into the organs of the mare the sperm cells did not live any longer than they would have lived under laboratory conditions at the same temperature. Whether further experiments will confirm these observations or not remains to be seen, but it is likely that with better facilities for making the observations slightly different results may be obtained.

Too frequent service of the stallion does a great deal to reduce the number and vitality of the sperm cells. In order to secure some information along this line four experiments were conducted and the data obtained is briefly stated below. Two stallions were used in the experiments, one a heavy draft horse, the other a grade draft. These horses were well kept as to feed and exercise, and the results obtained indicate very clearly that there is a marked reduction in the number of sperm cells present as well as a lessened vitality as a result of continuous service.

The first experiment extended over a period of nine days. A heavy draft stallion was used and was bred once each day, and samples of semen secured for laboratory work. The number of sperm cells present in the semen from the first service was 131,750 per cubic millimeter and live cells were found for ten hours after service when the semen was kept at from 31° to 35° C. The semen collected from the ninth service had only 5,840 sperm cells per cubic millimeter and the vitality of the cells was less than

one-half as compared with those obtained from the first service.

The second experiment was with the grade stallion and extended over a period of eleven days, giving one service daily. The semen was collected in a breeding bag. There were 232,500 sperm cells per cubic millimeter in the semen secured from the first service and 43,000 cells per cubic millimeter in the semen from the eleventh service. The difference shown in the vitality of the cells was about the same as indicated in the first experiment.

In experiment three a total of eighteen consecutive daily services were had with the grade stallion, and while the vitality and number of cells from the last service was very low the general results were about as indicated in the first two experiments.

In experiment four the grade stallion was again used and was bred twice daily for ten days. The number of sperm cells in semen from the first service was approximately the same as shown in the second experiment while the number of sperm cells from material secured from the twentieth service was 23,000 per cubic millimeter. The vitality of the cells when kept at from 18° to 23° C. dropped from seven to nine hours at the beginning of the test to three to five hours at the last of the test. The horse used in the three last experiments was of an active temperament and it is likely that these results are comparable with such as might be obtained from the best of stallions under similar conditions. Too frequent service of the stallion will necessarily result in a lessened vitality of the germ cells and in this way operate to reduce the per cent of foals. By the proper care of the horse as to feed and exercise, and care as to frequency of service, the vitality of the reproductive cells would be kept at the maximum for that individual. The successful handling of breeding stock is a feature of stock breeding that should be given more attention, and the mere matter of feed and exercise, while important, is by no means all that should claim our attention.

Some experiments were conducted to see if there was any difference in the length of time the sperm cells would live when the animal was bred out of heat as compared with those bred in heat. Hogs were used for this experiment and it was found that when sows were bred out of heat live sperm cells could be found for a greater length of time than when the sow was bred in heat.

Recent experiments with the hog has led to the conclusion that the ova are not liberated from the ovary until the last of the period of heat. This question could not be determined directly except by postmortem examinations, and this method was used to determine this with reference to hogs. Authorities on veterinary physiology state that as a rule the ova does not leave the ovary in the case of the mare until the last of the period of heat. Since it appears that the life of the sperm cell is relatively short it would seem that breeding during the last of the period of heat would likely give better results than breeding at the beginning of this period.

Many observations have been made with the hog to determine the length of time the sperm cell will live in the body of the female, the time the ova or egg cell leaves the ovary, the length of time the ova or egg cell retains its vitality, etc., and the almost uniform results obtained seem to warrant the following conclusions:

1. That in most cases the sperm cells are dead within twenty-four hours after service, and only exceptionally are they found alive longer than forty-eight hours.
2. That the egg cell is not capable of being fertilized until toward the last of the period of heat, as the egg has not escaped from the ovary until that time.
3. The ovum seems to soon lose its vitality, as breeding the first day after the period of heat has passed is without results in about 70 per cent of the cases tested, and no results followed breeding at a later date than one day after the period of heat had passed.

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