A comparative study of boar semen collected by electroejaculation and gloved-hand technique

by

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I. INTRODUCTION

Reproduction management including artificial insemination in swine has drawn much interest in many countries in the last 30 years. The collection of semen followed by its evaluation is an important and challenging part in this area of animal reproduction.

The study of semen from any species is dependent upon and influenced by the technique used. Therefore, it is important to have the techniques of semen collection well-known. There are three suitable techniques for collecting boar semen nowadays: gloved-hand, electroejaculation and artificial vagina.

Gloved-hand has been one of the most useful and practiced methods. Electroejaculation is less commonly utilized; however, it has been lately shown to collect satisfactory and representative boar semen samples. This is not a widely accepted method for semen collection and has been criticized because of low yields of semen.

The purpose of this thesis is to determine the volume, pH, color, morphology, motility, concentration and percent of live cells of boar semen collected by electroejaculation and compare these to samples collected by the gloved-hand technique.

II. REVIEW OF LITERATURE

A. Electroejaculation Technique

The collection of semen by electrical stimulus was first described by Batelli (1922). He obtained semen from the guinea pig by applying electrical shocks to the base of its brain. He used 2 electrodes, one in the mouth and the other under the skin of the dorsal surface of the neck, to apply 30 volts at a frequency of 47 cycles per second. Gunn (1936) was one of the first workers to use electroejaculation in large animals. He used two electrodes, inserting one electrode in the rectum and the other electrode in the Longissimus dorsi muscle of sheep. This avoided the hazard of brain stimulation. Gunn applied a maximum current of 160 to 190 milliamperes at a frequency of 50 cycles per second. Because the reaction of the animal was very severe, complete restraint was necessary. Gunn et al. (1942) and Bonadonna (1938) improved this technique by applying one electrode to the skin of the back, above the Longissimus dorsi. The area was soaked with saline solution to improve contact between the electrode and the sheep's back. Laplaud and Cassou (1945) found that inserting bipolar electrodes into the sheep's rectum gave better results than skin and muscle contact. Laplaud et al. (1948) and Ortavant et al. (1948) used the bipolar electrode method to collect semen from bulls, stallions, and rams where other collection techniques had failed. These workers evolved a cylindrical bipolar electrode probe in which a series of copper rings were set. The rings were connected respectively to the corresponding poles controlled by a potentiometer. In the bull, for example, the

voltage was in the range from 0 to 30 volts. The maximum current was between 100 and 1800 milliamperes with a frequency of 50 cycles per second. The concentration of the semen varied from 440,000 to 1,160,000 spermatozoa per cubic millimeter.

Electroejaculation has been performed and used more with bulls than any other species because of the expansion of artificial insemination in cattle. Thus, little research was done with electroejaculation collection in other species (Polge, 1956).

The electroejaculation technique was further improved by Dziuk et al. (1954a). They used a variable transformer making possible gradual changes in the voltage applied to the probe. The rectal probe was constructed of a stiff rubber hose. Each electrode was connected to the outside by a separate lead. Alternate electrodes were connected together to the source of stimulus, creating a difference in electrical potential between adjacent electrodes. The rectal probe used in the bull was 22 inches long and 1 1/2 inches in diameter with seven rings 1 1/2 inches apart. The probe was maintained on the ventral side of the rectum. Voltage was gradually increased, then reduced to zero over a five-second period followed by five seconds of no stimulation. The stimulus was repeated, and the voltage level of each succeeding stimulus was gradually increased until semen was obtained. Between 10 to 15 volts and 500 to 1000 milliamperes of current were used. The frequency ranged from 15 to 900 cycles per second, with the best results obtained using 90 cycles per second.

The quality of bull semen collected by electroejaculation has been studied by many other workers (Dziuk et al., 1954b; Hill et al., 1956;

Austin et al., 1961; Christian and Wolf, 1963; Cole and Cupps, 1969; Roberts, 1971; Foote, 1974). Although each study varied in its view of sperm concentration collected by this device, all agreed that it is necessary to have a certain experience in the manipulation of the electroejaculator in order to achieve consistent results.

Marden (1954) has shown this technique to be useful with bulls that are slow breeders or are injured and unable to jump. He described a cylindrical electrode probe containing four longitudinal metallic strips placed entirely inside the rectum. He used 55 volts, 900 milliamperes, and a frequency of 12 to 70 cycles per second. He obtained best results using between 20 and 30 cycles per second. He also found that electroejaculation causes a natural fractionation of the ejaculate. The fluids of the accessory glands are obtained first and can be discarded. The spermatozoa then can be obtained in a concentrated form. No harmful effects were observed in two bulls ejaculated for a period of 12 months. There was no lowering of fertility, and in some instances a slight improvement occurred.

Rollinson (1956), who used electroejaculation in Bos indicus (Zebu bull), considered maximum current to be between 120 and 200 milliamperes at 10 volts and 50 cycles per second. Semen was collected via a polyethylene funnel into a graduated sterile conical tube. In the experiment he used 6 bulls averaging 6 years old. Of 39 collections, only 9 were failures. The motility from some samples was determined by examination under the phase contrast microscope. He found the average concentration of 3 bulls was near 6 million cells per cubic millimeter, and the pH of the samples averaged 6.5. He used the Zebu semen collected by electrojacula-

tion to inseminate Zebu cows. The overall fertility rate for a total of five animals was 2.5 inseminations per conception.

Hill et al. (1956) used electroejaculation in 690 beef bulls. Before the probe was inserted, the rectum was irrigated with a warm saline solution to remove the fecal material to enhance the electrical conductivity to the rectal wall. No particular lubricant was applied, other than soap or water. The semen samples were scored on five physical criteria: volume, concentration, percentage alive, viability, and pH. The study indicated no appreciable differences between semen samples collected electronically and those collected with an artificial vagina. This is partially in agreement with results found by Roberts (1971) and Foote (1974). Carroll et al. (1963) described a total of 10,940 bulls examined for breeding soundness by physical examination and semen quality evaluation. Semen was collected from most bulls by electroejaculation technique. They used a method similar to Hill and co-workers and recorded motility, concentration, morphology and percentage of live cells. Carroll and co-workers (1963) found the morphological characteristic of sperm cells had the greatest correlation to the final semen quality classification. Also they found that a corkscrew configuration of the penis occurred due to an abnormal erection response obtained with the electroejaculator.

After Batelli (1922) found that electroejaculation could be used in animals and Gunn (1936) modified the technique, many other workers began to use it in other mammals. Healey and Weir (1967) performed electroejaculation in the unanesthetized chinchilla. The animal was restrained on its back by a leather strap over the neck and a canvas strap over the

thorax. The rectal pole was lubricated with soap solution and inserted into the rectum to a depth of 32 millimeters. The current was applied for 4 seconds out of every 10 seconds in a series of 10 shocks.

Snyder (1966) described the collection of mouse semen using electroejaculation. The mice were lightly anesthetized with ether to facilitate handling. A blunt greased electrode 2 millimeters in diameter was inserted into the rectum to a depth of 12 millimeters, and a needle electrode was held in the mouth. The stimulator was set to deliver intermittent charges of 80 volts at a frequency of 100 cycles per second with a duration of 0.2 milliseconds. Liquid and coagulate ejaculates were collected separately. Sperm concentration in the liquid ejaculates from 51 animals ranged from 25,000 to 2,280,000 spermatozoa per cubic milliliter with a total of 60,000 to 5,464,000 cells per ejaculate. He suggested that this method could be used as a practical means of obtaining ejaculated samples free of coagulum for reproductive research cultures.

Electroejaculation was also described by Dziuk et al. (1954a) who used rams and goats which required only three stimulations at a maximum of 8 volts to achieve ejaculation. They recommended this procedure to evaluate the animals' semen prior to the breeding season. In the 48 rams evaluated by Dziuk and co-workers (1954a), the volume of the samples varied from 0.5 to 8 cubic centimeters with an average of 1.7 cubic centimeters per collection. The percent of motile cells ranged from zero to 90 with an average of 55 percent. The cell concentration ranged from low to high. They gave no data on the morphology of the semen nor the percentage of live and dead cells from the samples.

