ARTIFICIAL INSEMINATION of SWINE

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INTRODUCTION

Achievements in dairy cattle artificial insemination during the past 15 years have stimulated considerable interest in the application of such techniques to other classes of livestock. If suitable boar semen preservation methods and swine artificial insemination techniques can be developed, certain advantages could be provided to the farmer that are impossible under present breeding methods. The utilization of a successful artificial insemination program could:

- (1) Increase the number of services per boar.
- (2) Improve the sire selection program.
- (3) Decrease the incidence of disease transmission due to mating.
- (4) Permit breeding of incompatible animals.
- (5) Reduce the time required to prove a boar by increasing the number of possible matings.

The Ohio artificial insemination research program has two major objectives.

- (1) Determine some of the characteristics of swine semen and factors which limit fertility.
- (2) Develop techniques which may be useful in swine artificial insemination.

The first objective has involved various studies of semen collection and scmen preservation. Boar semen has been extended with different diluents in an attempt to prolong the livability and fertilizing capacity of the boar sperm **in vitro**. The maintenance of suitable boar sperm livability and fertilizing capacity depends upon the constituents of the diluter and the refrigeration storage conditions.

The second objective has included an investigation of different types of insemination catheters and various approaches to penetration of the cervical canal at the time of estrus. The success of artificial insemination depends upon the ability to insert catheters into the uterus as well as the quality of the diluted semen deposited.

REVIEW OF LITERATURE

A limited, but growing interest in swine artificial insemination has developed in various countries during the past five or six years. Most of the research in the United States has been conducted on a laboratory basis, dealing with the physical and biochemical properties of semen. Some of these properties were excellently described by McKenzie (4, 5, 6).

(a) SEMEN PRESERVATION

Boar spermatozoan livability was suitably extended in a diluter containing 0.7 percent sodium chloride, 5-6 percent glucose and 10 percent sucrose (Ito et al.) (2). These researchers reported that boar semen dilution was not necessary for storage, but dilution should be made prior to insemination to increase volume. Du Mesnil Du Buisson (1) maintained the fertilizing capacity of boar semen for 5-6 hours and spermatozoan motility for 1-2 weeks when the ejaculate was diluted at the rate of 1:5 to 1:10 in a diluter containing milk or egg yolk with glucose and sodium carbonate, or glycine added and stored at 7°C.

Polge (7) artificially inseminated 61 gilts and sows with boar semen extended with egg yolk and glycine. A pregnancy rate of 30 percent resulted. The author expressed the opinion that even though spermatozoan motility is maintained for prolonged periods in an egg yolk glycine medium, the fertilizing capacity of the spermatozoa is reduced rather rapidly.

(b) PREGNANCY RATES FROM ARTIFICIAL INSEMINATION

In an investigation of swine artificial insemination, Wiggins et al. (10) obtained a pregnancy rate of 91 percent in gilts and 67 percent in sows when the boar semen was diluted with Kreb's solution at the rate of 3 parts of diluent to 1 part of semen.

Pregnancy rates of 54 percent for gilts and 68 percent for sows were obtained by Polge (8) when the spermatozoan concentration of the volume inseminated was about $2 \times 10^{\circ}$. The author prescribed the use of 100 ml. of semen for gilts and 200 ml. for sows.

Lasley (3) discovered that two inseminations per estrous period resulted in a pregnancy rate of 53 percent while single inseminations per estrus resulted in a 23 percent pregnancy rate. A pregnancy rate of 62.5 percent was reported by Ito et al. (2) when undiluted semen, stored at 20°C. for one day, was used. Storage of semen for two days reduced the pregnancy rate to 28.6 percent. Good results were not obtained unless 70 percent of the spermatozoa showed active progressive

motility. A reduction of 10-30 percent in pregnancy rates was noted when the results of field trials were compared with controlled laboratory artificial insemination. (Polge et al.) (9). The reduction in pregnancy rates under field conditions was thought to be due partially to the difficulty in detection of estrus in swine.

EXPERIMENTAL PROCEDURE

(a) SEMEN COLLECTION EQUIPMENT

The facilities used were suitable for housing and care of 6 boars and as many as 40 females. Females were used for the purpose of developing insemination techniques and to associate laboratory semen evaluations with fertility trials.

