The effect of progesterone in liquid semen extender on fertility and spermatozoa transport in the pig

by

Joaquin A. Becerril

A Thesis Submitted to the

Graduate Faculty in Partial Fulfillment of the

Requirements for the Degree of

MASTER OF SCIENCE

Department: Veterinary Clinical Sciences
Major: Veterinary Clinical Sciences
(Theriogenology)

Iowa State University
Ames, Iowa

1982

TABLE OF CONTENTS

I. INTRODUCTION

Artificial insemination offers great possibilities for pig production through the use of progeny-tested boars. The use of artificial insemination in swine is not yet as widespread as in cattle. During the past twenty years, diluents for preserving boar semen in the liquid state at 5 to 18°C have been improved and variable fertility results have been obtained with boar semen stored for two to six days in the liquid state as reported by Johnson et al. (1980, 1981) and Koh et al. (1976).

Several factors influencing sperm transport and fertility in artificially inseminated pigs have been described. Among them are the quality of semen itself, volume and number of spermatozoa inseminated, extender composition, role of anatomical structures of the genital tract and endocrine stage of the female (Baker et al., 1968; Bower et al., 1974; Einarsson, 1980; Einarsson and Viring, 1973a; First et al., 1968; Hancock and Hovell, 1961; Hunter, 1980a; Melrose, 1966; Polge, 1978; Thibault, 1973; Viring and Einarsson, 1980a,b).

Evans and Dawson (Department of Veterinary Clinical Sciences, Iowa State University, unpublished results) and Hunter (1972b) observed a local effect of progesterone on the edematous tissues of the uterotubal junction and oviductal isthmus in the pig. This relaxative effect of progesterone may increase the number of spermatozoa present at the site of fertilization and enhance the chances of successful fertilization.

The objective of the present study was to determine the effect of liquid extended semen treated with 5 mg of crystalline progesterone, on the number of piglets born after artificial insemination of 2×10^8 spermatozoa. The effect of the addition of 5 mg of progesterone to liquid extended semen on the number of sperm recovered from flushings of the genital tract of artificially inseminated pigs with 6×10^9 spermatozoa was also determined.

II. REVIEW OF LITERATURE

A. General Aspects of Artificial Insemination in the Pig

1. Historical background

Since the early years of artificial insemination three centuries ago when L. Spallangzani impregnated female dogs by injecting semen into the reproductive tract (Nishikawa, 1964), the use of artificial insemination in other domestic animals has become a common practice.

It has been surprising, that compared with other species, artificial insemination in pigs has been slower to develop (Melrose, 1966; Erickson, 1974). Polge (1956) cited that early investigations on the reproductive physiology of the pig were done almost fifty years ago. Reports by Aamdal (1964) and Polge (1956) described methods of semen collection and evaluation, dilution rate, insemination equipment, techniques of insemination, time of insemination and conception rates obtained with fresh diluted boar semen.

2. Semen collection

Several methods have been described for the collection of boar semen. The earliest recorded collection of boar semen was by McKenzie in 1931 (Polge, 1956; Basurto-Kuba, 1979). He used a very simple type of artificial vagina consisting of a soft rubber tube. A restrained sow was used as a mount for the boar. Polge (1956) observed better semen

quality when using a sow in heat to collect a boar. An ovariectomized teaser gilt or sow brought into estrus by estrogen treatments may also be used (Melrose, 1966). Aamdal (1964) considered the use of a dummy sow and a modified artificial vagina with relatively high internal pressure as the best method of collecting boar semen.

The gloved-hand technique of boar semen collection was developed through modification of the artificial vagina method (Hancock, 1959; Melrose and O'Hagan, 1959). In this technique, the stimuli which cause ejaculation appear to be essentially tactile. This procedure consists of grasping the extended penis with the gloved-hand and applying pressure to simulate the cervical constriction of the sow.

The training of the boar to mount a phantom was described by several authors (Polge, 1956; Aamdal, 1964; Dziuk, 1959; Clamohoy et al., 1960; Niwa, 1961). Boars can be easily trained to mount simple dummy devices and they will ejaculate readily with gloved-hand stimulation. Clamohoy et al. (1960) found that it took about seven days for inexperienced boars to be trained to mount a phantom and ejaculate freely. Males which had previously experienced some sexual activity by natural service, took an average of 14 days. Niwa (1961) recommended that training for collection purposes should start when the boar reaches 100 kg live-weight.

The electroejaculation technique described by Clark (1976) and Basurto-Kuba (1979) has also been used as a reliable method to obtain

representative samples from boars. When this technique was practiced, general anesthesia was used to achieve better collection results and to provide proper restraint of the animal at the time of electrical stimulation. This technique was also used to collect semen from boars that suffer physical disabilities or were unwilling to mount.

The process of semen collection in the boar is prolonged and depends on the technique used. It may take from three to 20 minutes to complete a full collection. Ejaculation pattern is distinguished as three fractions which were described by Rodolfo (1934a). The first fraction contains very little sperm and is called "pre-sperm" fraction; the second fraction contains a very high concentration of sperm and is generally termed "sperm-rich" fraction; the final fraction contains a much lower concentration of sperm and is called the "post-sperm" fraction. The second and third fractions are usually accompanied by a large quantity of tapioca-like lumps which are secreted by the bulbo-urethral glands (Polge, 1956). Usually an average of 82% of the sperm-atozoa is ejaculated within two minutes after the start of ejaculation (Niwa, 1961).

3. Frequency of semen collection

The frequency of semen collection and related factors, such as semen production capacity, have been reviewed by Melrose (1966). Polge (1956) proposed that a boar can be collected from two to three times per week for maximum production. Niwa (1961) recommended a five to

six-day collection interval, while Gerrits et al. (1962a) found that over a 20-day period 5, 10, and 20 ejaculations yielded an average total spermatozoa of 54.9 X 10^9, 39 X 10^9, and 23.7 X 10^9 respectively. Aamdal (1964) reported that using 48-hour or 72-hour collection intervals, the total number of spermatozoa obtained over time were similar. However, he found that few boars tolerated collection every 48 hours over a long period. DuMesnil du Buisson and Signoret (1970) observed that increasing the collection frequency from one to three per week, resulted in only 14% more spermatozoa and the farrowing rate obtained was not altered. They concluded that collection intervals from five to seven days were sufficient to obtain the maximum number of spermatozoa.

The effect of temperature and daylight rhythym on semen production was investigated by DuMesnil du Buisson and Signoret (1970). They found that semen obtained from animals kept in temperature-controlled pens gave significantly better farrowing rates during the summer months. They also observed that increasing the light phase from 10 to 16 hours daily impaired the farrowing rate. Hurtgen (1979) showed that evaporative cooling systems used to minimize the detrimental effects of heat stress on fertility rates did not alter the seasonal fluctuations in farrowing rate.

4. Semen evaluation

Fertility of boars has been measured by many different means

(Dziuk, 1977). These measurements include ability to copulate, number of sperm produced per unit of time or per unit volume of semen, morphological and biochemical characteristics of semen and sperm, sperm motility, conception rate, number of services per conception, and litter size. Conception rate and litter size are probably the most important biological and economical characteristics, whereas the other measures when examined alone are only indications of potential fertility.

The main characteristics of boar semen are its very large volume and rather low sperm density as compared with semen of most other domestic farm animals (Polge, 1956). There are wide variations in the composition of the ejaculate between different boars and from time to time in the same boar. The volume of the whole ejaculate may range from between 100 to 500 ml in mature animals, but the most common volume is about 200 ml per ejaculate. Similarly, the concentration of the sperm may vary between 10,000 and 1,000,000 per mm^3 with an average volume of about 100,000 per mm^3. The total number of sperm produced by the boar in a single ejaculate is near 20×10^9 cells, which is about five to ten times as many sperm as would be expected in a single ejaculate from a bull (Polge, 1956).

A breeding soundness evaluation for boars was described by Hurtgen et al. (1977). The recommended evaluation includes an estimation of total sperm cells per ejaculate, percent progressive motility and percent morphologically abnormal sperm (acrosome, head and midpiece morphology

and proximal cytoplasmic droplets). All these parameters were reported to be highly correlated with fertility (Singleton and Shelby, 1974). Infertility in the boar due to inadequate semen volume has not been reported (Hurtgen et al., 1977). Sperm cell concentration can be determined by using a red or white blood cell pipettes and a hemocytometer. This is a simple and efficient counting method. A spectrophotometer can also be calibrated for boar semen. The ejaculate of adult boars was found to contain 30 to 80 X 10^9 sperm cells (Swierstra, 1970). Hurtgen et al. (1977) recommended that the total acceptable number of spermatozoa for a young boar should not be less than 20 X 10^9 per ejaculate.

Koh et al. (1976) recommended that good quality boar semen should have at least 70% of individual cells with progressive motility. However, progressive motility estimates are very highly variable due to sperm concentration, motility of individual sperm, the volume collected, the temperature of the slide, the presence of blood, debris, or gel, and the size of the sample drop evaluated.

Studies have been conducted concerning the relationship of boar semen quality to fertility, but the majority of these studies have not included the evaluation of sperm morphology. Lagerlöf and Carlquist (1961) found that five to six month-old boars had a high percentage of abnormal spermatozoa due to sexual immaturity and that this condition may persist until the boar is eight months-old. Bane (1959)

found an acrosomal abnormality of certain sterile boars and drew attention to the importance of carrying out a careful morphological check of the semen of boars used for artificial insemination. Hurtgen et al. (1977) reported approximately the following percentage of sperm abnormalities may be expected in boars of normal fertility: less than 5% of abnormal acrosomes, 5% abnormal head shapes, 10% proximal cytoplasmic droplets, 5% abnormal midpiece segments and 5% coiled tails.

5. Number of spermatozoa per dose

Under natural conditions of mating, the boar deposits a very large volume of semen directly into the uterus of the female. The published recommendations on the threshold number of sperm cells required for normal fertility in the sow range from 2×10^9 to 10×10^9 spermatozoa (Melrose, 1966). Ito, Kudo, and Niwa (1959) found that each insemination dose must contain 5×10^9 to 7×10^9 spermatozoa if conception rate and litter size are to be normal. Polge (1956) observed that the conception rate was not affected if the dose contained more than 2×10^9 sperm cells. Paredis and Vandeplassche (1961) found that conception rate and litter size increased with increased number of sperm per insemination dose and concluded that the optimum number of spermatozoa per insemination for gilts was 7×10^9 and substantially higher for sows. Stratman and Self (1960) used doses containing between 2.5×10^9 and 10×10^9 sperm and did not observe significant differences among

treatments in fertility or litter size at three days post-insemination. Aamdal (1964) reported that the conception rate was about 60% when artificial insemination was done with a range of 2.4×10^9 to 3.4×10^9 spermatozoa. Satisfactory results were obtained by Melrose (1966) when he inseminated 2.5×10^9 spermatozoa, but that with half of this dose, the results were considerably impaired. However, studies done by Wiggins et al. (1951) indicate that 5.8×10^8 spermatozoa were required for gilts and 2.3×10^8 for sows.