Electroejaculation of boars has also been described by Dziuk et al. (1954a). Two methods of restraint were used. One method utilized a nose snare, while the other consisted of restraint between a gate and the adjacent side of the pen. The last method was unsatisfactory, because the boars always tried to back up. From ten collections there was marked absence of accessory secretions, and usually one or two of the thin tapioca-like pellets were obtained. Spermatozoa were dense enough so that the semen appeared milky and the swirling motion could be observed microscopically. The average volume of semen obtained was only 4.1 cubic centimeters with a range of 1 to 8 cubic centimeters. The estimated motility varied from zero to 85 percent with an average of 46 percent of progressive motility. In some cases, there was an increased sexual desire following electroejaculation.

Adams et al. (1969) and Clark (1976) have shown that collection of boar semen using electroejaculation is facilitated by having the animal anesthetized. They suggested that barbiturates may be used for the anesthesia procedure. This allows the examiner to collect an adequate volume while avoiding contaminations. A maximum of 60 milliliters of semen was collected by these workers, with an average being 20 to 25 milliliters. However, they did not record any data concerning motility, concentration, morphology or percentage of live spermatozoa in samples collected by electrical stimulation.

B. Gloved-Hand Technique

The artificial vagina has been considered the best device for collection of animal's semen. The earliest recorded collection of boar semen was by McKenzie (1931). He found using the artificial vagina that it was possible to observe and study the copulatory act, to examine the penis and to collect semen for examination. He used a very simple type of artificial vagina consisting of a soft rubber tube. One end of the rubber tube was fitted over the mouth of a test tube to serve as a container for the ejaculate. The other end of the tube was fitted over a key ring 1 5/8 inches in diameter with a clamp. The rubber tubing McKenzie used was soft and thin-walled, with an outside diameter of 1 1/4 inches. He said the sow used as a mount for the boar need not be in heat but must be restrained. However, Polgè (1956) said the sow must be in heat to obtain a good semen collection. McKenzie (1931) recorded that the average volume of 72 cubic centimeters of semen was collected from the 8-month-old boars. Milovanov (1932) and Rodolfo (1934) also collected boar semen using a dummy sow and the artificial vagina. Hutchings and Andrews (1945) described the collections of semen from a large number of boars. They used an artificial vagina similar to that used for bulls, only smaller.

Ito et al. (1948a,b) considered the use of a dummy sow and the artificial vagina as the best way of collecting boar's semen. They classified the boar ejaculates into five categories of 1, 2, 3, 4, and irregular. The average values they found for normal boars are as follows:

(1) Quantity of semen:

The entire ejaculate: 225.7 (65-680) cubic centimeters

The liquid portion: 178.8 (38-580) cubic centimeters

The gelatinous portion: 46.9 (10-205) grams

(2) Number of spermatozoa:

Per 1 cubic centimeter: 245 (43-730) millions

Per ejaculate: 43.79 (5.13-142.52) billions

(3) pH of fresh semen: 6.4-7.4

They found that an interval of 5 to 6 days between collections gave less variance in volume of semen, sperm number, sperm morphology, and duration of sperm motility. The proper storage temperature for the spermatozoa keeping their vigorous motility was between 15 to 20°C.

Herrick (1949) collected boar semen using a modified bovine artificial vagina. The volume of boar semen was 150 milliliters, and the gelatinous part was about half of this volume. Wallace (1949) in his study of castrated pigs and stilbestrol-treated pigs found total volumes of 100 to 350 milliliters, with a concentration of 1 to 2 x 10^8 spermatozoa per milliliter.

Glover (1955) classified boar semen in a manner much like that used by McKenzie et al. (1938). Glover, however, collected the sample in fractions. These were pre-sperm fraction, high sperm fraction, and post-sperm fraction. Ninety to 100 milliliters was the volume of the high sperm fraction, while the pre- and post-ejaculate volumes were between 10 and 30 milliliters. The concentration of semen found in the high sperm fraction was 40 x 10^6 spermatozoa per milliliter. The total concentration

of pre- and post-sperm fractions ranged from 5×10^6 to 30×10^6 cells per milliliter.

Polge (1956) found the average volume of boar semen produced to be 200 milliliters with a range from 100 to 500 milliliters. The concentration of semen samples in Polge's studies was 1.0×10^8 spermatozoa per milliliter. Aamdal and Hogset (1957) found volumes of boar semen averaged 261 milliliters with the gelatinous part averaging 59 milliliters. They reported 2.61×10^8 spermatozoa per milliliter corresponding to the sperm-rich fractions, and the total spermatozoa per ejaculate was 52.8×10^9.

Hancock (1959a) obtained in the first ejaculate an average of 173.7 milliliters of boar semen and in the second ejaculate an average of 156.9 milliliters of semen. However, he did not give the concentration values for the samples collected. Foote et al. (1959) found in boars from 47 to 52 weeks old an average of 245 milliliters of semen collected, with 68 milliliters corresponding to the gelatinous part. The concentration was 2.75×10^8 cells per milliliter or an average of 470×10^8 spermatozoa per ejaculate.

According to Dziuk (1959), Niwa (1961), Aamdal (1964), and Campbell and Lingam (1965), boars can be trained to mount the dummy sow in a short period of time. However, various training methods were used by these authors. Niwa (1961) suggested three important characteristics have to be considered in the fabrication of a dummy sow. It should be adjustable to variable heights, be stable, and be free of obstruction underneath. Ito et al. (1948a,b), Polge (1956), Aamdal and Hogset (1957), and Aamdal et al. (1958) have shown in their studies some improvements in the dummy

sow and artificial vagina. Dziuk (1958) substituted the dummy for a live sow after the boar had established a considerable association with the collection procedure and surroundings.

DuMesnil duBuisson and Dauzier (1959), Niwa (1961), and Campbell and Lingam (1965) achieved better results in the collection of boar semen using the dummy smeared with urine, vaginal secretions, or semen from other boars. Campbell and Lingam (1965) suggested the more aggressive the animal, the more readily it reacted to the dummy association.

The gloved-hand technique is another method of boar semen collection. This was developed through modification of the artificial vagina. Since Hancock and Hovell (1959) and Melrose and O'Hagan (1959) described this technique, it has become accepted as one of the best methods for collecting boar semen. Herrick and Self (1962) also described the gloved-hand technique for collecting boar semen. This technique consists of grasping the extended penis with the gloved-hand and applying pressure to simulate the cervical constriction of a sow. Singleton and Shelby (1972), Salamon (1973), and Crabo and Hurtgen (1977) also reported that gloved-hand technique was a satisfactory method for collecting boar semen.

The frequency of semen collection has been discussed by several authors. McKenzie et al. (1938) found that collecting every 48 hours or longer, the volume of semen remained above 200 milliliters and the total number of spermatozoa stayed above 20×10^9 cells. However, Dziuk and Henshaw (1958) suggested collecting at 72-hour intervals resulted in good and representative semen samples from boars ranging in age between 7 months and 4 years old.

Niwa et al. (1959) found that if the collection intervals are shortened, the quantity of semen and the number of spermatozoa decreases. Also, motility and viability of sperm are affected, especially the appearance of abnormal spermatozoa is increased. Later, Niwa in 1961 found that semen collected at intervals of more than 3 days had only 70 to 80 percent of normal semen quality. Better sample quality occurred when the interval of collection was 5 to 6 days. This was in partial agreement with Gerrits et al. (1962) whose results are summarized in Table 1.

Table 1. Volume and concentration of boar semen affected by the collection intervals (Gerrits et al., 1962)

Collection time	No. of ejaculates	Volume ml	Sperm/ml $x10^6$	Total sperm $x10^9$
Every 4 days	5	286.3	277.5	54.9
Every other day	10	237.3	192.0	39.5
Daily	20	193.1	142.9	23.7

Young et al. (1960) explained the turbidometric method for estimating sperm concentration in ram and bull semen, which was adapted to evaluate boar semen concentration using a photelometer. No significant differences were found between the average sperm counts made by the hemocytometer and transmittancy values. Semen dilution rates of 1:9 and 1:19 gave readings most of which were within the more sensitive range on the photelometer scale. Correlations of 0.95 or higher were obtained between hemocytometer counts of sperm concentration and photelometer readings expressed as 2-log of the photelometer reading. The regression equation

for estimating sperm concentration ($\hat{Y}$) in million/ml in boar semen at 1:9 dilution rate was $\hat{Y}$ = 862.1 x -72.4 and for 1:19 dilution $\hat{Y}$ = 1328.9 x -21.0, where x equals 2-log of the photelometer readings.