Boar semen was collected by the use of an artificial vagina as the boar mounted a "dummy sow". The artificial vagina (Figure 1) was made from a three inch section of radiator hose (1.5 inch in diameter), a six inch piece of 1.25 inch rubber tubing (water jacket), and a three foot section of 1.25 inch rubber tubing (semen receptacle). Water,



Fig. 1.—The artificial vagina was constructed from a three inch section of radiator hose, a six inch piece of 1.25 inch rubber tubing and a three foot section of 1.25 inch tubing. that was warmed to 105-110°F. was added prior to semen collection. The addition of warm water to the artificial vagina was not necessary during the hot summer days.

The "dummy sow" (Figure 2) was constructed from 1.25 inch steel pipe. The rear portion of the dummy was heavily padded with burlap and periodically saturated with the urine of a sow in estrus.

(b) TRAINING BOARS TO MOUNT

Boars that have mounted on first association with the dummy have not required additional training. On a few occasions, some boars have required considerable coaxing before they would allow semen to be collected in the artificial vagina. Three boars have been slaughtered because they could not be trained to mount.

Training can be facilitated by allowing the boar to remain in proximity of the dummy for short periods of time. These periods may be extended if the boar shows interest in the dummy. Some boars have allowed semen to be collected on first association with the dummy, but



Fig. 2.—This "dummy sow" was constructed from 1.25 inch steel pipe. The rear portion was heavily padded with burlap.

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others required three to four visits. The age of the boar at the time of training has not made any apparent difference in the response to the presence of the dummy and the use of artificial vagina.

From a comparative standpoint, boars are much easier to train to mount than bulls or rams. Boars have been known to mount bales of straw, feed tubs or fence posts without coaxing.

Hundreds of collections have been made with only two failures. These were attributed to hot weather in one case and a feverish boar in the other.

No serious penis injuries have resulted from artificial collection although an occasional small blood vessel may rupture due to the contact of the penis with the burlap covering on the "dummy sow".

(c) COLLECTION OF SEMEN

The volume of semen collected from a single mounting may range from 200 to 500 ml., but an average of 200 to 300 ml. can be expected.

During semen collection the boar is ejaculated until he withdraws on his own volition. Cessation of sexual interest can be determined by the degree of turgidity of spiral ridges of the boar's penis. Complete ejaculates may be obtained within 15 minutes after collection begins.

(d) BOAR SEMEN DILUTION

Early experiences in the handling of boar semen indicated that it was necessary to remove the gelatinous fraction of the ejaculate prior to dilution. The retention of the "gel" in the diluted semen sample can impair the function of the insemination equipment. Freshly collected ejaculates were filtered through cheesecloth, diluted and slowly cooled to 5° C. within 45 minutes.

The livability of boar sperm in vitro has been of concern to most researchers since the success of artificial insemination of swine may depend on the prolongation of boar sperm livability without the loss of fertilizing capacity. Preliminary work was conducted to find an extender suitable for boar semen dilution and storage. Microscopic evaluations were made on the boar semen diluted with various extenders. The microscopic evaluations were the percent of motile spermatozoa (0-100) and the degree of progressive spermatozoan motility evaluations (0-10). Semen samples were diluted with the various extenders at the rate of 4 parts of diluter to 1 part of semen and stored at 5°C. Evaluations were made at 1, 2, 3 and 4 days storage. A splitsample technique was not employed in this particular study. The results of this study are shown in Table I.

		%	6 Motil	e Speri	m	Sper	m Mo	tility	Rate
Diluter*	No. Samples	Days Stored at 5°C.			Days	Days Stored at 5 $^{\circ}$			
		1	2	3	4	1	2	3	4
EYG	9	63.3	21.1	0.0	0.0	7.0	4.0	1.3	0.0
MG	7	62.8	24.2	4.0	0.0	7.1	4.2	1.1	0.0
EYGM	14	76.0	37.4	13.5	0.0	7.3	4.4	1.3	0.0
EYGMS	35	76.0	41.0	5.0	0.0	7.2	5.8	4.7	2.1
EYGS	56	85.0	63.8	45.3	27.2	7.4	5.7	4.6	2.3
EYCS†	150	83.6	63.3	44.2	26.6	8.6	7.2	7.0	4.4
EYS†	39	85.1	71.4	58.7	39.8	8.5	7.3	7.1	3.9

TABLE I.---The Evaluation Means of Boar Semen Diluted with Various Extenders and Stored at 5°C for 1, 2, 3 and 4 Days

* EYG ---egg yolk (30%) - glycine (1%).