With regard to the volume of semen and number of spermatozoa per insemination, Graham et al. (1978) found the early literature extremely contradictory. An insemination volume of 30 to 50 ml was generally considered necessary to initiate uterine contractions and optimal sperm transport (Bower et al., 1974). Stratman and Self (1960) using 10, 20 or 50 ml of semen containing either 2.5×10^9, 5×10^9 or 10×10^9 spermatozoa observed that there were no significant differences among treatments in fertility or litter size at three days, but at 25 days the group inseminated with 50 ml volume had a higher rate of embryo survival and conception rate than the 10 or 20 ml volume groups regardless of the number of sperm used. Baker et al. (1968) studied the effects of volume inseminated (20, 100 or 200 ml) and the number of sperm (1×10^9, 5×10^9 or 10×10^9) in all combinations on the transport of sperm and fertilization rate. They found that gilts inseminated with 100 ml of semen had a significantly higher proportion

of eggs fertilized than the gilts inseminated with 20 or 200 ml of semen. Hancock and Hovell (1961) inseminated 48 sows with sperm rich fraction diluted to 20 ml to contain 10×10^9, 1×10^9 or 0.1×10^9 sperm cells. In half of these animals, insemination of the 20 ml diluted semen was followed by insemination of 100 ml of extender. No significant differences in fertility were found between treatments.

6. Liquid semen extenders

The development of artificial insemination in swine depends to a large extent on being able to preserve the semen *in vitro* for several days without causing a serious reduction in the fertilizing capacity of the sperm cells (Graham *et al.*, 1978; Pursel, 1979a). When whole semen was kept at body temperature in the presence of air, the sperm cells maintained a high degree of motility for six to eight hours, but after this time they soon became less motile and started to loose their fertilizing capacity (Polge, 1956). The addition of suitable diluting fluids or extenders to semen increases greatly the length of time for which the sperm can be kept alive *in vitro*.

The major factors that the diluent and the preservation environment provide are adequate nutrients for metabolism, protection against rapid cooling, buffering against pH shifts, proper osmotic pressure, balanced electrolytes, inhibition of bacterial growth, and volume for multiple inseminations (Foote, 1980; Graham *et al.*, 1978). In addition, providing an environment that prevents lipid peroxide formation may be

one of the key functions of a diluent in maintaining sperm viability during in vitro preservation (Pursel, 1979a). Jones and Mann (1977a,b) reported the serious debilitating effects of oxidation of the polyunsaturated fatty acids. Most semen extenders contain constituents that aid in minimizing lipid peroxidation, and certain naturally occurring semen constituents have chelating properties that prevent metal ions from acting as catalysts in the peroxidative process. Peroxidation of the plasmalogens, polyunsaturated fatty acids and palmitaldehyde in the phospholipid of cellular membranes results in the formation of lysolecithin and other highly toxic compounds that destroy the fertilizing capacity of spermatozoa (Pursel, 1979a).

Historically most diluents used for storage of liquid bull semen have also been tested for boar semen (Graham et al., 1978). Examples are citrate, glycine, bicarbonate or glucose solutions with or without the addition of skim milk and or egg yolk (Borton, 1965; as cited by Graham et al., 1978). Citrate has generally been toxic for boar spermatozoa, but Aamdal and Hogset (1957) obtained satisfactory results with boar semen extended in citrate buffers. Furthermore, Illinois variable temperature (IVT) buffers which are based on citrate are widely used in commercial swine artificial insemination throughout the world. Other buffers used in liquid semen extenders are bicarbonate-glucose and those based in milk. Skim milk heated to 92°C for five minutes is also used where the semen is inseminated the day of collection

or the following day. Koh et al. (1976) reported that a skim milk-glucose buffer gave a pregnancy rate of 79.9% for approximately 20,000 inseminations. It appears that most buffers currently used in swine artificial insemination are essentially based on glucose with added buffering material (citrate, bicarbonate or milk) and, when sodium bicarbonate or citrate are used, potassium is also added. Proteins in the form of egg yolk or milk proteins are sometimes added but appear not be essential (Graham et al., 1978).

The Beltsville L-1 diluent or BL-1 diluent reported by Pursel et al. (1973b), maintained boar sperm fertilizing capacity for 102 hours of in vitro storage at 15°C to 18°C. Another extender widely used is the Kiev extender (Plishko, as cited by Pursel, 1979a) also known as Varohm, and Guelph diluent. Fertility results of field trials (Johnson et al., 1980) comparing BL-1 and Kiev extenders indicate that the Kiev extender was superior (69.3% farrowing rate) to the BL-1 extender (60.5% farrowing rate) for use in routine artificial insemination of swine.

Farrowing results with artificial insemination reported by the Association for Swedish Livestock Breeding and Production was 66.2% in 50,351 sows after first service. Of these sows 18.7% were double inseminated and 11.2 pigs were born per litter. The farrowing rate was 60.5% in 14,236 gilts with a litter size of 9.1 pigs. Double insemination was used in 12.9% of these gilts. Each dose contained 2×10^9 to

2.5×10^9 spermatozoa in IVT extender and was used within three days of collection (Crabo, 1977).

Koh et al. (1976) using a glucose-skim milk diluent with 5×10^9 spermatozoa per dose on the first day of heat and again the following day, obtained an overall farrowing rate of 79.9%. They also observed a significant difference between purebred breedings (65.4% farrowing rate) and crossbred breedings (78.2% farrowing rate). Johnson et al. (1981) in a field study obtained a farrowing rate of 79.1% for sows inseminated with fresh semen using Kiev extender, and an average number of 10.6 piglets per litter. The total number of spermatozoa used per dose was 3×10^9.

There are other diluents used for storing boar spermatozoa in the liquid state. Among them are the Dziuk diluent (Dziuk, 1958) also known as EGB (egg yolk-glucose-bicarbonate); Kharkov diluent also known as Trilon-B diluent (Serdiuk, 1968); SCK-7 (patented composition) and the Spermlife I[1] diluent (patented composition).

Even though many of these diluents have preserved the fertilizing capacity of the spermatozoa for up to six or seven days when stored in the liquid state at 15°C to 18°C, in actual practice most semen is used for insemination within two or three days after collection and dilution (Pursel, 1979a).

---

[1] Immuno Genetics Inc., Dover, New Hampshire.

7. Heat detection and timing of insemination

Heat detection and time of insemination may be among the factors responsible for the greatest variability in artificial insemination results (Crabo and Hurtgen, 1977; Graham et al., 1978). In the absence of a boar, detecting estrus and inseminating at the correct time depend on close observation of the females for changes in behavior. The degree of vulvar swelling alone was unreliable (Melrose, 1966). A technique was described by Madden (1961) to select the female for insemination in which the operator placed the flat of his hands on the loins of the animal and exerted moderate pressure and if she did not move away, he then attempted to mount the female in the pelvic region and sat astride her loins, removing his feet from the ground. A positive response was obtained when the female stood rigidly and insensibly to be mounted. DuMesnil du Buisson and Signoret (1970) observed that the presence or absence of the "standing reaction" in the female at the time of insemination was very important because the farrowing rate was decreased by 20 to 30% in sows without the response. Approximately two thirds of females on heat will respond to back pressure or riding test when the boar is absent (Crabo and Hurtgen, 1977). Signoret and DuMesnil du Buisson (1961) demonstrated that both the sound and smell of the boar can make the signs of estrus much more definite. Teaser boars increase the detection rate. Hughes and Varley (1980) recommend that heat detection should be carried out twice a day.

Incorrect timing of insemination is probably the biggest single factor responsible for poor conception rates (Hunter, 1980a). The onset of estrus is characterized by gradual changes in behavioral patterns (e.g. restlessness, mounting other animals, lordosis response), vulva responses (e.g. swelling, pink-red coloring) and occasionally a mucous discharge (Melrose, 1966; Anderson, 1980). Sexual receptivity lasts an average of 53 hours (range 40 to 60 hours). Estrus in gilts is usually shorter than in sows. Breed, seasonal variation and endocrine abnormalities affect the duration of heat (Anderson, 1980; Hunter, 1980a). Ova are released 38 to 42 hours after onset of estrus and the ovulatory process requires 3.8 hours (Signoret, 1972).

Niwa (1961) considered that the proper time for insemination of a sow was before or around ovulation, or 10 to 30 hours after she allows the boar to mount. Stratman and Self (1962) reported that no significant difference in percent conception or percent fertility was noted between groups of sows mated naturally and those bred artificially on the second day of the first post-lactation estrus. Conception rates and litter size were higher when sows were bred on the second day of the second post-lactation estrus than when bred on the first day of the second post-lactation estrus.

When once daily estrus detection was used and low numbers of sperm cells were inseminated, a single breeding or service 24 to 36 hours after the onset of estrus was recommended by Polge (1978). Hunter (1967c),

observed that artificial insemination of gilts 10 to 14 hours after induced ovulation resulted in fewer gilts with fertilized ova than did artificial insemination in gilts six through two hours before ovulation. Dziuk (1970) related that the optimum time for insemination appears to be 12 hours before ovulation. Foote (1980) recommended insemination either late on the first day or early on the second day of estrus. An advantage of about 10 to 15% is gained by inseminating on both the first and second days of estrus (Graham et al., 1978; Foote, 1980; Hunter 1980a). Insemination 12 hours after the onset estrus and reinsemination 12 hours later if the female continues to show estrual behavior seems to be a practical rationale for fresh semen (Crabo and Hurtgen, 1977). An interval of between 8 and 16 hours from the first to the second insemination is currently suggested as offering the best fertility level (Hughes and Varley, 1980).

B. Hormonal Control of Estrus and Ovulation in the Pig

If a technique for controlling estrus and ovulation in gilts could be developed without any reduction in litter size or fertility, the difficulties and consequent financial loss with mating and farrowing gilts at irregular and unpredictable intervals would be overcome. Such a development could undoubtedly lead to a wider use of artificial insemination in the pig since the difficulties of heat detection and the economic loss through poorly timed inseminations would be lessened

(Melrose, 1966).

The time of estrus and ovulation can be controlled by administering compounds which interrupt the normal estrous cycle. These hormonal compounds may act to suppress the release of gonadotropins and consequently delay estrus or others may hasten estrus by inducing regression of the corpora lutea. Following administration of such treatments, follicular development and estrus usually occur spontaneously with animals exhibiting heat four to eight days after treatment (Webel, 1977).

1. Progesterone and synthetic progestogens

Progesterone or synthetic progestogens have been the compounds most commonly used to suppress the estrous cycle. Daily injections of progesterone inhibited estrus and, if adequate doses were given, resulted in normal fertility (Ulberg et al., 1951; Baker et al., 1954; Gerrits et al., 1962b). Likewise, several synthetic progestogens administered either orally or by injection in sufficiently high doses inhibited follicular growth and estrus. However, consistently satisfactory results have not usually been obtained because of poor synchronization of estrus, low fertility and the formation of cystic follicles (Webel, 1977).

Rather large amounts of 6-methyl-17-acetoxyprogesterone (MAP) inhibited estrus without producing cystic follicles, but estrus was usually not well synchronized and litter size was often reduced

(Baker et al., 1954; Dziuk, 1969; Nellor et al., 1961; Dziuk and Baker, 1962).

Oral administration of other progestational compounds including 6-chloro-delta-6-17-acetoxy progesterone (Wagner and Seerley, 1961; Veenhuizen et al., 1965; Ray and Seerley, 1966), and 17-alfa-acetoxy-6-methylpregna-4, 6-dien-3, 20-dione (Pond et al., 1965) produced similar results to those obtained with MAP.

More recently a new progestogen has been reported (Davis et al., 1976; Knight et al., 1976; Schutze and Mayer, 1977; Zerobin, 1977) which appeared to control the time of estrus without reducing litter size or producing cystic ovaries.

In general, the administration of progesterone or progestational compounds has not been a satisfactory treatment for controlling estrus and ovulation because of the increased incidence of cystic follicles, decreased fertility at the first post-treatment estrus and a lack of precise synchronization (Webel, 1977).