Hurtgen et al. (1977) and Crabo and Hurtgen (1977) have suggested that the concentration of boar semen may be found by either use of spectrophotometer or by the hemocytometer counts. However, they have discussed that with some experience the semen concentration can be estimated by the color of the samples. They found that creamy-colored boar semen contained about 1 billion cells per milliliter; milky-colored semen about 300 to 500 million cells per milliliter; and opalescent or watery-colored semen to have about 50 to 200 million cells per milliliter. Yellow and pink colors indicated contamination or presence of blood in the semen collected.

The method used most often for evaluating motility was described by Herrick and Self (1962). Nevertheless, Stevermer et al. (1964) claimed that the motility is an unreliable indicator of fertility in artificial insemination of swine. However, Dziuk (1977) and Graham (1978) suggested motility is one among many other patterns to be measured to estimate the level of fertility of the boar.

The morphology of freshly ejaculated boar semen and semen exposed to various experimental treatments has been studied in stained smears by several workers. Aamdal (1964) found a significant correlation between the percentage of unstained spermatozoa and the conception rate using 10 percent anilin-blue.

The following staining techniques are also used to determine the morphology of boar spermatozoa:

1. Bodians protargol method (Dawson and Barnett, 1944)
2. Gomon's reticulum stain (Mallory, 1938)
3. Negrosin-eosin stain (Hancock, 1952)
4. Giemsa (Hancock, 1957)
5. Periodic acid-Schiff (PAS) (Pearse, 1960)
6. Silver impregnation (Hancock and Trevan, 1957)

The differentiation between live and dead spermatozoa from boars and bulls has been deeply investigated by several authors, among them are: Mercier and Salisbury (1947); Hancock (1959b); Herrick and Self (1962); Shelby and Foley (1964); and Crabo and Hurtgen (1977). Radford (1961) used nigrosin-eosin stain to differentiate live from dead boar spermatozoa; however, the dead cells were only faintly stained. Nevertheless, Shelby and Foley (1964) using different stains found no clear difference between live and dead cells.

III. MATERIALS AND METHODS

Eight 12-month-old Hampshire boars averaging 120 kilograms in body weight were used in this experiment. All the boars were housed in one concrete pen, using sawdust as bedding. They were hand-fed daily at the rate of 2.27 kilograms of feed containing 14 percent protein.

The experiment commenced by applying gloved-hand to all animals. The boars had 72 hours of rest between techniques. This allows the animals to sufficiently recuperate (Dziuk and Henshaw, 1958). A total of eight collections in each boar were made while alternating the collection techniques.

A. Collection of the Sample

1. Anesthesia

The drug used to anesthetize the boars was thiamyl sodium (Surital[1]), which has shown excellent results in this species (Clark, 1976). The recommended dosage is 4.4 milligrams per kilogram up to 180 kilograms, and 2.4 milligrams per kilogram thereafter. Maximum dose should not exceed 8.0 milligrams per kilogram.

In this study, 1 gram of Surital diluted in 10 milliliters of physiological saline was given to each boar (1 gm/160 kg). Before this was achieved, the boar was restrained with a snout snare so that the drug could easily be injected. The period of immobilization was about 30 minutes. However, additional drug of about 1/2 of a gram was given to

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[1]Surital, Parke Davis and Co., Detroit, Michigan.

sustain the anesthesia in boars which weighed more or in boars which were unduly aroused by the stimulations.

The marginal ear vein was the route of administration of the anesthetic. Careful injection into the anterior vena cava was equally effective; however, the danger of intra-arterial (carotid artery) or extravascular injections made this route less desirable.[1] The marginal ear vein was raised and its dorsal border was marked at the point the needle puncture would be made (Figure 1). A sterile 20 gauge, 1 inch needle or a butterfly catheter[2] was used to administrate the drug. Three-fourths of the anesthetic was given rapidly; seconds later the rest of it was infused. Most of the boars were immobilized within 30 seconds after the total drug was injected. With this procedure a stage of light anesthesia was achieved.

2. Electroejaculation

An electroejaculator machine (Pulsator II[3]) and a specially designed rectal probe[4] were used on the boars.

The probe was made of flexible rubber hose, approximately 45 centimeters in length and 3.75 centimeters in diameter. Onto it were placed 6 metallic electrode rings, which supplied the electrical stimulations.

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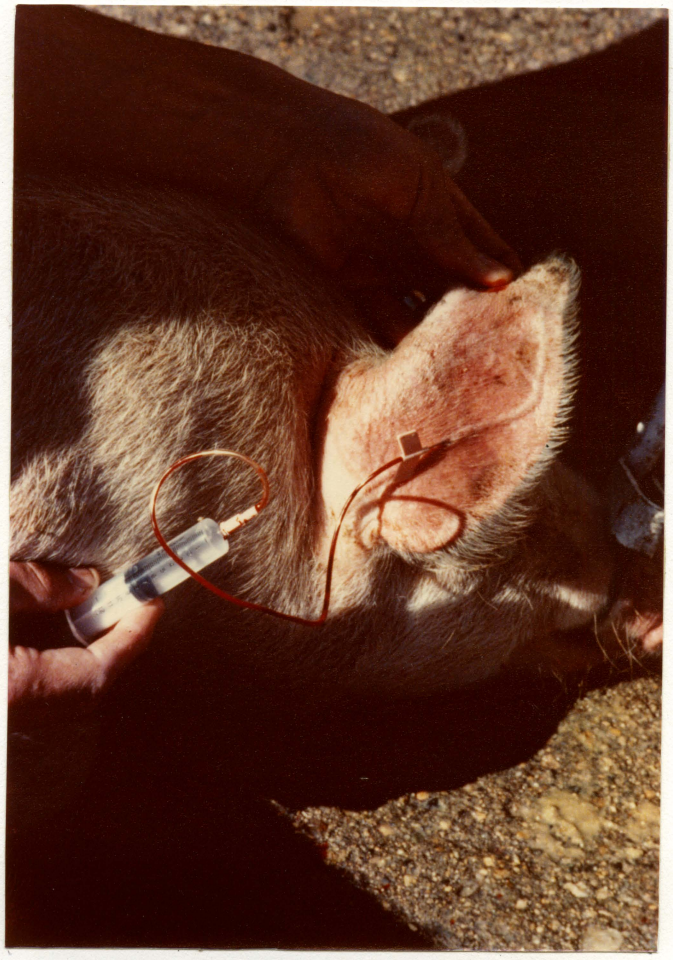
[1]Private communication, Dr. L. E. Evans, Iowa State University, Ames, Iowa.

[2]E-Z Set, Desert Pharmaceutical Co., Inc., Sandy, Utah.

[3]Pulsator II, Lane Manufacturing, Inc., Denver, Colorado.

[4]Boar probe, special order, Tracy Clark, Iowa State University, Ames, Iowa.

Figure 1. Marginal ear vein is the place where the needle puncture would be made to infuse the anesthetic.



Each ring was 0.7 centimeters wide and placed with a 3.5 centimeter space from adjacent rings (Figure 2).

The normal curvature of the probe allows good contact with the pelvic plexi nerves stimulating the reproductive organs.

3. Preparation of the animals

Once the boar was anesthetized, the long hair around the sheath was clipped. The fluid contents of the preputial diverticulum was discarded before collection was made, followed by drying the boar's sheath with a paper towel. At the same time, the excessive fecal material was removed from the last portion of the boar's rectum. The last procedure allowed better contact between the probe and the pelvic nerves. Before the probe was placed into the boar's anal orifice it was lubricated with jelly.[1] This aided in the insertion of the probe and avoided injury to the rectum.

The probe was inserted 25 to 30 centimeters until the last ring was just inside the anal sphincter.

It is extremely important to have the boar's penis out of the sheath prior to ejaculation in order to avoid contamination of the semen sample with fluid from the preputial diverticulum.

To achieve protrusion of the penis from the sheath an atraumatic forceps[2] was used. It was inserted into the prepuce until the nonerect penis was grasped and pulled out from its place.