MG -milk - glycine (1%).

EYGM -egg yolk (30%) - glycine (1%) - milk.

EYGS —egg yolk (30%) - glycine (1%) - glucose (1%). EYCS —egg yolk (30%) - sodium carbonate (0.1%) - glucose (3%). EYGMS—egg yolk (30%) - glycine (1%) - glucose (1%) - milk.

---egg yolk (30%) - glucose (3%). EYS

† Gratitude is expressed to Dr. Phillip Dziuk, University of Illinois, for suggesting the use of the EYCS and EYS extenders.

On the basis of the significantly higher percent of motile spermatozoa, the egg yolk (30 percent)—glucose (3 percent) extender was used as a standard for all future comparisons. No significant differences were found between the mean motility rates of semen diluted with the egg yolk-glucose and the egg yolk-carbonate-glucose diluters when 1, 2, and 3 day-old semen evaluations were tested.

ARTIFICIAL INSEMINATION OF GILTS AND SOWS

One of the more difficult problems associated with artificial insemination of swine is the intra-uterine deposition of boar semen. The condition of the sow's cervical canal at the time of estrus is not too favorable for artificial insemination. Since it has been determined that the penis of boar threads the cervical canal during copulation, suitable insemination equipment must be designed to accomplish similar penetration while the gilt or sow is in estrus.

A cattle plastic insemination rod, modified to resemble the penis of the boar, was used for insemination of all gilts and sows. The plastic rod was heated over an alcohol flame until it became soft and pliable.

One end was formed into two left-handed spirals while the plastic was soft. A 16 gauge hypodermic needle was sealed in the straight end of the spiralled rod and served as an adaptor for a 30 or 50 ml. Leur-Loc syringe.

Insemination was performed during the second day of estrus. A 100 ml. volume of diluted semen was used for each insemination. Due to the nervous disposition of some females during estrus, it was often necessary to use fore-play prior to insemination. Fore-play consisted of massage of the clitoris and the udder for a few minutes before insemination. The rod was inserted by gently rotating the spirals through the vagina and as far through the cervical canal as possible. Due to anatomical differences in the reproductive tracts, it has been more difficult to perform intra-uterine insemination in some females than others. Although the number of sows inseminated has been limited, it has been observed that they are much easier to inseminate than gilts. The ease of insemination of sows may be due to the conditioning of the cervix, developed through farrowing, as well as the characteristics of the sow's estrous period. Improper insemination technique can be easily detected by a back-flow of diluted semen from the vagina. Care should be taken not to excite the female prior to or during the insemination since relaxation is important in gaining entrance into the cervical canal.

Fertility trials, using artificial insemination, have been conducted. These trials were divided into two parts:

- (1) 1, 2 or 3 day-old semen. The trial included 64 gilts and sows. The objective of this trial was an investigation of the mechanics of the insemination technique. Females were inseminated during the second day of estrus with 100 ml. of diluted semen. The semen dilution rate was standardized at 4:1. The diluted semen available at the time of estrus was used for insemination.
- (2) A 2 day-old versus 3 day-old semen trial. This trial included 50 gilts and sows. Females were inseminated with 100 ml. of diluted semen during the second day of estrus. The semen was diluted to approximately 50 million sperm/ml. and stored at 5°C. for 2 or 3 days. Split-samples were used whenever possible.

RESULTS FROM ARTIFICIAL INSEMINATION

Pregnancy rates in both fertility trials were determined through slaughter of the females 21 to 75 days after insemination. The number of corpora lutea and fetuses was recorded from observation of the recovered reproductive organs.

The results obtained from the artificial insemination of gilts and sows with fresh diluted boar semen are presented in Table II.