New orally active progestogens have reportedly controlled the time of estrus in pigs without producing follicular cysts or reducing subsequent fertility (Davis et al., 1976; Knight et al., 1976; Webel, 1977; Mayer and Schutze, 1977a; O'Reilly et al., 1979; Kraeling et al., 1981). In an experiment reported by O'Reilly et al. (1979), they obtained a significantly ($P < 0.001$) shorter interval to estrus and better synchronization in the treatment groups than with the control

group. There was no significant difference between treated and control groups in regard to fertility. Treated animals were fed for 19 to 20 days with either 12.5 or 15 mg of the compound 17 alpha-allyl-estratriene-4-9-11, 17 beta-ol-3-one; also called allyl-trenbolone or RU-2267. Pursel et al. (1981) used the same compound and obtained a similar farrowing rate between treated and untreated animals. Kraeling et al. (1981) determined that the most effective doses of allyl-trenbolone for synchronizing estrus and ovulation without inducing development of follicular and/or luteal cysts, were 20 and 40 mg per day during 18 days.

In contrast to the problems associated with progestogens, effective control of the estrous cycle has been obtained by inhibiting ovarian function with a non-steroidal compound ICI-33828 (a dithiocarbamoyl-hydrazine derivative). A high proportion of sows or gilts exhibited estrus five to eight days after withdrawal of the compound following an 18 to 20-day treatment period with the compound incorporated in the feed. Furthermore, fertility was not affected following treatment with this compound (Polge et al., 1968). But the use of this compound was curtailed and regulatory approvals withdrawn in many countries following reports of teratogenic effects in pregnant gilts. Presently its use is limited to a very few countries (Webel, 1977).

2. Gonadotrophic preparations

Pituitary gonadotrophic preparations, pregnant mare serum

gonadotrophin (PMSG), human chorionic gonadotrophin (HCG) and hypothalamic releasing hormones or combinations of these hormones have been widely used to induce follicular growth or ovulation. They have been used respectively in prepuberal gilts; during the luteal and follicular phase in the cycling animal; in anestrus gilts or sows, in lactating or early weaned sows and following suppression of the estrous cycle with other exogenous hormones. In addition, these gonadotrophic preparations have been used to induce superovulation (Webel, 1977).

3. Induction of ovulation

As early as 1935, Casida demonstrated that ovulation could be induced in prepuberal gilts by giving multiple injections of PMSG or purified pituitary preparations. These observations were confirmed by Dziuk and Gehlbach (1966); and Baker et al. (1968). Although injection of PMSG followed by HCG 48 to 96 hours later induced a fertile ovulation, pregnancy was not usually associated with such a treatment unless progestogens or gonadotrophins were given after breeding, because the corpora lutea normally regressed by 20 to 25 days of pregnancy (Rampacek et al., 1976). Other workers using a combination of PMSG and HCG given as a single injection have reported synchronized estrus and perhaps improved pregnancy rates in prepuberal gilts (Schilling and Cerne, 1972).

Superovulation can be induced in swine by injection of the

appropriate gonadotrophins (Tanabe et al., 1949; Hunter, 1966). However, litter size at farrowing has not consistently been increased and may only be measurably increased by PMSG in sows which have lower than average litter sizes when untreated (Schilling and Cerne, 1972).

The time of ovulation can be precisely controlled by injection of HCG 48 to 96 hours after PMSG (Dziuk and Baker, 1962; Hunter, 1966; Guthrie, 1979) or gonadotrophin-releasing hormone (GnRH) may be used in place of HCG (Webel and Rippel, 1975). However, attempts to induce follicular stimulation, superovulation or estrus with GnRH have apparently been unsuccessful (Webel, 1977). The ability of PMSG to stimulate follicular development and of HCG to precisely control the time of ovulation have been utilized to synchronize ovulation and hence to allow insemination at a fixed time. The combined treatment (PMSG + HCG) has been used following inhibition or suppression of the estrus cycle with compounds such as ICI 33828 (Polge et al., 1968; Webel, 1977) or following oral progestagen (Dziuk and Baker, 1962; Dziuk and Polge, 1962). This three stage sequence of treatments (ICI 33828/ Progestagen + PMSG + HCG) is effective in either controlling the time of ovulation in order to allow a single insemination or inducing superovulation. Other uses of PMSG and HCG have been to synchronize estrus in sows by injecting PMSG on the day of weaning and hence shorten the interval to the first heat and injection of HCG 80 to 96 hours later in order to synchronize ovulation and permit a single

insemination (Longenecker and Day, 1968; Christenson and Teague, 1975). Injection of PMSG or a combination of PMSG-HCG as a single injection to sows at weaning or to non-cycling gilts can overcome anestrus problems (Schilling and Cerne, 1972; Hunter, 1980a). Pregnancy has been induced in lactating animals but with quite variable results, especially if used in early post-partum sows. The ovarian response became greater as the interval from parturition to injection increased, but consistently good results were not obtained (Crighton, 1970). Normal fertility has been obtained in lactating sows with an injection of PMSG at 25 days post-partum followed 96 hours later by HCG (Kuo et al., 1976).

4. Induction and regression of corpora lutea

Another method for regulating the estrous cycle in the pig is by inducing accessory corpora lutea. Injection of PMSG followed by HCG will induce ovulation at any stage of the estrous cycle and the accessory corpora lutea induced will regress after an approximately normal life span with estrus occurring 18 to 24 days after the HCG injection (Neill and Day, 1964). However, this method is not very precise because of the variability in the duration of luteal function and the early regression of accessory corpora lutea induced during the first six days of the cycle (Webel, 1977).

Injection of estrogens during metestrus has a luteotrophic effect in the pig (Gardner, et al., 1963), and will not shorten the life span

of the corpus luteum as in the bovine. It is not, therefore, an effective treatment for interrupting the estrous cycle.

Prostaglandins are not luteolytic in the pig until after day 11 or 12 of the cycle and so do not offer a practical means of synchronizing estrus in randomly cycling animals (Guthrie and Polge, 1976a,b).

A sequence of treatments in which corpora lutea are maintained by the injection of estrogen or accessory corpora lutea are induced by the injection of gonadotrophins, offers a possible means of utilizing prostaglandins. In studies reported by Guthrie (1975) and Guthrie and Polge (1976a,b), 10 mg of estradiol-benzoate were administered on days 10 to 14 of the cycle in order to maintain corpora lutea, or PMSG and HCG were given during the luteal or follicular phase to induce accessory corpora lutea. Regression of these corpora lutea could then be induced by the injection of prostaglandins 5 to 20 days later and estrus occurred four to six days after the injection of prostaglandin with normal fertility ensuing. Guthrie (1979), in a similar study, reports that up to 83% of sows treated had a synchronized estrus four to seven days after prostaglandin ($PGF_2\alpha$) injection. This treatment consisted of induction of corpora lutea by an injection of 1500 IU of PMSG followed 72 hours later by 750 IU of HCG. On days 13 and 14 after HCG injection, they were treated with 10 mg of $PGF_2\alpha$ or its analog and at the time of the second $PGF_2\alpha$ injection, the sows were again treated with 100 IU of PMSG followed by 500 IU of HCG. A total of 75% of the

sows inseminated with 6 X 10^9 spermatozoa in a Beltsville L-1 extender on the second day of estrus, farrowed.

C. General Aspects of Sperm Transport in the Pig

Fertilization is just one of the essential links in the entire chain of reproductive events leading to the production of offspring. Efficient transport of spermatozoa to the upper reaches of the oviducts of females in estrus is an essential preliminary to fertilization.

1. Deposition and transport of semen

The volume of the ejaculate and the site of its deposition, vary between species. In the pig, the voluminous ejaculate is propelled through the cervix into the uterine lumen during coitus. In artificial insemination, the semen is deposited by means of a catheter into the uterus. The large volume of semen is then rapidly distributed along the uterine horns and the oviducts (Einarsson, 1980; Polge, 1978). This transport is facilitated by the contractile activity of the reproductive tract (Hunter, 1977). By insemination of non-cellular particles into the uterus of cows at estrus, Rowson (1955), demonstrated indirectly a very rapid transport of seminal plasma along the uterine horns and into the oviducts. In similar experiments in estral pigs, Polge (1978) found no radio-opaque material within the oviducts. Mann et al. (1956), using biochemical methods, detected measurable amounts of ergothionine, fructose and citric acid in the oviductal flushing from

mares but not in the oviducts from pigs. It was therefore concluded by Polge (1978) that there is not bulk transport of fluids into the oviducts of pigs. For tracing low concentrations of seminal plasma constituents Einarsson et al. (1980) preferred to utilize radio-labelled compounds suspended in seminal plasma to study the possible entrance of different size molecules into the oviducts of estrous gilts. Of the compounds used, quantities were found in all oviducts measured. After simultaneous insemination of two different compounds there was a close agreement between their distribution in the genital tract. All compounds thus entered the oviducts regardless of their molecular size. They also found a difference in total amount of recovered radioactivity between left and right uterine horns, but this was not reflected in similar differences between oviducts. Rigby (1964) and Viring (1980) demonstrated, in opposition to First et al. (1968), a consistent disproportion of spermatozoa between left and right uterine horns in pigs. Viring and Einarsson (1980a) did not find any relationship between numbers of large follicles and numbers of recovered spermatozoa in the adjacent horn after natural mating. By using radio-labelled compounds as tracers, Viring et al. (1980a) demonstrated a transuterine transport of radio-labelled molecules suspended in seminal plasma in the pig after insemination. This transuterine transport had already begun in gilts slaughtered five minutes after deposition in the uterine body.

2. Sperm reservoirs

Within a few hours of mating or insemination, most of the spermatozoa and seminal fluid have been removed from the uterus and only a relatively small number of spermatozoa persist in the region of the uterotubal junction (UTJ) and lower part of the isthmus (Rigby, 1964; First et al., 1968; Polge, 1978; Einarsson, 1980; Hunter, 1980b, 1981). Rigby (1964), studying the fate of spermatozoa in the genital tract of the sow up to 48 hours after artificial insemination, reported that most of the semen could be recovered from the uterus up to one hour after insemination. At two hours, no measurable amount of fluid was recovered from the tract. The backflow of whole semen through the cervix after insemination is comparatively high. Approximately one quarter to one-third disappears this way (Einarsson, 1980). Horne and Thibault (1962) found evidence that spermatozoa may pass into the peritoneal cavity of women. They obtained live spermatozoa from the peritoneal fluid of women at laparatomy performed 24 hours after coitus. Viring (1980) demonstrated extremely large numbers of spermatozoa in the very proximal part of the ampulla of sows two hours after insemination. Using labelled compounds of different sizes, Einarsson et al. (1980) found the highest recovered amount in the very proximal part of the porcine oviduct one hour after insemination, indicating that spermatozoa and fluid pass through the oviducts into the abdominal cavity.

Lovell and Getty (1968) reported that there seem to be several mechanisms involved in the process of immobilization and disappearance of spermatozoa from the uterus like phagocytosis, clumping, adherence to the epithelium in glandular tubules and at the uterotubal junctions. Also, some enzymes or antibodies are released by leukocytes, epithelial cells, or cilia that would tend to cause immobilization and lysis of spermatozoa and thus facilitate phagocytosis and that seminal plasma was absorbed through the epithelial layers into vascular or lymph channels in the pig. Mattner (1969) suggested that in the cow live spermatozoa are more likely than are dead spermatozoa to be engulfed by leukocytes present in the vaginal and cervical mucus.