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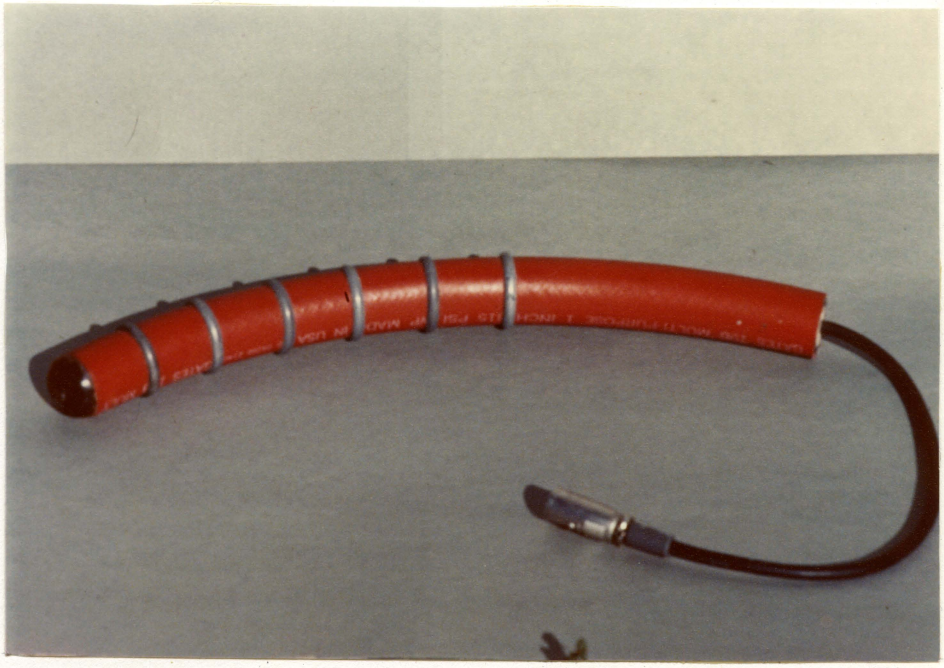
[1] K-Y Jelly, Johnson and Johnson, New Brunswick, New Jersey.

[2] Bozeman uterine forceps, Arista Surgical Supply Co., New York.

Figure 2. Rectal boar probe. Note that 6 metallic electrode rings are located on the curvature of the probe. The cord is plugged into the electroejaculator.



Sometimes it was necessary to give electrical stimulations before the forceps were introduced into the sheath. This caused some penile erection so that it could be grasped with the forceps easily.

There was no evidence of serious traumatic injury to the penis with this method. Once exteriorized, the penis was grasped with a sterile 4 x 4 gauze pad and gently extended out of its original place.

4. Stimulation and collection

The pattern of electrical stimulation used depended upon the response of each boar. The lowest voltage output was used to begin the stimulation. The boar was stimulated for 5 to 6 seconds and then rested for 8 to 15 seconds which allowed the boar to take 3 to 4 respiratory breaths before restimulation. Usually a total of 5 to 6 stimulations within each voltage selection was used. The amplitude of the stimulation was gradually increased until the desired ejaculate was collected. Each new stimulation was done slowly (Figure 3).

The semen was collected in a 250 milliliter insulated pre-warmed (37°C) plastic thermos bottle fitted with a sterile plastic bag. The opening was covered with sterile 4 x 4 gauze, to separate the gelatinous parts from the sperm-rich fraction. The tip of the penis was left uncovered with gauze so that the examiner could see the ejaculate during collection (Figure 4).

5. Training of boars

The eight virgin boars were trained to mount the dummy sow for semen collection. The training period took almost 1 month for all of the boars to be trained. The surface of the dummy was covered with carpeting to

Figure 3. In this picture, note the rigidity of the posterior limbs of the boar. This is seen when the stimulation is applied. Note the rectal probe in the right place.

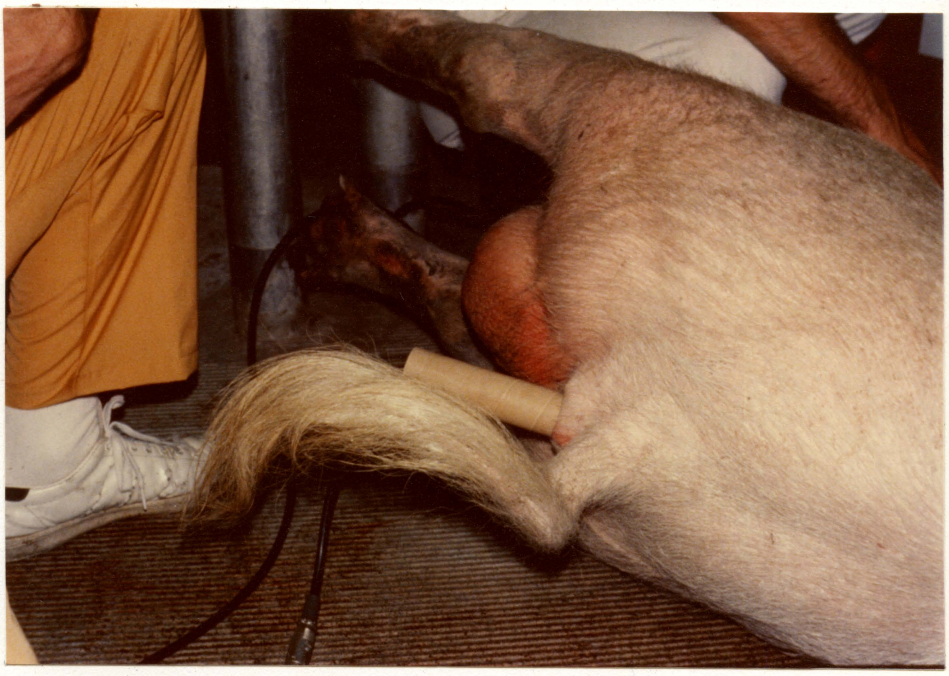


Figure 4. The collection of boar semen takes place with the boar in lateral recumbency when electroejaculation is used. The tip of the penis is uncovered. The thermos bottle is a perfect container to collect the sample.

preserve the odor from previous collections (Polge, 1956; Niwa, 1961). The dummy was smeared with urine, vaginal mucus from a sow and semen from other boars. The boar's pen was arranged in such a manner that all boars could see the collections being made. This familiarized the boars with the collection area. Campbell and Lingam (1965) considered this exposure very important, because the boar builds up a conditioned reflex which greatly facilitates collections.

6. Gloved-hand collection

Semen was collected from boars using the technique described by Hancock and Hovell (1959). The sperm-rich fraction was collected by gloved-hand for comparative studies with semen samples collected by electroejaculation. Two pre-warmed (37°C) thermos bottles covered with sterile 4 x 4 gauze to separate the gelatinous part were used to collect the semen. A sterile plastic bag was placed in one thermos bottle to collect the sperm-rich fraction. The other bottle was used to collect the pre- and post-sperm rich fractions together. Both collections were made in order to check the total number of spermatozoa per ejaculation.

B. Sample Analysis

After each collection of semen was made, using the gloved-hand or electroejaculation methods, the sample was taken immediately to the laboratory. Volume, pH, color and concentration were determined macroscopically, while motility, morphology and percentage of live cells were determined microscopically using the light microscope.

1. Volume, pH, color and concentration

From each semen sample the following characteristics were determined. Volume was measured in a transparent graduated container. The pH was assessed by litmus paper. Color was recorded after visual examination. The concentration was determined by spectrophotometry (Table 7).

These characteristics were checked for the sperm-rich fraction and for the nonsperm-rich fraction when the gloved-hand technique was used.

2. Motility

The motility of the semen samples was subjectively graded in percentage. However, the samples were also given a number from 1 through 5 according to the scoring used in bulls by Asdell in 1955 (Table 2).

Table 2. Scoring used for motility of boar semen

Score	Description	Percentage (%)
1	All spermatozoa nonmotile	0-30
2	Weak oscillatory motion	31-50
3	About equal proportion with progressive and oscillatory motion, 25% nonmotile	51-70
4	Most with progressive motion	71-90
5	Nearly all with high progressive motion	91-100

3. Morphology and percentage of live cells

These parameters were evaluated using the light microscope. The spermatozoa abnormalities were classified according to Aamdal's (1964) proposal.