	Number insemi- nated	% pregnant	Mean number of corpora lutea	Range in number of corpora lutea	Mean number ⊧of fetuses	Range in number of fetuses
Gilts	44	68.2	14.1	1-18	10.1	1-18
Sows	20	85.0	15.5	10-21	10.2	5-15
Total or average	64	73.4	14.6	1-21	10.2	1-18

TABLE II.—Observations Made on the Reproductive Organs of Gilts and Sows* Artificially Inseminated with Fresh Diluted Boar Semen (All Services)

*Females were provided by the Department of Animal Science, Ohio Agricultural Experiment Station and the Ohio State Mental and Hygiene Correction Institution, Apple Creek, Ohio.

The group of 64 gilts and sows required 72 inseminations. Fortyeight of the females were inseminated with 1 day-old semen, 13 with 2 day-old semen, and only 3 with 3 day-old semen. Forty-seven of the females were found to be pregnant on slaughter. The evidence suggests that some of the inseminated females did not return to estrus even though they were not pregnant. This is partially explainable since females are not restrained for insemination and only those that would stand for insemination were recorded as being in estrus.

The average number of fetuses present in the pregnant females compares favorably with the average number of fetuses expected from natural services. In one pregnancy there was only one fetus present.

Results of the trial may be divided into first and second services. These are shown in Table III.

Pregnancy rate in sows was higher than in gilts when first service results were compared although the number inseminated was small.

	No. 1st services	% pregnancy	No. 2nd services	% pregnancy
Gilts	44	61.3	4	75
Sows	20	75.0	4	50

TABLE III.—Pregnancy Rate of Gilts and Sows Resulting from First and Second Inseminations

The fact that a higher pregnancy rate was observed in sows than gilts, in both first and repeat services, is supportive evidence that sows are much easier to inseminate.

The quality of each semen sample intended for insemination was microscopically evaluated within an hour before its use. Semen evaluation included the percent of motile sperm and the degree of progressive motility. The evaluation means of semen used for insemination and their relationship to fertility are shown in Table IV.

TABLE IV.—The Evaluation of Boar Semen as Related to Fertility

	No, females inseminated	Mean % sperm in se	, of motile amples used*	Mean sperm motility of samples used†		
		Females pregnant	Females non-preg.	Females pregnant	Females non-preg.	
Gilts	44	70.3	65.0	8.60	7.27	
Sows	20	75.8	70.8	8.00	8.00	
Total or average	64	72.3	70.8	8.40	7.30	

* Percent motility evaluations were scored from 0-100.

† Motility rates were scored from 0-10.

In samples used, where pregnancy occurred, the means of the percent of motile spermatozoa and motility rates were higher. The higher rates of pregnancy existed when the semen samples used were in the 80-90 percent of motile spermatozoa range. A greater degree of relationship existed between motility rate and pregnancy than percent of motile sperm and pregnancy. Higher pregnancy rates resulted when the spermatozoan motility rates ranged from 8-9. The greatest decline in pregnancy rate resulted from semen with spermatozoan motility rates below 8. When the inseminated females were divided into two groups:

one in which semen possessed spermatozoan motility rates above 7 and the other below 7, the pregnancy rates were 77.3 percent and 52.6 percent respectively.

The results obtained from the 2 day-old versus 3 day-old semen trial are presented in Table V.

		2 Day-a	old Semen	3 Day-old Semen				
	Numbet services	% preg- nant	Mean number of corpora lutea	Mean number fetuses	Number services	% preg- nant	Mean number of corpora lutea	Mean number fetuses
Gilts	23	47.8	120	10.5	21	28.5	11.5	9.0
Sows	13	61.5	12.7	10.2	8	37.5	11.9	7.6

TABLE V.—Artificial Insemination of Gilts and Sows with Diluted Boar Semen Stored at 5°C. for 2 or 3 Days (All Services)

A comparison of the two groups revealed a marked decline in pregnancy rate and also the mean number of fetuses when the semen used for insemination had been stored for 3days. The decrease in the average number of fetuses, where 3 day-old semen was used, may be attributed to the lower concentration of live sperm and the lower motility ratings in these samples. The comparison of split-sample insemination where 2 or 3 day old semen was used are presented in Table VI.