After the removal of most of the spermatozoa and seminal fluid, small numbers of spermatozoa persist in the UTJ and lower part of the isthmus. In pigs this reservoir is established about 12 hours after insemination (Rigby, 1964, 1966) or even earlier. Viring (1980) found approximately the same numbers of sperm cells at the UTJ six hours and twelve hours after insemination. Thereafter, there is a direct correlation between the number of spermatozoa at the UTJ and the number in the corresponding isthmus and this reservoir persists relatively unchanged for 24 hours and declines over the next 48 hours (Rigby, 1966). Thibault (1973) observed that the spermatozoa were accumulated essentially in the longitudinal non-coiled folds of the UTJ where no leukocyte invasion took place. This may explain the

longer survival time of spermatozoa at this site. Viring et al. (1980b) using a similar technique found that most spermatozoa survived within a 3 mm-area of the UTJ in pigs. Most of the sperm cells were present in primary and secondary diverticulae between the edematous polyp-like projections of the mucosa.

In a scanning electron microscope study of the UTJ and isthmus by Flechon and Hunter (1981), they confirmed that those structures regulate the transport of spermatozoa through the oviduct. The UTJ appears to form a mechanical valve strongly limiting the number of sperm cells entering the oviduct. The isthmus, especially its proximal part, which has few ciliated cells, is a storage place for spermatozoa. It is also postulated that the transport of spermatozoa in the isthmus towards the site of fertilization depends in part upon ciliary motion. No leukocytes which would ingest degenerated spermatozoa were observed in the lumen of the UTJ or in the isthmus, but they were found in aggregations at the border between the UTJ and the uterine horn.

It seems probable that the epithelium of the UTJ produces an environment favorable for storage of spermatozoa without loss of their fertilizing capacity (Viring et al., 1980b). They may stay in such reservoirs due to the constriction of the lumen by the thick muscular wall of the duct, or to some chemotactic attraction of tubal secretions, or simply due to adhesion on the epithelium (Flechon and Hunter, 1981).

3. Factors affecting the transport and survival of spermatozoa

There are several factors affecting transport and survival of spermatozoa within the female genital tract. Rodolfo (1934b) found sperm cells in the oviducts of a sow within 40 minutes of artificial insemination. Austin (1964) found that few spermatozoa were in the oviducts within 15 to 30 minutes of mating in the pig. Overstreet and Cooper (1978a,b) recovered rabbit spermatozoa from the upper ampulla and the fimbrial and ovarian surfaces of all females examined at one minute post coitum. This rapid transit phase of sperm transport was an asymmetric phenomenon and most of the spermatozoa were non-motile and with disrupted acrosomal membranes. The sustained phase of sperm transport was first detected at 90 minutes post coitum when motile sperm were recovered. The tubal isthmus was the most important region restricting spermatozoa ascent to the site of fertilization. There was little migration of sperm cells beyond the isthmus until six hours after mating and the majority of the spermatozoa reached the site of fertilization during the periovulation period.

Hunter and Hall (1974a) using a technique of post-coital separation of the oviducts and uterus to define the timing of entry of spermatozoa into the oviducts, found that sufficient sperm cells to fertilize a proportion of eggs, were present in the oviducts within 30 minutes of mating. In a similar experiment by Hunter (1981) after the transection of the caudal region of the isthmus, sufficient sperm cells

were already present in the oviducts within 30 minutes after mating to ensure a high proportion of ovum fertilization. An increase in the fertilization rate observed at 60 minutes after mating confirmed a progressive colonization in the isthmus from sperm reserves in the uterus. He concluded that a population of spermatozoa adequate to promote 100% fertilization would be established in the oviducts of most gilts within one to two hours of mating.

The increased myometrial activity caused by the volume of fluid inseminated might contribute to the initial rapid transport of spermatozoa into the oviduct in the pig (Bower et al., 1974). The stimuli known to elicit sexual behavioral responses, back pressure, presence of a boar, mounting by a boar and introduction of inseminating catheter, had no effect on myometrial activity whereas manual stimulation of the vulva and clitoris occasionally resulted in a simple contraction (Bower, as cited by Einarsson, 1980). Thibault and Winterberger-Torres (1967) observed that stress delays transport of spermatozoa in ewes susceptible to environmental alterations. Bower et al. (1974) reported that exciting the animal or injection of 0.2 mg epinephrine had a transient effect on the activity lasting only a few minutes. The addition of 1 to 2 mg of carbamylcholine to the inseminating fluid greatly increased myometrial activity. Injection of 10 USP units oxytocin increased activity during estrus but had no effect during diestrus.

Numerous experiments have been carried out to determine the optimum volume of semen and number of spermatozoa inseminated which may be compatible with high fertility in the pig. Hancock and Hovell (1961) provided evidence that a very large volume of semen within the uterus was not necessarily required for adequate sperm transport and suggested that the conception rate might be related to the concentration of spermatozoa inseminated. Baker et al. (1968) proposed that the volume of semen inseminated affects the fertility rate, but that the number of spermatozoa reaching the oviducts is depending on the concentration of spermatozoa in the inseminate.

When considering the relationship between conception rate and number of spermatozoa inseminated, the interval between insemination and ovulation must also be considered. The conception rate in pigs inseminated during the early part of estrus could be improved by increasing the sperm concentration in the insemination dose. A better alternative might be to inseminate twice during estrus to ensure that pigs are inseminated close to the time of ovulation (Einarsson, 1980). Baker and Degen (1972) found an approximately ten-fold difference in concentration of spermatozoa between the uterine and tubal fluids collected through cannulae inserted in vivo into the uterine apex and oviduct in the pig.

The UTJ, as well as the lower isthmus, have been proposed as major barriers to sperm transport (Hunter, 1981; Thibault, 1973; Polge,

1978; Einarsson, 1980; Flechon and Hunter, 1981; Hunter, 1981). Dead as well as live sperm cells enter the oviducts (First et al., 1968; Baker and Degen, 1972; Viring, 1980), and the transport of dead spermatozoa is as rapid but less efficient than that of live spermatozoa (Baker and Degen, 1972). Krzanowska (1974) reported that the UTJ in mice acts as a barrier against morphologically abnormal spermatozoa. Einarsson et al. (1980), inseminating radio-labelled molecules of different sizes into estrus pigs, found that all compounds entered the oviducts regardless of their molecular size. Perez et al. (1981) reported that following unilateral microsurgical resection of the UTJ in 15 rabbits, fourteen became pregnant on the UTJ resected side and 14 on the control side following artificial insemination and induction of ovulation. They also examined ovum transport and there was no significant difference in ovum transport between UTJ resected and control animals. They concluded that the UTJ is not necessary for normal fertility and ovum transport in the rabbit.

To the seminal plasma itself has been ascribed a promoting effect on sperm transport in the reproductive tract of the female pig (Viring and Einarsson, 1980b). This effect is localized mainly to the isthmus part of the oviduct, close to the UTJ. Viring and Einarsson (1980b) reported that boar seminal plasma did not affect the contractility of the uterine horn more than electrolyte buffers. By recording the motility of the isthmus part of the oviduct with the use of a balloon-

tipped catheter in estrus gilts, Viring and Einarsson (1980b) demonstrated a decreased motility caused by boar seminal plasma almost immediately after its deposition in the lumen. This relaxant effect of seminal plasma on the myometrium of the isthmus and of the UTJ probably facilitates sperm transport into the oviducts.

According to Poyser (1974) and Niwa and Hashizume (1978) small amounts of prostaglandins $F_2\alpha$ and E_2 are present in boar seminal plasma, and both prostaglandins stimulate the smooth circular muscle activity of the isthmic as well as of the ampullar part of the porcine oviduct, therefore the relaxative effect of the boar seminal plasma on the porcine isthmus must be caused by a still unidentified substance (Viring and Einarsson, 1980b). Evans and Dawson (Department of Veterinary Clinical Sciences, Iowa State University, unpublished results) studied the effect of progesterone and $PGF_2\alpha$ on gravity flow at the uterotubal junction of estrous gilts and observed that both compounds increased the flow rate. Intrauterine catheters were placed surgically on the first day of estrus in sows, and the flow rate was measured during the next 24 hours. Progesterone caused flow rates to increase in approximately four hours subsiding by six to seven hours after treatment in the test fluids (5% dextrose solution in 0.45% sodium chloride solution). Flow rate in treated animals was nearly twice to three times that of control animals during that period. Similarly, $PGF_2\alpha$ increased the flow rate in approximately one hour and this increased rate of flow lasted for three to four hours at nearly four

times that of the control.

Pursel et al. (1978a) reported that the oviductal sperm population was greater in gilts inseminated with fresh semen two hours before ovulation than in gilts inseminated twelve hours before ovulation. Insemination of ewes during late estrus resulted in fewer spermatozoa in the uterus and in the oviducts than insemination during early estrus (Mattner and Braden, 1969). Also in ewes, Robinson (1973) reported that in a slightly abnormal endocrine state such as that following progestogen-induced synchronization of ovulation, penetration and initial establishment of a cervical sperm population does occur, but it fails to persist beyond 12 hours and a normal oviductal sperm population does not develop. There is, moreover, an extensive loss of spermatozoa through phagocytosis due to an unfavorable environment (Hawk and Conley, 1971; Robinson, 1973). Hunter (1981) cited that the formation of a reservoir in the isthmus of gilts is more rapid even six to eight hours after ovulation than when the insemination is performed early in the period of estrus. Ito et al. (1959) also reported that transport of spermatozoa to the oviducts of pigs is accelerated in the latter part of estrus. The mechanisms underlying this change in oviductal activity are thought to be programmed by ovarian hormones, possibly mediated in part by a local rather than a systemic vascular pathway (Hunter, 1980b). Specific modifications in the pig could involve not only the muscular and ciliary activity

of the oviducts, but also a reduced state of edema at the UTJ and in the longitudinal folds of the isthmus, permitting a greater permeability to sperm passage as ovulation approaches (Hunter, 1972b). Because the swollen condition of these structures in pre-ovulatory pigs can be reduced by local microinjections of progesterone in oil, preovulatory secretion of progesterone by the ovarian follicles may be involved in the physiological regulation of this change (Hunter et al., 1972).

The seminal fluids also may actively influence sperm transport. Mellish and Baker (1970) inseminated gilts with fresh heterospermic semen from two boars. When heterospermic inseminations were used, more spermatozoa were attached to the zona pellucida than when equal numbers were inseminated from just one boar. Differences in fertilizing capacity of frozen-thawed semen between boars were reported by Larsson and Einarsson (1976) and by Polge (1978). Saacke et al. (1980) demonstrated the effectiveness of heterospermic insemination to study the correlation between semen quality and fertility.

Another factor that influences sperm transport is the quality of semen itself. Frozen-thawed spermatozoa are removed from the genital tract more readily after insemination than are freshly ejaculated spermatozoa (Mattner et al., 1969; Pursel, 1979a). The medium used for diluting and thawing fresh and frozen boar semen also influences the

number of spermatozoa recovered from different parts of the tract (Einarsson and Viring, 1973a). Hunter and Dziuk (1968) reported that sperm penetration of pig ova can occur as early as two to three hours after insemination with undiluted fresh semen.

The effects of volume of semen, number of sperm and drugs on transport of sperm in artificially inseminated pigs have been studied by Stratman et al. (1959); Stratman and Self (1960); Self (1961); and Baker et al. (1968). Stratman et al. (1959) reported that intravenous injections of gilts with 10 IU of oxytocin produced a beneficial effect on the percent of ova recovered that were fertilized when 2.5×10^9 sperm cells suspended in 20 ml were inseminated. The addition of 10 IU of oxytocin to the semen had a detrimental but non-significant effect on litter size and number of fertilized ova. Sergeev (1964) observed that oxytocin when introduced with semen into the porcine uterus hastened uterine motility, increased conception rate and reduced embryonic death.