Before the evaluation commenced, all equipment such as slides, coverslips, stains, and pipets, were pre-warmed to 37°C. The morphology of the spermatozoa was determined from the raw semen. The semen was placed on the slide at the rate of one drop of sperm-rich fraction and one or two drops of saline and then covered with a coverslip. Two staining methods were used; one differentiated the live cells, nigrosin-eosin stain (Hancock, 1957) (Table 8) and the other stain differentiated acrosomal damage, buffered formal saline (Hurtgen et al., 1977) (Table 9).

The slides were marked with the boar number, date of collection and technique used for their identification. Stained smears of each sample were examined at 450x and 1000x magnification.

The microscopic examination covered five to ten microscopic fields. A total of 500 cells were examined for the percentage of live cells and spermatozoa abnormalities.

C. Statistical Analysis

The data from this study were analyzed statistically by the use of a computer. An analysis of variance of whole plot and split plot design was utilized in examining each variable involved in this experiment. Therefore, interval collection; pig; and pig*interval collection corresponded to whole plot, while technique; tecnnique*pig; and pig*technique*interval collection corresponded to split plot. F-tests were used to test the effect(s) upon the variables used.

IV. RESULTS

The volume, total sperm concentration, and pH were compared with sperm-rich samples collected by electroejaculation and gloved-hand technique. The results are listed in Table 3.

The results from Table 3 show that larger volumes of semen and total spermatozoa were obtained by gloved-hand total collection (GH/T). This included sperm-rich fraction plus pre-sperm and post-sperm fractions.

It is very important to say, however, that during this experiment most comparisons were made between sperm-rich fractions collected by electroejaculation (EE/SR) and gloved-hand (GH/SR).

Table 3 also shows that of a total of eight boars, only two boars (boar Nos. 2 and 8) had a greater volume of sperm-rich fraction using electroejaculation rather than gloved-hand technique. However, a higher total sperm production per ejaculate occurred in four boars (boar Nos. 2, 4, 5 and 6) in which electroejaculation was used. This demonstrates that for some boars there may be a greater advantage to use electroejaculation instead of gloved-hand technique for semen collections.

The average pH from the samples collected by electroejaculation (EE/SR) was slightly higher (7.01) compared with those collected by gloved-hand (GH/SR) (6.86). One possible explanation to this may be that samples with higher sperm concentration tend to have a lower pH value. However, there is always exception as demonstrated by 2 boars (Nos. 2 and 5 in Table 3).

The values of volume, total sperm and pH of boar semen are respectively represented as histograms in Figures 5, 6 and 7.

Table 3. Comparison of average values of volume, total sperm, and pH of 8-boar semen collected by electroejaculation (EE) and by gloved-hand (GH) techniques; spectrophotometer was used to determine the sperm counts

Boar No.	Volume (ml)			Total sperm x 10^9			pH		
	EE/SR[a]	GH/SR[b]	GH/T[c]	EE/SR	GH/SR	GH/T	EE/SR	GH/SR	GH/T
1[d]	22.50	33.75	91.25	24.29	26.93	34.33	7.12	6.90	7.07
2	63.75	39.50	89.50	57.05	29.56	39.93	7.02	6.90	7.05
3	20.00	49.25	149.25	24.05	49.66	53.73	7.02	6.80	6.95
4	35.00	41.25	113.75	28.19	25.56	38.13	7.02	7.02	7.02
5	16.25	17.50	45.00	21.06	17.30	25.16	6.90	6.80	7.01
6	31.25	56.25	126.25	46.81	32.33	46.66	6.80	6.90	7.00
7	30.00	61.75	170.50	19.44	44.56	51.20	7.20	6.80	7.00
8	43.75	34.50	109.50	41.20	44.86	50.06	7.01	6.80	7.00
Mean	32.81	41.71	111.87	32.76	33.84	42.40	7.01	6.86	7.01
Range	(16.25-63.75)	(17.50-61.75)	(45.00-170.50)	(19.44-57.05)	(17.30-49.66)	(25.16-53.73)	(6.80-7.20)	(6.80-7.02)	(7.07-6.95)

[a]EE/SR - electroejaculation/sperm-rich fraction.

[b]GH/SR - gloved-hand/sperm-rich fraction.

[c]GH/T - gloved-hand/total (sperm-rich and nonsperm-rich fraction).

[d]Four collections of each technique for each boar.

Figure 5. The histogram shows the differences in semen volume from 8 boars. Comparisons were made between EE/SR and GH/SR. Values for GH/T are also shown.

Figure 5

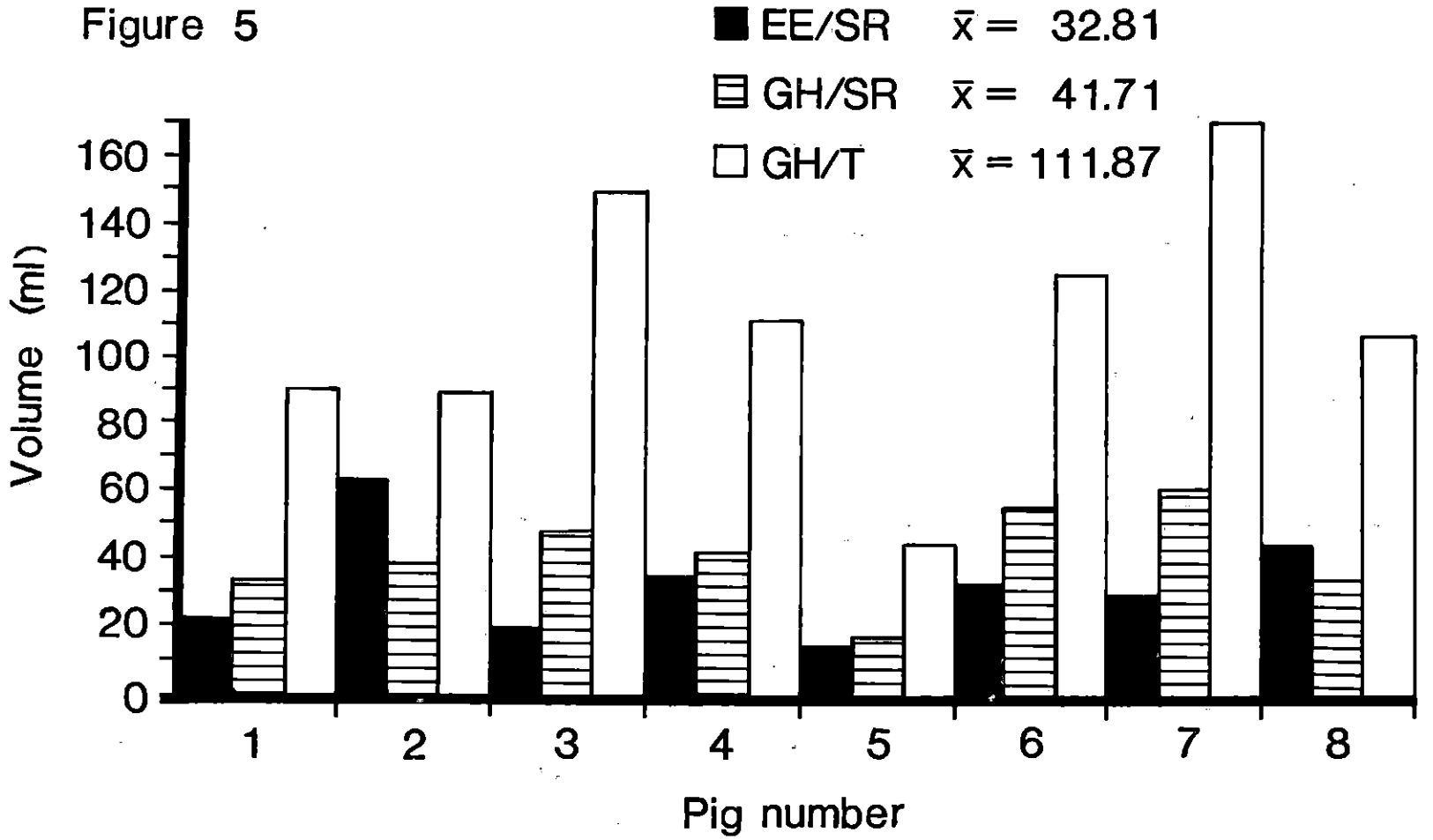

EE/SR x̄ = 32.81
GH/SR x̄ = 41.71
GH/T x̄ = 111.87
Volume (ml)
160
140
120
100
80
60
40
20
0
1
2
3
4
5
6
7
8
Pig number

Figure 6. The histogram shows the differences in total semen concentrations from 8 boars. Comparisons were made between EE/SR and GH/SR. Values for GH/T are also shown.