TABLE VI.—A Comparison of Pregnancy Rate where Split-Sample Inseminations Were Made Using Diluted Semen Stored Either 2 or 3 Days

Number insemi- nated	2	day-old se	men		3 day-old semen			
	% preg- nant	Mean number of corpora lutea	Mean number of fetuses	Number insemi- nated	% preg- nant	Mean number of corpora lutea	Mean number of fetuses	
15	53.3	13.8	10.4	16	31.2	12 6	9.9	

Due to the small number of females inseminated, the data, resulting from the use of 2 or 3 day-old semen where a split-sample technique was employed, were grouped to include all services. These limited data indicate a reduction in pregnancy rate and the mean number of fetuses when females inseminated with 2 versus 3 day-old semen.

A comparison was made of the evaluation means of 2 day-old semen and 3 day-old semen with fertility. These results are shown in Table VII.

			3 Day-ol	d Semen				
	Non-pregnant		Pregnant		Non-pregnant		Pregnant	
	% motile sperm	Motility rate	% motile sperm	Motility rate	% motile sperm	Motility rate	% motile sperm	Motility rate
Gilts	74.0	7.4	65.0	7.3	52.6	5.6	56.6	6.3
Sows	68.0	7.4	64.3	7.0	50.4	5.5	60.0	6.3

TABLE VII.—The Relation of 2 Day-Old and 3 Day-Old Semen Quality to Fertility

The lack of association between the quality of 2 day-old semen and the pregnancy rate when the 2 day-old semen was used may be attributed to the higher evaluation means and a higher incidence of difficult breeding females associated with this group. High correlations may not exist when the evaluation means of semen samples used are within the higher ranges (60 to 80 percent) for percent motility.

SUMMARY AND CONCLUSIONS

(1) Boar senien can be readily obtained through the use of an artificial vagina and a "dummy sow". Ejaculate volume ranges from 200 to 500 ml.

(2) Egg yolk (30 percent)-glucose (3 percent) has been the most successful semen extender of the diluents tested.

(3) Artificial insemination with 1-2 day-old diluted semen can be of practical value. Fertility, measured through slaughter information, is drastically reduced when the semen used is over 2 days old. The decline in pregnancy rate may be due either to an inadequate number of motile sperm to maintain higher levels of fertility or a partial loss of fertilizing capacity of the sperm due to other factors.

(4) Due to anatomical differences in the reproductive tract of gilts and sows, some are more difficult to inseminate than others.

The development of a successful and practical swine artificial insemination program will depend on the discovery of more suitable semen diluters, semen storage methods, and improved insemination techniques.

LITERATURE CITED

- Du Mesnil du Buison, F. Preservation of Boar Sperm After Dilution and Maintenance of Its Fertility Ability. Anim. Breed. Abstr., 25: 1378, 1956.
- Ito, S., Niwa, T., Kudo, A. and Mizuho, A. Studies on Artificial Insemination in Pigs. II. Observations on Semen and Its Storage. Anim. Breed. Abstr., 19:755, 1948.
- Lasley, J. F. Artificial Insemination of Swine and Storage of Boar Semen. (Cited by Weaver and Bogart in Mo. Agr. Exp. Sta. Bull. No. 461, 1943).
- 4. McKenzie, F. F. A Method for the Collection of Boar Semen. J. Amer. Vet. Med. Assn., **78:**244-246, 1931.
- McKenzie, F. F., Terrill, C. E., Warbutton, V. and Nahm, L. J. The Migration of the Spermatozoa Through the Genital Tract of the Sow. Res. Bull. Mo. Agr. Exp. Sta. No. 358, 20, 1935.
- McKenzie, F. F., Miller, J. C. and Bauguess, L. C. The Reproductive Organs and Semen of the Boar. Res. Bull. Mo. Agr. Exp. Sta. No. 279, 122, 1938.
- Polge, C. Techniques for Artificial Insemination in Pigs. Pap. 3rd. Int. Congr. Anim. Reprod. (Camb.), Sec. 3, 59-62, 1956.
- 8. Polge, C. Artificial Insemination in Pigs. Vet. Rec., 68:62-76, 1956.
- Polge, C. and Rowson, L. E. A. The Development of an Artificial Insemination Service for Pigs. Animal Breed. Abstr., 24, 209-217, 1956.
- Wiggins, E. L., Grummer, R. H. and Cassida. The Minimal Volume of Semen and Number of Sperm for Fertilization in Artificial Insemination in Swine. J. Anim. Sc., 10:138-143, 1951.