D. General Aspects of Progesterone

Progesterone is a steroid hormone secreted by the ovary, placenta, and adrenal cortex. The major site of production occurs within the corpus luteum. Is readily soluble in organic solvents, only sparingly soluble in plasma and still less in water. The biological half-life in the cow exhibits a biphasic curve; phase one lasting 3 minutes and

phase two lasting 28 minutes. Progesterone is also an intermediate in the synthesis of androgens and estrogens. After a period of estrogen stimulation, the endometrium will proliferate in the presence of progesterone. The tubular glands undergo changes in length and tortuosity and epithelial cells enlarge and secrete glycogen. During pregnancy, progesterone is responsible for preparation of the uterus for reception of the fertilized ovum and later for maintenance of the myometrial quiescence. Progesterone is required for myometrial hypertrophy and after parturition its withdrawal facilitates uterine involution. The presence of functional corpora lutea is associated with both the lack of estrus and ovulation.

1. Historical background

Frankel in 1903, as cited by Duncan (1960), was the first to demonstrate that removal of corpora lutea from pregnant rabbits was responsible for failure of rabbit enbryos to survive. Corner (1928) confirmed Frankel's observations, pointing strongly to the existence of a special action of the corpora lutea upon endometrium proliferation necessary not only for implantation, but also for nutrition of the free blastocyst. Corner and Allen (1929) prepared lipid extracts of corpora lutea of the sow and injected these extracts into ovariectomized rabbits to establish some criteria for a biological response. In addition to accomplishing a bioassay, they demonstrated that these extracts could maintain pregnancy in the ovariectomized rabbit. In

1934, Allen and Wintersteiner; Butenandt et al., and Slotta et al., as cited by Duncan (1960), each independently succeeded in crystallizing the active hormone from lipid extracts of sow corpora lutea. In 1934, Fernholz, as cited by Tausk (1971), was able to establish the chemical structure, and the next year Allen, Butenandt, Corner and Slotta proposed jointly that the hormone should be called "progesterone" (Tausk, 1971). In 1937, Makepeace et al., discovered that progesterone was responsible for the inhibition of ovulation in the rabbit. In 1938, Inhoffen and Hohlweg, as cited by Tausk (1971), reported a synthetic steroid now known as ethisterone, that produced the same histological effects characteristic for progesterone. This new steroid, which was called progestational, was active in relatively low doses after oral administration in rabbits and in women. For some time, this was the only oral substitute for progesterone until Hertz et al. (1954) discovered a new compound that had five times the activity of ethisterone. Since these early discoveries, numerous new progestational compounds have been produced.

2. Chemical nature of progesterone

Over 1800 biologically active steroids have been isolated from biological sources or are produced synthetically (Cole and Cupps, 1977). Naturally occurring progestogens including progesterone (4-pregnen-3, 20 - dione) are biosynthetized from acetate and cholesterol in the gonads, adrenals, and placenta. Progesterone has the dual functions

of a hormonal substance and an important biosynthetic intermediate in the formation of corticoids, androgens and estrogens (Dorfman, 1973). All these hormones are chemically based on a structure called a cyclopentanophenanthrene nucleus (also called gonane or sterane), containing four carbon rings linked together to give a total of 17 carbon atoms. This basic tetracyclic compound is initially synthesized as part of the cholesterol molecule. The active hormones are formed from the cholesterol molecule by specific enzymes present in the particular endocrine glands. Some conversion may also occur in peripheral tissues such as the liver (Bentley, 1980).

3. Distribution of progesterone

Progesterone is secreted by the ovary, placenta and adrenal cortex (Cole and Cupps, 1977). The principal source of progesterone in the ovary is the corpus luteum, but it is also biosynthesized in other parts of the ovary including the follicle and the interstitial tissue (Dorfman, 1973). Progesterone has been identified in the corpus luteum of many species including cows (Sweat et al., 1960); pigs (Duncan, 1960) and mare (Short, 1962). Duncan (1960) demonstrated the formation of progesterone by porcine luteal tissue in vitro. The ovarian follicle of various species contains progestagens, although the concentration is considerably less than that found in the corpus luteum (Dorfman, 1973). The concentration of progesterone in the ovarian follicle is highest just prior to ovulation (Short, 1964).

The liver is the principal site for the metabolism of progesterone. This involves both conjugation reactions and the modification of the steroid nucleus. Some of the products may be secreted in the bile and thus enter the intestine, from which it may be reabsorbed due to hydrolysis and liberation of the free steroid. The kidney has also an important role in the clearance of progesterone (Bentley, 1980).

4. Actions of progesterone

The physiological effects of progesterone do not appear to be elicited alone, but are superimposed or are dependent on the priming effects of estrogens. The latter may promote the formation of progesterone receptors at the target organ. Progesterone and estrogens usually act on the same organs and tissues. Some of their actions appear to be diametrically opposite, but in other important respects they complement each other (Bentley, 1980).

Progesterone has long been established as an indispensable steroid mediator of pregnancy. The primary physiological effect of progesterone appears to be the induced transformation of uterine endometrial cells so that implantation of the blastocyst is facilitated (O'Malley and Strott, 1973). Csapo (1956) studied the effects of progesterone on the myometrial cell and proposed that during pregnancy, progesterone is the factor responsible for myometrial quiescence.

Korenman and O'Malley (1968) reported that after a single exposure

to progesterone, the goblet cells of an estrogen-stimulated oviduct synthesized the protein avidin, which serves as a specific intracellular biochemical marker for the action of progesterone in the reproductive target tissue of the chicken.

Progesterone promotes the secretion of a form of mucus by the cervix, whose composition and properties inhibit the transport of sperm. It is of small volume and is very viscous. This effect is the basis for a contraceptive action of progestagens (Bentley, 1980).

Considering ova transport rate through the oviduct, progesterone has been reported to have no effect (Black and Asdell, 1959; Greenwald, 1961), while other works indicate that it may inhibit movement (Austin, 1949) or increase rate of movement (Harper, 1964). Longley et al. (1968) reported that estrogen when given alone or in combination with autonomic drugs, had the greatest influence on the position at which the ova were located, whereas progesterone did not influence ova position. Hafez et al., (1968), as cited by Cole and Cupps (1977), found that progesterone accelerates ova transport in superovulated cows. Day and Polge (1968) observed that an accelerated rate of egg transport through the oviducts was caused by subcutaneous injection of 100 mg of progesterone 36 hours before ovulation in pigs. Chang (1966) also found that the administration of progesterone before ovulation speeds the transport of eggs through the reproductive tract of rabbits. Hunter (1972b) injected microdroplets of progesterone (1 mg) beneath

the serosa of the tubal isthmus and uterotubal junction 8 to 12 hours before ovulation and found polyspermic fertilization in 32.3% of the eggs recovered. He concluded that relaxation of the edematous tissues of the isthmus and uterotubal junction occurred and that a larger proportion of spermatozoa than normally accumulate in the region of the uterotubal junction in pigs after mating, may pass directly into the isthmus and ampulla with very little quantitative regulation.

The effect of progesterone on the epithelial lining of the oviduct was studied by Mastroianni et al. (1961). They found a decrease in volume of tubal secretions under the influence of progesterone.

Austin et al., as cited by Hunter (1972b), have suggested the involvement of progesterone in development of acrosomic reaction. The possibility of steroid involvement in the capacitation of porcine spermatozoa has been cited by Hunter (1972b). Thus, progesterone may have an effect on sperm transport, fertilization and ovum passage in the oviduct.

III. MATERIALS AND METHODS

A. Experimental Animals

Fifty animals were used in this experiment. In the first part (experiment A), 20 crossbred sows and 20 crossbred gilts were used. In the second part (experiment B), four sows and six gilts were used.

All animals were maintained in total confinement at the swine unit of the College of Veterinary Medicine of Iowa State University. The animals were fed twice daily a complete ration of 14% protein based on corn and soybean meal. One boar was used as semen donor and was subjected to the same management.

B. Experimental Groups

In experiment A, 20 sows and 20 gilts were allocated to eight groups (Table 1) of five animals each. Each group consisted of either sows or gilts exclusively. Any gilt that had shown estrus for the third time was assigned randomly to one of four groups. Sows showing their first heat after weaning were randomly assigned to one of four other groups. All animals were inseminated with 2×10^8 spermatozoa diluted in 70 ml of liquid semen extender (Spermlife I[1]). Only fresh extended semen was used. None of the animals were synchronized with exogenous hormones.

---

[1]Immuno Genetics Inc., Dover, New Hampshire.

Table 1. Design of experimental groups indicating treatments[a]

	Gilts		Sows	
	Single Service	Double Service	Single Service	Double Service
Liquid semen extender with 5 mg of progesterone	Group A 5 animals	Group B 5 animals	Group E 5 animals	Group F 5 animals
Liquid semen extender without 5 mg of progesterone	Group C 5 animals	Group D 5 animals	Group G 5 animals	Group H 5 animals

[a]A single service consisting of 2×10^8 spermatozoa with or without 5 mg progesterone was defined as an insemination dose.

In half of the animals 5 mg of crystalline progesterone[2] (4-pregnen-3, 20-dione) were added to the extended semen. Group A consisted of five gilts receiving just one insemination on the second day of standing heat (24 hours after they first showed estrus signs). Gilts in group B were inseminated 12 hours after they showed standing estrus and again 24 hours later. Groups A and B received extended semen with the addition of 5 mg of progesterone. Group C was comprised of five gilts receiving a single insemination 24 hours after they showed standing heat. Gilts in group D were inseminated 12 hours after they showed standing estrus and again 24 hours later. Both groups (C and D) were inseminated without the addition of progesterone to the extended semen. In group E, five sows were inseminated only once 24 hours after they showed standing heat. Sows assigned to group F were artificially bred 12 hours after they showed standing estrus and again 24 hours later. Both groups E and F were artificially inseminated with the addition of 5 mg of progesterone to the extended semen. Group G was comprised of five sows artificially bred once 24 hours after they showed standing heat. Sows in group H were inseminated 12 hours after they showed standing estrus and again 24 hours later. Groups G and H were inseminated with extended semen without the addition of progesterone. All these animals were maintained in the same facilities and the number of piglets born was recorded.

In experiment B, 10 animals were randomly allocated to either

---

[2]United States Biochemical Corporation, Cleveland, Ohio.

one of two groups of five animals each with three sows and two gilts per group. They were inseminated once with a total of 6 X 10^9 spermatozoa diluted in liquid semen extender (Spermlife I). Half of the animals received the insemination dose with 5 mg of crystalline progesterone suspended in the diluent, whereas the remainder were inseminated without the addition of progesterone to the extended semen. Artificial insemination was carried out 18 to 20 hours after the injection of 500 IU of HCG.

C. Experimental Procedure

Experiment A. The incidence of heat was checked twice daily with a mature boar (0900 and 1800h). A teaser boar was walked outside the pen and the back-pressure test was performed to determine standing estrus. When a female first exhibited behavioral estrus in the presence of a boar, it was scheduled for insemination. A boar of known fertility was used for the present study. Semen was collected by the gloved-hand technique (Hancock and Hovell, 1959; Melrose and O'Hagan, 1959) after the boar mounted a dummy sow. The semen was collected in an insulated plastic bottle and taken immediately to the laboratory for evaluation and dilution. Volume, motility and morphology were checked and sperm concentration was determined with a hemocytometer as described by Koh et al. (1976), and Hurtgen et al. (1977). Only semen exhibiting at least 70% of motile spermatozoa and with less than 5% of abnormal acrosomes, 5% abnormal head shapes, 10% proximal cytoplasmic droplets, and 5% coiled tails, was used.