Figure 6

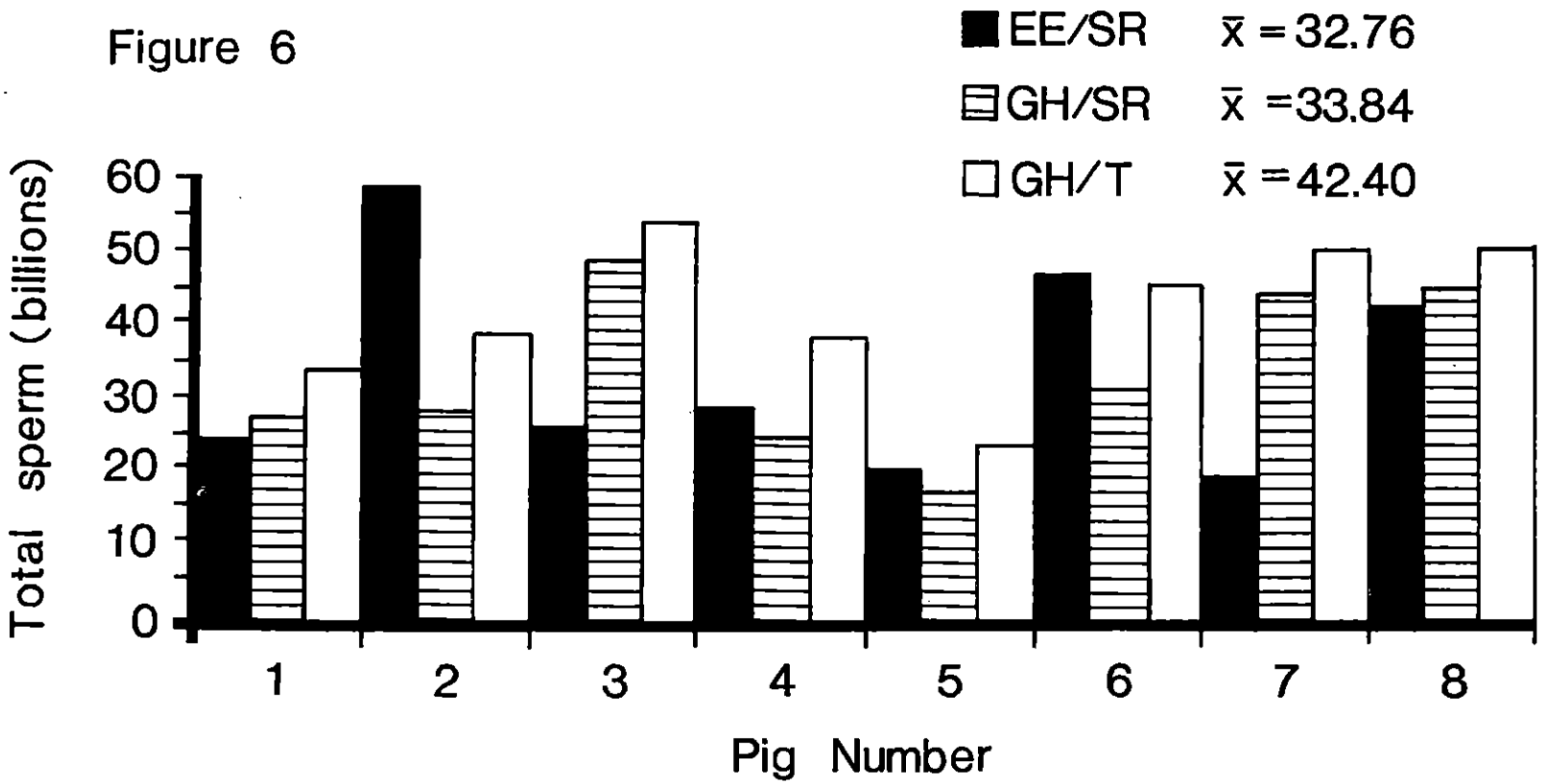
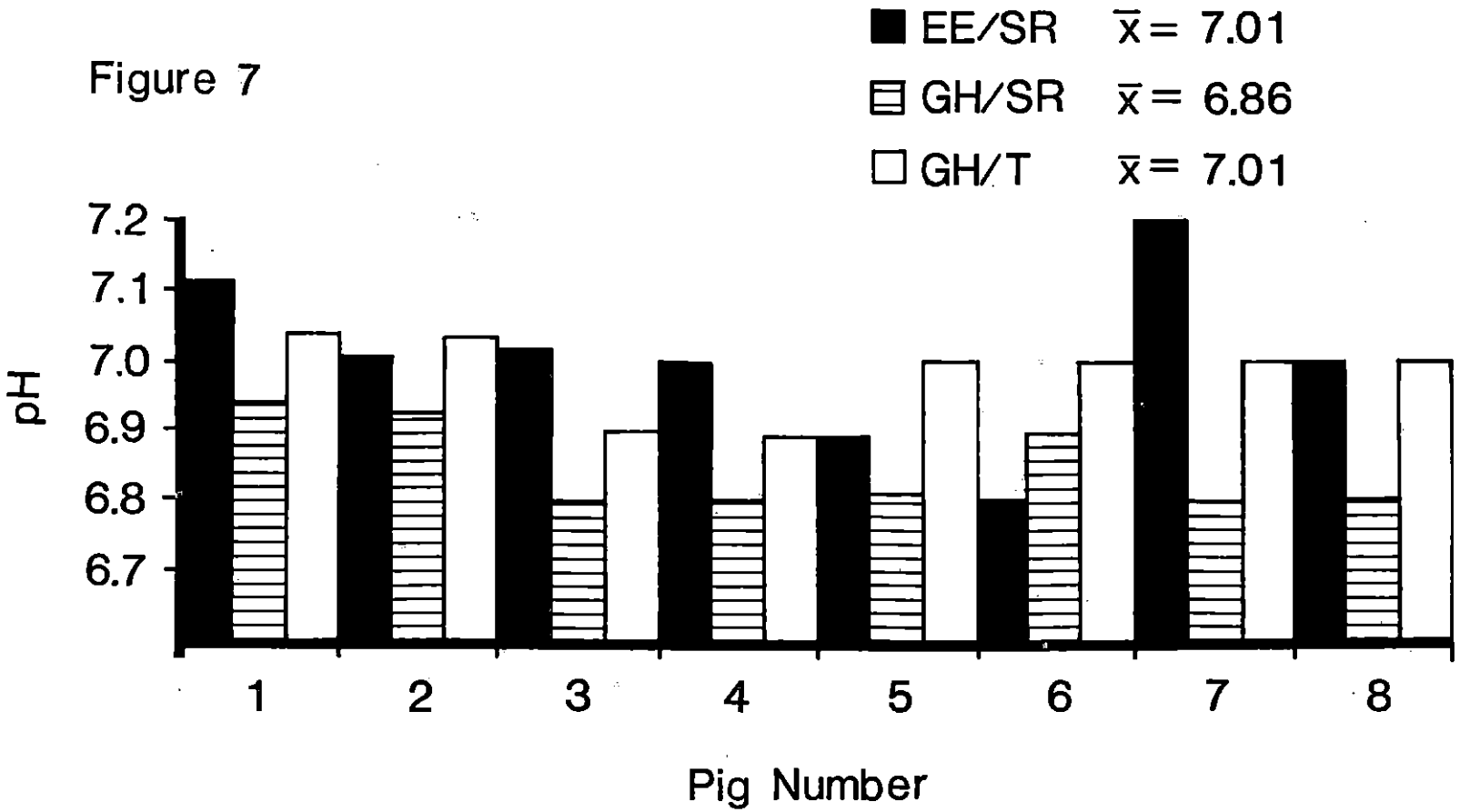

EE/SR x̄ = 32.76
GH/SR x̄ = 33.84
GH/T x̄ = 42.40
Total sperm (billions)
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
Pig Number

Figure 7. The histogram shows the differences in pH semen from 8 boars. Comparisons were made between EE/SR and GH/SR. Values of GH/T are also shown.

Figure 7


EE/SR x̄ = 7.01
GH/SR x̄ = 6.86
GH/T x̄ = 7.01
7.2
7.1
7.0
6.9
6.8
6.7
pH
1
2
3
4
5
6
7
8
Pig Number

Results in Table 4 show that there was not a significant difference in total sperm produced among pigs or the interval between collections. In the same manner, there was not a statistical difference in total sperm produced between techniques used. However, there was a statistically significant difference ($P < .05$) in total spermatozoa between techniques and pigs together. Although this was relatively unimportant for some boars it resulted because of marked difference for others. In other words, there were boars that performed better using electroejaculation rather than gloved-hand techniques and vice-versa. See also Table 10.

Table 4. Analysis of variance for the linear model of 6 traits[a]

Source of variance	Degrees of freedom	Sum of squares	Mean square	F-value
Whole plot				
Interval collection	3	173.001	57.667	0.332
Pig	7	2952.038	421.720	2.428
Error (a): Pig*interval collection	21	3648.207	173.724	1.127
Split plot				
Technique	1	63.980	63.980	0.415
Technique*pig	7	3297.878	471.125	3.057[b]
Error (b): Pig*tech*interval collec.	24	3698.597	154.108	-
Corrected total	63	13833.701	-	-

[a]Standard deviation = 12.839; overall mean - 27.424

[b]Statistically significant at 5% probability level.

Neither technique used in the collection of boar semen significantly influenced the percentage of live cells, nor the production of abnormal spermatozoa. But it was observed that there were fewer spermatozoa with coiled tails, malformed middle pieces and malformed heads using electroejaculation than with gloved-hand collections. On the other hand, the higher percentages of distal and proximal droplets and fewer dag tails corresponded with the use of electroejaculation. However, the total abnormalities mentioned in this study never exceeded 20 percent of the total cell counts regardless of collection technique (Table 5).

Motility was subjectively evaluated by light microscope using the sperm-rich fraction only. The progressive motility was estimated under 100x and 450x magnification. The samples were rated with a number from 1 to 5 ranging from poor to excellent motility, respectively. The results are partially in agreement with those described by Dziuk et al. (1954a) and Foote (1974). They found no significant difference in motility of boar semen collected by electroejaculation or by gloved-hand techniques. However, in this study there was a slight increase in the percentage of cells in progressive motility using electroejaculation. This value was obtained by visual estimations, a factor subject to considerable error. The comparison of motility between electroejaculation and gloved-hand techniques are shown in Table 6.

Table 5. Morphological study of 8-boar semen; percentage counts of 500 cells per sample from each technique

Boar	Date	Technique	Distal droplets	Proximal droplets	Coiled tails	Dag tails	Malformed middle piece	Malformed heads
1	7/6/78	GH	5	0	2	0	0	1
	7/13	GH	5	1	2	2	1	0
	7/20	GH	3	2	2	1	0	0
	7/27	GH	5	2	1	2	0	1
	7/10	EE	2	1	1	0	0	0
	7/17	EE	3	1	2	4	2	0
	7/24	EE	10	5	2	2	0	1
	7/31	EE	4	3	1	2	0	0
2	7/6	GH	2	1	2	0	3	0
	7/13	GH	2	0	2	2	0	0
	7/20	GH	3	2	2	1	0	0
	7/27	GH	5	3	1	1	0	0
	7/10	EE	2	1	1	0	0	0
	7/17	EE	2	0	2	2	0	0
	7/24	EE	3	1	1	2	0	1
	7/31	EE	3	2	1	1	0	0
3	7/6	GH	2	0	1	2	0	0
	7/13	GH	1	1	2	0	0	0
	7/20	GH	5	3	2	4	0	1
	7/27	GH	9	3	2	1	0	0
	7/10	EE	2	1	2	1	0	0
	7/17	EE	3	0	2	1	0	0
	7/24	EE	5	4	2	5	0	0
	7/31	EE	5	2	0	1	0	0
4	7/6	GH	2	1	2	1	0	0
	7/13	GH	2	1	2	0	1	0
	7/20	GH	5	3	1	1	0	0

Table 5. (Continued)

Boar	Date	Technique	Distal droplets	Proximal droplets	Coiled tails	Dag tails	Malformed middle piece	Malformed heads
	7/27	GH	2	1	6	2	0	0
	7/10	EE	1	2	2	2	0	0
	7/17	EE	5	1	3	2	0	0
	7/24	EE	3	2	1	1	0	0
	7/31	EE	7	4	1	4	0	0
5	7/6	GH	1	2	1	0	0	0
	7/13	GH	2	1	2	1	0	1
	7/20	GH	2	2	4	9	0	0
	7/27	GH	5	3	2	1	0	0
	7/10	EE	3	2	2	1	0	0
	7/17	EE	5	1	2	2	0	0
	7/24	EE	6	5	1	1	0	0
	7/31	EE	7	4	3	2	0	0
6	7/6	GH	2	2	1	0	0	0
	7/13	GH	3	5	5	2	0	0
	7/20	GH	2	2	1	2	0	0
	7/27	GH	8	1	1	1	0	0
	7/10	EE	3	2	2	0	0	0
	7/17	EE	5	2	1	2	1	0
	7/24	EE	8	3	1	1	0	0
	7/31	EE	5	6	1	1	0	0
7	7/6	GH	2	2	2	2	0	0
	7/13	GH	3	4	1	1	0	0
	7/20	GH	3	3	4	5	1	2
	7/27	GH	6	2	3	3	0	2
	7/10	EE	3	3	2	1	2	1
	7/17	EE	3	3	2	3	0	0
	7/24	EE	5	2	5	7	0	0
	7/31	EE	7	4	1	1	0	0

Table 5. (Continued)

Boar	Date	Technique	Distal droplets	Proximal droplets	Coiled tails	Dag tails	Malformed middle piece	Malformed heads
8	7/6	GH	2	3	1	1	1	1
	7/13	GH	5	2	0	1	2	0
	7/20	GH	3	1	2	3	0	0
	7/27	GH	4	2	2	3	0	0
	7/10	EE	1	2	1	1	1	1
	7/17	EE	10	1	2	1	0	0
	7/24	EE	6	2	2	1	0	0
	7/31	EE	3	3	0	2	0	0
% average and range		EE	4.2 (1-10)	2.3 (0-6)	1.6 (0-5)	1.7 (0-7)	0.1 (0-2)	0.1 (0-1)
		GH	3.4 (1-9)	1.9 (0-5)	2.0 (0-6)	1.6 (0-9)	0.28 (0-3)	0.28 (0-2)

Table 6. Comparison of motility of 8-boar semen collected by electroejaculation and gloved-hand techniques

Boar No.	Electroejaculation	%	Gloved-hand	%
1	4[a]	75[b]	4	75
2	4	85	5	95
3	4	85	5	95
4	5	95	4	71
5	5	95	5	95
6	5	95	4	85
7	3	51	4	85
8	5	95	3	51
Mean	4.37	83	4.25	81

[a]Asdell's (1955) classification. (See Table 2.)

[b]Subjective values (average motility; see Table 2).

V. DISCUSSION

Semen evaluation is one of the most critical and yet subjective procedures in work with boar semen. Though the ultimate criterion is fertility, it was not possible to accomplish this evaluation on the samples collected in this study, because no females were impregnated with the semen.

The observations made in this study of boar semen collected by electroejaculation or gloved-hand found no significant difference between samples in volume of sperm-rich fractions, cell morphology, pH, total sperm numbers and percentage of live cells. However, the most significant difference found in this study compared with results found by Dziuk et al. (1954a), Roberts (1971), Foote (1974), and Hurtgen et al. (1977) was the volume of semen collected by electroejaculation. They were only able to collect small volumes of semen using electroejaculation in boars. This difference may be in part explained by the type of boar probe utilized and the method used to restrain the animals.

The probe used in this study had the improvement of flexibility and good conductivity. This produced better stimulations to the pelvic nerves which resulted in a high volume of semen collected without changes in its quality. The pattern of stimulations given to the boars was variable and depended on the individual. However, it was observed that when the boars were subjected to heavy stimulations, meaning high peaks of voltage, there was usually emission of high volumes of semen.