Based on this criterion, only one ejaculate was discarded. A total of 2×10^8 spermatozoa were diluted in 70 ml of liquid semen extender (Spermlife I) under isothermal conditions. When it was required, 5 mg of crystalline progesterone were added to the extended semen. The doses were transported in an insulated box to the swine unit for insemination. All inseminations were performed in the presence of a teasing boar outside of the female's pen. A rubber spiral catheter (Melrose and O'Hagan 1961) was used for all inseminations. The catheter was passed through the vagina and then twisted in an anti-clockwise direction until it locked firmly in the cervical folds. The semen was drawn into a 35 ml plastic syringe. The syringe was connected to the end of the catheter and the liquid extended semen was infused slowly.

Experiment B. In this experiment, four gilts and six sows were used. Heat detection, collection, evaluation and handling of semen, as well as the insemination technique were identical to that in experiment A. All animals in this experiment were brought into estrus by hormonal treatments. This was done to synchronize breeding with available slaughter dates and to assure the collection of specimens 24 to 28 hours after breeding. Four cycling gilts were treated with a subcutaneous injection of 5 mg of estradiol cypionate (ECP[1]) on day 11 of the cycle to delay luteolysis (Guthrie, 1975), and

[1]ECP, The Upjohn Company, Kalamazoo, Michigan.

6 to 10 days later, they were induced into heat by the subcutaneous administration of 1000 IU of pregnant mare serum gonadotrophin (PMSG) and an intramuscular injection of 10 mg of prostaglandin $F_2\alpha$ (Lutalyse[2]) and then, the next day another injection of 10 mg of prostaglandin $F_2\alpha$ was given. Approximately 72 hours after the PMSG treatment, an intramuscular injection of 500 IU of human chorionic gonadotropin (HCG) was given. Four sows were treated one day after weaning with a subcutaneous injection of 1000 IU of PMSG, followed 72 hours later by the intramuscular administration of 500 IU of HCG. Two cycling sows were treated in the same manner as the group of gilts. Animals in all three groups were assigned at random in equal numbers to the two treatments.

In all animals of experiment B, artificial insemination was carried out 18 to 20 hours after the injection of HCG. A total of 6×10^9 spermatozoa were diluted in 70 ml of liquid semen extender (Spermlife I), and in five of the animals 5 mg of crystalline progesterone were added to the extended semen. This larger number of spermatozoa was utilized in experiment B, because preliminary work (6 animals) with 2×10^8 sperm cells yielded too few spermatozoa to count in the oviduct and UTJ. Six to eight hours after insemination, the sows and gilts were slaughtered at the Meat Laboratory facilities of the Animal Science Department, and the reproductive tracts were removed immediately and transported to the laboratory for recovery of

---

[2]Lutalyse, The Upjohn Company, Kalamazoo, Michigan.

spermatozoa. The oviducts and uterotubal junctions were carefully trimmed away from the mesosalpinx and each oviduct was then flushed with 10 ml of warm physiological saline solution (0.89% NaCl). The oviducts were stripped of the saline solution twice during flushing. Flushings from the two oviducts were pooled and centrifuged to concentrate the sperm cells for counting as described by Hawk and Conley (1975). Both uterotubal junctions were placed in a Petri dish that contained 10 ml of physiological saline solution. The Petri dish was then gently shaken and maintained with intermittent agitation for one hour. Aliquots of the flushings of the oviducts and washings of the uterotubal junctions were counted at 200X in a hemocytometer under a phase contrast microscope.

D. Experimental Analysis

In experiment A, five replicates of animals were assigned to a randomized 2 X 2 X 2 factorial arrangement. The factors were two ages (sows and gilts), two services (single and double), and with or without progesterone treatment. An analysis of variance was carried out with five observations per group. The dependent variable was the total number of piglets born. Animals that did not become pregnant were scored as zero. Data obtained in this experiment were transformed to square roots as described by Snedecor and Cochran (1967) to make the variances relatively independent of the means and therefore closer to a normal distribution. Since there was a substantial number of non-pregnancies (40%), the data for pregnant animals were analyzed

separately using a 2 X 2 X 2 factorial non-orthogonal analysis of variance. F-tests were used to determine the effects upon the variable used.

In experiment B, the individual sperm cell counts at the uterotubal junctions were transformed to $\log_{10}$ to normalize the distribution (Snedecor and Cochran, 1967). When no sperm cells were found in the washings, the log of the lowest number of cells that would have been detected was used. The transformed data were analyzed statistically by the t-test (Snedecor and Cochran, 1967) to determine if there was a difference between treated and non-treated animals. No sperm cells were recovered from many of the oviducts; therefore, data for the oviducts were omitted from the statistical analysis.

IV. RESULTS

Experiment A. The overall farrowing rate after artificial insemination with a low spermatozoa concentration ($2 X 10^8$) diluted in 70 ml of liquid semen extender (Spermlife I), with or without the addition of 5 mg of crystalline progesterone was 60%. The number of piglets born in each group is illustrated in Table 2.

In the initial analysis of variance (Table 3) carried out to determine differences between groups, sows and gilts inseminated twice during estrus with or without the addition of progesterone to the extended semen had a higher ($P < .017$) number of piglets born than gilts or sows inseminated with only one dose of extended semen with or without the addition of progesterone. Non-significant differences in number of pigs born were found between progesterone-treated animals and non-progesterone treated animals in either gilts or sows. The interactions of progesterone * reproductive age, progesterone * number of services, and between reproductive age * number of services * progesterone were also non-significant ($P > .10$) when total number of pigs per group is considered.

The data from the pregnant animals were separately analyzed for litter size using a non-orthogonal analysis procedure (Winer, 1971). The results of this analysis of variance are given in Table 4.

In the full model analysis of variance of litter size for pregnant animals shown in Table 4, the total effect for the interaction was non-significant when using a α - level of 0.25 in order to minimize

the probability of type II error (Sum of squares = 22.934; F = 0.825), so the main effects were analysed, and this analysis is shown in Table 5. The effects of reproductive age (F - 5.97; P = 0.024) and progesterone (F = 4.87; P= 0.039) were significant.

These results suggest that double insemination during estrus is required to produce a higher number of pregnancies, and that reproductive age and the addition of progesterone to extended semen had a significant effect on the litter size. However, these results are not considered conclusive and replication is recommended.

Experiment B. The total number of spermatozoa recovered from the washing of the uterotubal junctions and their transformation to $\log_{10}$, as well as the values for the t-test, are shown in Table 6. There was a non-significant difference between the number of spermatozoa recovered from animals inseminated with extended semen treated with the addition of 5 mg of progesterone and the group of animals inseminated with extended semen without treatment of progesterone. These results indicate that progesterone did not inhibit sperm transport up to the uterotubal junction.

Table 2. Number of piglets born from animals inseminated with 2 X 10^8 sperm cells diluted in a liquid semen extender

	Gilts				Sows			
	Single Service		Double Service		Single Service		Double Service	
	Gilt Number	Piglets Born	Gilt Number	Piglets Born	Sow Number	Piglets Born	Sow Number	Piglets Born
Liquid	111	0	121	10	131	0	141	16
semen	112	9	122	0	132	0	142	13
extender	113	0	123	8	133	11	143	11
with	114	0	124	12	134	12	144	0
5 mg of	115	0	125	14	135	0	145	10
progesterone								
Totals		9		44		23		50
Liquid	211	0	221	0	231	0	241	7
semen	212	11	222	9	232	0	242	9
extender	213	0	223	0	233	0	243	13
without	214	5	224	8	234	13	244	10
5mg of	215	9	225	2	235	12	245	11
progesterone								
Totals		25		19		25		50
Combined Totals		34		63		48		100

Table 3. Analysis of variance for the total of piglets born to all groups[a]

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob > F
Treatments	7	15.319	2.188	1.68	0.149
Age	1	2.327	2.327	1.79	0.191
Services	1	8.275	8.275	6.36	0.017
Progesterone	1	0.001	0.001	0.00	0.983
Age * Serv	1	0.700	0.700	0.54	0.469
Age * Prog	1	0.114	0.114	0.09	0.770
Serv * Prog	1	1.691	1.691	1.30	0.263
Age * Serv * Prog	1	2.211	2.211	1.70	0.202
Error	32	41.662	1.302		
Corrected total	39	56.981			

[a]Data were transformed to square roots of litter size +1.

Table 4. Full model analysis of variance of litter size for pregnant animals[a]

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob > F
Treatments	7	94.625	13.518	1.98	0.123
Age	1	41.592	41.592	6.09	0.025
Services	1	0.668	0.668	0.10	0.756
Progesterone	1	13.869	13.869	2.03	0.174
Age * Serv	1	0.668	0.668	0.10	0.756
Age * Prog	1	4.365	4.365	0.64	0.436
Serv * Prog	1	16.708	16.708	2.45	0.138
Age * Serv * Prog	1	0.074	0.074	0.01	0.918
Error	16	109.333			
Corrected total	23	203.958			

[a]Overall mean and standard deviation are 10.21 ± 2.61.

Table 5. Analysis of variance of main effects on litter size for pregnant animals[a]

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob > F
Treatments	3	71.692	23.897	3.61	0.031
Age	1	39.490	39.490	5.97	0.024
Services	1	1.397	1.397	0.21	0.651
Progesterone	1	32.181	32.181	4.87	0.039
Error	20	132.267	6.613		
Corrected total	23	203.958			

[a]Overall mean and standard deviation are 10.21 ± 2.61.

Table 6. The total number of spermatozoa recovered from the uterotubal junction of animals inseminated with 6 X 10^9 sperm cells diluted in a liquid semen extender

Extended semen plus progesterone–5 mg			Extended semen without progesterone		
Animal Number	Spermatozoa Recovered (10^3)	Log_{10}	Animal Number	Spermatozoa Recovered (10^3)	Log_{10}
301	0.72	2.8573	401	0.72	2.8573
302	965.00	5.9845	402	737.00	5.8675
303	2200.00	6.3424	403	50.00	4.6990
304	1750.00	6.2430	404	375.00	5.5740
305	75.00	4.8751	405	0.72	2.8573
		$\bar{y} = 5.2605$[a]			$\bar{y} = 4.3710$[a]

[a]Calculated "t" = 0.966 N.S. Table "t"$_{0.05,8}$ = 2.306.

V. DISCUSSION

In experiment A, the overall farrowing rate obtained after artificial insemination with a low sperm concentration was 60%. This percentage is lower than the average expected when inseminating fresh liquid extended semen. Koh et al. (1976) reported farrowing rates up to 78.2% using 5 X 10^9 spermatozoa diluted in 50 ml of liquid semen extender. Johnson et al. (1981) obtained an overall farrowing rate of 79.1% using 3 X 10^9 spermatozoa diluted in 100 ml of extender. In the present study the total number of spermatozoa used per dose was 2 X 10^8. This was ten-fold smaller than the minimal number of sperm cells recommended for artificial insemination with fresh semen (Melrose, 1966). This low number of spermatozoa was used in order to have a more accurate assessment of the fertility response in the artificially inseminated animals. Saacke et al. (1980) cited the theory that when a high dose of spermatozoa is used, optimum fertility of the female population could be obtained without regard to the semen quality, but semen quality characteristics would have the greatest impact on fertility when sperm doses were minimal and the average fertility of the female population had not been attained.

Groups of animals inseminated twice during estrus had a 80% farrowing rate and thus produced significantly ($P < .017$) more piglets per group than single inseminated animals which had only a 40% farrowing rate (Table 2, Table 3). Similar results have been reported by Crabo

and Hurtgen (1977). A single insemination 24 to 36 hours after the onset of estrus as recommended by Polge (1978), may not place adequate numbers of spermatozoa in the oviducts at the right time to maximize the chances of fertilization. The timing of insemination in relation to estrus has been based upon the assumption that the onset of the preovulatory luteinizing hormone (LH) surge coincided with the onset of estrus. This assumption is now open to question because the preovulatory LH surge in many gilts and sows starts 24 to 36 hours before estrus is detected (Findley et al., and Aherne et al., as cited by Guthrie 1979). Therefore, a single insemination on the second day of estrus may have been made after the animal had already ovulated. The use of a single insemination of low numbers of spermatozoa will probably require ovulation control with gonadotrophins or gonadotrophin-releasing hormones as suggested by Johnson et al. (1981).