Anesthesia used in this study immobilized the boars and led to the collection of considerably more sperm-rich fraction which was nearly free

of mucus. At the same time, this allowed the sample to be collected free of contaminations, while contamination frequently occurred with gloved-hand collections. Therefore, electroejaculation is desirable when the examiner needs to culture the semen.

If the anesthesia was injected slowly the animal became excited and slightly ataxic before the total muscular relaxation became sufficient to cause lateral recumbency. Therefore, it was advisable to inject the anesthetic rapidly. The dosage of the drug used was sufficient to achieve muscular relaxation allowing the collection of semen samples to be taken with minimum or no restraint.

Rapid recovery and very few undesriable clinical signs were seen in the boars with the application of this anesthesia. It was observed, however, that heavier boars required additional amounts of the drug. This often amounted to an extra one-half to one gram of the anesthesia. In these cases the time required to recover was usually longer.

The anesthesia was utilized a total of 32 times in this study without evidence of adverse effects in the animals. When used at the dose specified on the label, the ultra short acting barbiturates appeared to be very safe in boars.

The time required for semen collection with each technique was not recorded, but it appeared that electroejaculation was more efficient in most boars.

Normally the gloved-hand technique required only a few minutes for collection. However, it was frequently noted that four boars displayed poor libido and required considerably more time and alteration in the

collection arrangements to stimulate interest in the dummy mount. In contrast, electroejaculation usually required 10 to 12 minutes. Furthermore, when electroejaculation was used the collection did not depend upon the behavioral activity of the boar. Therefore, electroejaculation also allows the collector to predict more closely the time the semen would be in the laboratory. In addition, electroejaculation can be used in shy or mean boars and those boars which are unable to mount. Their semen may be used in the herd for artificial insemination.

It was also observed that after using electroejaculation the libido of the animals was not harmed. On the contrary, some boars showed sexual interest after recovery from anesthesia and tried to mount the dummy sow.

Progressive motility and concentration are two important parameters in the study of boar semen; they are correlated in conjunction with other factors with the fertility of boar semen (Graham, 1978). As shown in Tables 2 and 3 the gross motility and total sperm are often closely related.

Since the boar semen has a relatively low concentration of spermatozoa per milliliter, volume was considered an important parameter when electrojaculation was performed.

The results of this experiment have shown that the average volume of the sperm-rich fractions obtained by electroejaculation and gloved-hand collections were 32.81 milliliters and 41.71 milliliters respectively and were not significantly influenced by collection technique performed (Table 3).

There was considerable variation in the total number of spermatozoa in sperm-rich fractions obtained by electroejaculation from the same boar on different occasions (boar No. 3). This was in part expected as normal individual difference and partly to variance in the amount and rate of stimulations. However, the same variance occurred using gloved-hand. Two boars (boar Nos. 1 and 6) had wide variance in concentration in their sperm-rich fractions throughout the experiment.

Morphological evaluation of semen from the sperm-rich fractions was determined with the light microscope. Although the contrast microscope and the electron microscope were considered in their use, they were not needed in this study because abnormalities occurred infrequently.

The morphology of the spermatozoa was observed with the nigrosin-eosin stain. It was noted that using this stain the attachment of the tail to the sperm-cell head was often in an off center position. This phenomenon, "abaxial attachment," was described by Hancock (1959b) as a normal configuration of boar semen when stained.

Semen collected by electroejaculation has shown to have fewer abnormal heads, malformed middle pieces and coiled tails, compared with the collections made by gloved-hand technique. But a greater percentage of distal droplets, proximal droplets and dag tails defects occurred in collections by electroejaculation.

Proximal and distal droplets have been considered as "normal" morphology in boar semen because they have no detrimental effect on fertility (Graham, 1978; Crabo and Hurtgen, 1977), although this has not been fully clarified.

A further consideration affecting semen volume and quality was the change in environmental condition as summer progressed. This can be seen in Table 5. Distal droplets and proximal droplets in the semen samples seem to increase as summer temperature increased. Coiled tails and dag tails were slightly reduced when the collection periods advanced. On the other hand, malformed middle pieces and malformed heads showed no significant changes. However, from a total of eight normal boars examined in this study, five of them had increased numbers of cell abnormalities in their semen as summer advanced.

During this experiment the morphology of the spermatozoon was not appreciably affected when electroejaculation was used. This is in agreement with results reported by Dziuk et al. (1954a). Furthermore, the same occurred in bulls and sheep which seems to have no morphological effect in their semen collected by electroejaculation (Rowson and Murdock, 1954; Mattner and Voglmayr, 1962).

Less important parameters are pH and color which were influenced in part by the method of collection and total volume. However, the difference in color and pH between electroejaculation and gloved-hand technique were not significantly different. Nevertheless, the little difference which may exist between collections may be associated with the time of season, environmental changes and individual boar differences.

VI. CONCLUSIONS AND SUMMARY

Electroejaculation and gloved-hand techniques were compared as methods for obtaining satisfactory semen samples. Various parameters frequently utilized in assessing semen quality were applied to samples collected by each technique.

In this study collections by either technique had no significant influence on the proportion of live spermatozoa, cell morphology, progressive motility, cell concentration, volume color and pH of boar semen.

It was observed that the percentage of malformed heads, malformed middle pieces, and coiled tails was usually lower when semen was collected by electroejaculation than when the gloved-hand collection was used. However, using electroejaculation the percentage of distal and proximal droplets was higher. No explanation was found for these differences.

The two methods of collection were evaluated clinically as techniques of general use of boar semen studies.

When electroejaculation was practiced, anesthesia was used to achieve better collection results. This allowed the proper restraint of the animal at the time of stimulation, followed by smooth, rapid and safe recovery of the animal.

Electroejaculation can also be used to collect semen of boars that suffer some physical disabilities or are unwilling to mount. If their semen is good, it may be used for insemination and breeding. The practitioner will also be able to examine the boar's prepuce and penis when semen is being collected. Once finished with the collection, he may check the testicles as well as the whole body. Therefore, electroejaculation

technique should be recognized as a valid and suitable method for semen collection in the boar.

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IX. APPENDIX

Table 7. Spectrophotometer[a] used in the counts of boar semen cells

1. Power switch on for 10 minute warm-up period.
2. Instrument set at 550 millimicrons wavelength.
3. Zero instrument with Zero Control knob with no sample in the instrument and sample holder closed.
4. Fill blank standard tube with 5 ml distilled water. Insert blank with line of tube to front. Close cover and adjust glavanometer with Light Control knob so needle reads 100% transmittance.
5. The apparatus was calibrated by diluting the semen in sephadex columns to a dilution 1:20.
6. Aspirate 0.20 ml of rich semen fraction with a micro-pipette.[b] Wipe the tip of the pipette with tissue paper and transfer to a tube which previously had 4.8 ml of 2% acetic acid.
7. Mix by inversion 5x; do not shake.
8. Wipe tube with a cloth or by 4 x 4 gauze.
9. Insert the tube in the knob. Close the cover, make the reading immediately in % transmittance.
10. The results were in millions of cells per cubic millimeter.

[a] Spectronic 20, Baush and Lomb.

[b] Micro-pipette, Micro-Folin-Pipets DADt Division, Miami, Florida.

Table 8. Stain formula used in the differentiation of live spermatozoa (Hancock, 1957)

Compounds	Quantity
Nigrosin solution[a]	150 ml
Eosin Y (G. T. Gurr)	5 gm
Stock buffer solution	30 ml
Stock glucose solution	30 ml
H_2O	to 300 ml

[a]20 gm nigrosin (G. T. Gurr) are added to 100 gm water and dissolved by boiling under a reflux condenser.

Table 9. Stain formula to differentiate acrosomal abnormalities, buffered formal saline (Hurtgen et al., 1977)

Compound	Quantity
$Na_2HPO_4 2H_2O$	6.19 gm (4.93 g Na_2HOPO_4)
KH_2PO_4	2.54 gm
38% formaldehyde	125 ml
NaCl	5.41 gm
Distilled water	add to 100 ml

Table 10. Statistical hypothesis from only four main variables

Variable	Discussion
Pig	No statistical difference among pigs.
Technique	F-value not significant, thus no difference between them.
Technique*pig	Was statistically significant 5% probability.
Interval collection	No statistical difference along with the repetition altering both techniques.