When the data of animals that became pregnant were separately analyzed for litter size, sows had a higher ($P < .025$) number of piglets than gilts (Table 4). All sows were inseminated during their first postweaning estrus. Similar results comparing litter size in sows and gilts have been reported by Graham et al. (1978); Foote (1980); and Hughes and Varley (1980).

Sows and gilts inseminated with liquid extended semen treated with 5 mg of crystalline progesterone, farrowed more piglets per group than gilts and sows inseminated without progesterone in the extended semen, but this difference was non-significant ($P > .98$) when considering

all animals in the experiment (Table 3). When the data for pregnant animals were analyzed for litter size using only the main effects model (Table 5), progesterone significantly ($P < .039$) increased the litter size of treated animals. This effect of progesterone was not significant ($P < .174$) for litter size when the data were analyzed with the full model analysis of variance.

Thus it appears that progesterone in the insemination dose may have an effect to increase litter size but this effect was confounded by other variables in this study.

Hunter (1972b) reported that after a local injection of progesterone beneath the serosa of the tubal isthmus and the uterotubal junction, the myometrial cells of the edematous uterotubal junction may relax and a larger proportion of spermatozoa may have passed directly into the isthmus and ampulla with very little quantitative regulation. Evans and Dawson (Department of Veterinary Clinical Sciences, Iowa State University, unpublished results), found that progesterone when added to test fluids, produced an increase in the flow rate through the uterotubal junction of treated animals.

On the basis of the results obtained in the present experiment and those obtained by Hunter (1972b) and Evans and Dawson (Department of Veterinary Clinical Sciences, Iowa State University, unpublished results), a positive effect on litter size might be expected with the addition of progesterone to liquid semen of low sperm concentration. Whether or not progesterone-treated semen may alter uterine myometrium

and transport of semen from the cervix to the uterotubal junction must be considered since both progesterone trials mentioned previously were applied locally at the uterotubal junction or upper uterine horns. Day and Polge (1968) reported that after a subcutaneous injection of 100 mg of progesterone 24 to 36 hours before ovulation, an accelerated rate of egg transport through the oviducts was produced in gilts. Further experiments are required to determine more precisely the role of progesterone on sperm and ovum transport in the pig.

In experiment B, the effect of progesterone on sperm transport in animals artificially inseminated with 6 X 10^9 spermatozoa in liquid semen extender and treated with the addition of 5 mg of crystalline progesterone was tested.

Factors influencing sperm transport have been described by Baker et al. (1968); Bower et al. (1974); Einarsson (1980); Einarsson and Viring (1973a); First et al. (1968); Hancock and Hovell (1961); Hunter (1972b, 1980a, 1981); Polge (1978); Thibault (1973); and, Viring and Einarsson (1980a,b). The role of the uterotubal junction (UTJ) as a barrier that effectively regulates the passage of spermatozoa into the oviducts has been described by Hunter (1980a) and Flechon and Hunter (1981). Viring and Einarsson (1980b) reported that seminal plasma decreased the spontaneous motility of the isthmus close to the UTJ and this relaxative effect facilitates sperm transport into the oviducts, but the effectual constituents are still unidentified. They concluded that sperm transport through the UTJ is essentially of a passive nature.

In early trials of experiment B, six animals were inseminated with 2 X 10^8 spermatozoa diluted in 70 ml of liquid semen extender, but no sperm cells were recovered from the oviducts. Consequently, sperm cell numbers were increased to 6 X 10^9 and diluted in 70 ml of liquid semen extender. Ten females were inseminated with this dose. Although the number of spermatozoa recovered from the UTJ was increased with the latter insemination dose, no sperm cells were recovered from the oviducts of seven animals. Therefore, data from the flushings of oviducts were omitted from the statistical analysis.

The results of washings of the UTJ to recover the spermatozoa, indicate that more sperm cells were recovered from the UTJ of animals inseminated with progesterone-treated extended semen but this difference was non-significant (Table 6). The numbers of sperm cells recovered from the UTJ are comparable to figures reported by Viring (1980) and Viring and Einarsson (1980a) when the recovery was done six hours after insemination with 14.6 X 10^9 live spermatozoa. They also reported that there was a positive correlation between the number of spermatozoa in the UTJ and those found in the oviduct.

All animals used in experiment B were brought into heat by hormonal treatment to synchronize breeding with available slaughter dates and to assure the collection of specimens at 24 to 28 hours after breeding. The animals were inseminated 18 to 20 hours after an intramuscular injection of 500 IU of HCG. They were bred at this time in order to avoid the possible release of ova which could interfere with the counting

of sperm cells. When counting sperm in the oviduct after ovulation, the number of spermatozoa attached to the zona pellucida of each egg may be as many as 100 or more (Hunter, 1972b), and this would require special techniques to separate the spermatozoa from the zona. Viring (1980) reported that the sperm count in the UTJ remained relatively unchanged up to 12 hours after insemination with live spermatozoa. Whether or not the hormonal treatment may have played a negative role on sperm transport and on the sperm recovery from the oviducts was not determined. Guthrie (1979) suggested that injection of HCG at 72 hours after PMSG treatment may terminate follicular estradiol secretion. This premature injection of HCG could interfere with gamete transport by altering estradiol production.

In this study, the number of spermatozoa in the oviducts six to eight hours after artificial insemination could not be determined. Therefore, the studies of Evans and Dawson (Department of Veterinary Clinical Sciences, Iowa State University, unpublished results), and Hunter (1972b) on the relaxative effect of progesterone at the UTJ could not be substantiated by sperm counts in the oviducts. However, the results of the present experiment suggest that progesterone-treated extended semen does not inhibit sperm transport to the UTJ. Results of experiment A suggest that more piglets per litter could be obtained when progesterone is included in extended semen of low spermatozoa concentration.

VI. SUMMARY AND CONCLUSIONS

Artificial insemination of sows and gilts with 2 X 10^8 spermatozoa diluted in 70 ml of liquid semen extender produced an overall farrowing rate of 60%. It was found that double insemination of gilts and sows resulted in twice as many pregnant animals and significantly more piglets born ($P < .017$) than when only one insemination was carried out. When analyzing reproductive age effect for pregnant animals, it was found that sows farrowed significantly larger litters ($P < .025$) than gilts. Among the animals that became pregnant, sows and gilts artificially inseminated with extended semen treated with 5 mg of crystalline progesterone farrowed larger litters than sows and gilts inseminated with extended semen without progesterone. Litter size was significantly different ($P < .039$) when analyzing only the main effects model but non-significant when all interactions were included in the analysis.

The number of spermatozoa recovered from the uterotubal junction of hormonally-induced estrous animals, six to eight hours after artificial insemination with 6 X 10^9 sperm cells diluted in liquid semen extender and treated with 5 mg of crystalline progesterone, was not significantly different from that obtained after artificial insemination with non-treated extended semen.

It can be concluded that the treatment of liquid extended semen with 5 mg of crystalline progesterone does not inhibit sperm transport to the uterotubal junction and may increase the number of piglets born when inseminating with a low sperm concentration.

VII. REFERENCES

Aamdal, J. 1964. Artificial insemination in the pig. Proc. 5th Int. Congr. Anim. Reprod. Artif. Insem. (Trento) 4:147-177.

Aamdal, J., and I. Hogset. 1957. Artificial insemination in swine. J. Am. Vet. Med. Assoc. 131:59-64.

Anderson, L. L. 1980. Reproductive cycles: Pigs. Pages 358-386 in E.S.E. Hafez, ed. Reproduction in farm animals. 4th ed. Lea & Febiger, Philadelphia.

Austin, C. R. 1948. Number of sperms required for fertilization. Nature (London) 162:534-535.

Austin, C. R. 1949. Fertilization and transport of gametes in the pseudo pregnant rabbit. J. Endocrinol. 6:63-70.

Austin, C. R. 1964. Behavior of spermatozoa in the female genital tract and in fertilization. Proc. 5th Int. Congr. Anim. Reprod. Artif. Insem. (Trento) 3:7-22.

Baker, R. A., and P. J. Dziuk. 1964. Fertilization in artificially inseminated gilts. J. Anim. Sci. 23:1224. (Abstr.)

Baker, R. D., and A. A. Degen. 1972. Transport of live and dead boar spermatozoa within the reproductive tract of gilts. J. Reprod. Fertil. 28:369-377.

Baker, R. D., and C. Polge. 1976. Fertilization in swine and cattle. Can. J. Anim. Sci. 56:105-119.

Baker, R. D., P. J. Dziuk, H. W. Norton. 1968. Effect of volume of semen, number of sperm and drugs on transport of sperm in artificially inseminated gilts. J. Anim. Sci. 27:88-93.

Baker, L. N., L. C. Ulberg, R. H. Grummer and L. E. Casida. 1954. Inhibition of heat by progesterone and its effect on subsequent fertility in gilts. J. Anim. Sci. 13:648-657.

Bakst, M. R. 1981. Sperm recovery from oviducts of turkeys at known intervals after insemination and oviposition. J. Reprod. Fertil. 62:159-164.

Bane, A. 1959. Some results of studies on the semen of young boars and on artificial insemination in pigs. Ann. Zootech. 8 (Suppl.): 15-20.

Bareither, M. L., and H. G. Verhage. 1981. Control of the secretory cell cycle in cat oviduct by estradiol and progesterone. Am. J. Anat. 162:107-118.

Barr, A. J., J. H. Goodnight, J. P. Sall, W. H. Blair, and D. M. Chilko. 1979. SAS User's guide. SAS Institute, Inc., Raleigh, N.C.

Basurto-Kuba, V. M. 1979. A comparative study of boar semen collected by electro ejaculation and gloved-hand technique. M.S. Thesis. Library, Iowa State University, Ames, Iowa. 61 pp.

Bedford, J. M. 1971. The rate of sperm passage into the cervix after coitus in the rabbit. J. Reprod. Fertil. 25:211-218.

Beir, H. M. 1974. Oviducal and uterine fluids. J. Reprod. Fertil. 37:221-237.

Bentley, P. J. 1980. Endocrine pharmacology. Cambridge University Press, Cambridge, London. 496 pp.

Bhela, S. L., B. P. Sengupta, and M. N. Razdon. 1981. Effect of exogenous testosterone, estradiol, and progesterone on oxidative and glycolitic metabolism of buffalo spermatozoa. Indian J. Exp. Biol. 19:915-917.

Black, D. L., and S. A. Asdell. 1959. Mechanism controlling entry of ova into rabbit uterus. Am. J. Physiol. 197:1275-1278.

Blandau, R. J. 1961. Biology of eggs and implantation. Pages 797-882 in W.C. Young, ed. Sex and internal secretions. Vol. 2. 3rd edition. Williams and Wilkins Co., Baltimore, MD.

Blandau, R. J., and P. Gaddum-Rosse. 1974. Mechanism of sperm transport in pig oviducts. Fertil. Steril. 25:61-66.

Bower, R. E. Jr., B. G. Crabo, and E. F. Graham. 1974. Factors affecting myometrial activity. J. Anim. Sci. 39:200. (Abstr.)

Braden, A. W. H., and C. R. Austin. 1954. The number of sperms about the eggs in mammals and its significance for normal fertilization. Austr. J. Biol. Sci. 7:543-551.

Butakov, A. K. 1973. Distribution of spermatozoa in the uterus of heifers in relation to its morphological and functional changes (in Russian). Anim. Breed. Abstr. 41:102. (Abstr.)

Campbell, D. A. R. 1961. Observations on certain aspects of artificial insemination in pigs. Proc. 4th Int. Congr. Anim. Reprod. and Artif. Insem. (The Hague) 4:847-850.

Campbell, E. A., and S. A. Lingam. 1965. Artificial insemination of pigs in Australia. Austr. Vet. J. 41:147-150.

Casida, L. E. 1935. Prepuberal development of the pig ovary and its relation to stimulation with gonadotrophic hormones. Anat. Rec. 61:389-396.

Catchpole, H. R. 1959. Endocrine mechanisms during pregnancy. Pages 469-501 in H. H. Cole and P. T. Cupps, eds. Reproduction in domestic animals. Academic Press, New York.

Catchpole, H. R. 1969. Hormonal mechanisms during pregnancy and parturition. Pages 415-440 in H. H. Cole and P. T. Cupps, eds. Reproduction in domestic animals. Academic Press, New York.

Chang, M. C. 1966. Effects of oral administration of medroxiprogesterone acetate and ethinyl estradiol on transportation and development of rabbit eggs. Endocrinology 79:939-948.

Christenson, R. K., and H. S. Teague. 1975. Synchronization of ovulation and artificial insemination of sows after lactation. J. Anim. Sci. 41:560-563.

Clamohoy, L. L., J. P. Abilay, and D. A. Paled. 1960. A study of two procedures in training boars for semen collection. Phillip. Agric. 44:348-354.

Clark, T. L. 1976. Electroejaculation in the anesthetized and non-anesthetized boar. Proc. Int. Pig Vet. Soc Congr. (Ames) 4:D-14.

Cole, H. H., and P. T. Cupps. 1977. Reproduction in domestic animals. 3rd ed. Academic Press, New York. 665 pp.

Corner, G. W. 1928. Physiology of the corpus luteum. I. The effect of very early ablation of the corpus luteum upon embryos and uterus. Am. J. Physiol. 86:74-81.

Corner, G. W., and W. M. Allen. 1929. Physiology of the corpus luteum. II. Production of a special uterine reaction (progestational proliferation) by extracts of the corpus luteum. Am. J. Physiol. 88:326-339.

Crabo, B. G. 1977. Frozen boar semen. Pages 33-38 in Proc. Ann. Meet. Soc. for Theriogenology, St. Paul, MN. AVSSBS, Hastings, NE.

Crabo, B. G., and J. P. Hurtgen. 1977. Artificial insemination methods in swine. Pages 29-32 in Proc. Ann. Meet. Soc. for Theriogenology, St. Paul, MN. AVSSBS, Hastings, NE.

Crighton, D. B. 1970. Induction of pregnancy during lactation in the sow. J. Reprod. Fertil. 22:223-231.

Cross, B. A. 1959. Recent progress in endocrinology of reproduction. Edited by C. W. Lloyd. Academic Press, New York.

Csapo, A. I. 1956. The mechanism of effect of the ovarian steroids. Recent Progr. Horm. Res. 12:405-431.

Davis, D. L., D. B. Killian, and B. N. Day. 1976. Control of estrus in gilts with compound A-35957. J. Anim. Sci. 42:1358. (Abstr.)

Day, B. N. and C. Polge. 1968. Effects of progesterone on fertilization and egg transport in the pig. J. Reprod. Fertil. 17:227-230.

Day, B. N., L. L. Anderson, L. N. Hazel, and R. N. Melampy. 1959. Synchronization of estrus and ovulation in swine. J. Anim. Sci. 18:909-917.

Dorfman, R. I. 1973. Biosynthesis of progestogens. Pages 537-546 in R. O. Greep and E. W. Astwood, eds. Handbook of Physiology. Section 7, Endocrinology. American Physiological Society, Washington, D.C.

DuMesnil du Buisson, and J. P. Signoret. 1970. Reproductive physiology and artificial insemination in pigs. Vet. Rec. 87:562-568.

Duncan, G. W. 1960. In vitro synthesis of progesterone by swine corpora lutea. Ph.D. Dissertation. Iowa State University, Ames, Iowa. 63 pp.

Dziuk, P. J. 1958. Dilution and storage of boar semen. J. Anim. Sci. 17:548-553.

Dziuk, P. J. 1959. Influence of storage of boar semen on its subsequent fertilizing ability. Ann. Zootech. 8 (Suppl.):21-26.

Dziuk, P. J. 1969. Influence of orally administered progestins on estrus and ovulation in swine. J. Anim. Sci. 19:1319-1320. (Abstr.)

Dziuk, P. J. 1970. Estimation of optimum time for insemination of gilts and ewes by double-mating at certain times relative to ovulation. J. Reprod. Fertil. 22:277-282.

Dziuk, P. J. 1972. Swine artificial insemination. Whether and Whither. Pages 75-76 in Proc. 4th Tech. Conf. Artif. Insem. Reprod. NAAB, Chicago, IL.

Dziuk, P. J. 1977. Assessment of fertility in boars. Pages 18-28 in Proc. Ann. Meet. Soc. for Theriogenology, St. Paul, MN. ABSSBS, Hastings, NE.

Dziuk, P. J., and R. D. Baker. 1962. Induction and control of ovulation in swine. J. Anim. Sci. 21:697-699.

Dziuk, P. J., and G. Henshaw. 1958. Fertility of boar semen artificially inseminated following in vitro storage. J. Anim. Sci. 17:554-558.

Dziuk, P. J., and G. D. Gehlbach. 1966. Induction of ovulation and fertilization in the immature gilt. J. Anim. Sci. 25:410-413.

Dziuk, P. J. and C. Polge. 1962. Fertility in swine after induced ovulation. J. Reprod. Fertil. 4:207-208.

Einarsson, S. 1980. Site, transport and fate of inseminated semen. Proc. 9th Int. Congr. Anim. Reprod. and Artif. Insem. (Madrid) 2:147-158.

Einarsson, S., and S. Viring. 1973a. Distribution of frozen-thawed spermatozoa in the reproductive tract of gilts at different time intervals after insemination. J. Reprod. Fertil. 32:117-120.

Einarsson, S., and S. Viring. 1973b. Effect of boar seminal plasma on the porcine uterus and the isthmus part of oviduct in vitro. Acta. Vet. Scand. 14:639-641.

Einarsson, S., B. Jones, K. Larsson, and S. Viring. 1980. Distribution of small- and medium-sized molecules within the genital tract of artificially inseminated gilts. J. Reprod. Fertil. 59:453-457.

Ellicott, A. R., and P. J. Dziuk. 1973. Minimum daily dose of progesterone and plasma concentration for maintenance of pregnancy in ovariectomized gilts. Biol. Reprod. 9:300-304.

Erickson, W. E. 1974. Update on swine artificial insemination. Pages 82-84 in Proc. 5th Tech. Conf. Artif. Insem. Reprod. NAAB, Chicago, IL.

First, N. L., F. W. Stratman, and L. E. Casida. 1963. Effect of sperm age on embryo survival in swine. J. Anim. Sci. 22:135-138.

First, N. L., R. E. Short, J. B. Peters, and F. W. Stratman. 1965. Transport of spermatozoa in estrual and luteal sows. J. Anim. Sci. 24:917. (Abstr.)

First, N. L., R. E. Short, J. B. Peters and F. W. Stratman. 1968. Transport and loss of boar spermatozoa in the reproductive tract of the sow. J. Anim. Sci. 27:1037-1040.

Flechon, J. E., and R. H. F. Hunter. 1981. Distribution of spermatozoa in the utero-tubal junction and isthmus of pigs, and their relationship with the luminal epithelium after mating: a scanning electron microscope study. Tissue & Cell 13:127-139.

Foote, R. H. 1980. Artificial insemination. Pages 521-545 in E. S. E. Hafez, ed. Reproduction in farm animals. Lea and Febiger, Philadelphia.

Gaddum-Rosse, P. 1981. Some observations on sperm transport through the uterotubal junction in the rat. Am. J. Anat. 160:333-341.

Gardner, M. L., N. L. First, and L. E. Casida. 1963. Effect of exogenous estrogens on corpus luteum maintenance in gilts. J. Anim. Sci. 22:132-134.

Gerrits, R. J., E. F. Graham, and C. L. Cole. 1962a. Effect of collection interval on the characteristics of the ejaculate in the boar. J. Anim. Sci. 21:1022. (Abstr.)

Gerrits, R. J., M. L. Fahning, R. J. Meade, and E. F. Graham. 1962b. Effect of synchronization of estrus on fertility in gilts. J. Anim. Sci. 21:1022. (Abstr.)

Graham, E. F., B. G. Crabo, and M. M. Pace. 1978. Current status of semen preservation in the ram, boar and stallion. XII. Biennial Symposium on Animal Reproduction. J. Anim. Sci. 47 (Suppl. II):80-118.

Greenwald, G. S. 1961. A study of the transport of ova through the rabbit. oviduct. Fertil. Steril. 12:80-94.

Guthrie, H. D. 1975. Estrus synchronization and fertility in gilts treated with estradiol-benzoate and prostanglandin $F_{2\alpha}$. Theriogenology 4:69-76.

Guthrie, H. D. 1979. Fertility after estrus cycle control using gonadotropin and prostaglandin F-2α treatment of sows. J. Anim. Sci. 49:158-162.

Guthrie, H. D., and C. Polge. 1976a. Luteal function and estrus in gilts treated with a synthetic analogue of prostaglandin F-2α (ICI 79,939) at various times during the estrus cycle. J. Reprod. Fertil. 48:423-425.

Guthrie, H. D., and C. Polge. 1976b. Control of estrus and fertility in gilts with accessory corpora lutea by prostaglandin analogues, ICI 79,939 and ICI 80,996. J. Reprod. Fertil. 48:427-430.

Guthrie, H. D., D. M. Henricks, and D. L. Handlin. 1972. Plasma estrogen, progesterone and luteinizing hormone prior to estrus and during early pregnancy in pigs. Endocrinology 91:675-679.

Guthrie, H. D., D. M. Henricks, and D. L. Handlin. 1974. Plasma hormone levels and fertility in pigs induced to super ovulate with PMSG. J. Reprod. Fertil. 41:361-370.

Hafez, E. S. E. 1980. Reproduction in farm animals. 4th ed. Lea & Febiger, Philadelphia. 626 pp.

Hancock, J. L. 1959. Pig insemination technique. Vet. Rec. 71: 523-527.

Hancock, J. L. 1961. Fertilization in the pig. J. Reprod. Fertil. 2:307-331.

Hancock, J. L., and G. J. R. Hovell. 1959. The collection of boar semen. Vet. Rec. 71:664-665.

Hancock, J. L., and G. J. R. Hovell. 1961. The effect of semen volume and number of spermatozoa on the fertility of intrauterine inseminations of pigs. Anim. Prod. 3:153-161.

Hancock, J. L., and G. J. R. Hovell. 1962. Insemination before and after the onset of heat in sows. Anim. Prod. 4:91-96.

Harper, M. J. K. 1964. The effects of constant doses of estrogen and progesterone on the transport of eggs through the reproductive tract of ovariectomized rabbits. J. Endocrinol. 31:1-19.

Hawk, H. W., and H. H. Conley. 1971. Loss of spermatozoa from the reproductive tract of the ewe and intensification of sperm "breakage" by progesterone. J. Reprod. Fertil. 27:339-347.

Hawk, H. H., and H. H. Conley. 1975. Involvement of the cervix in sperm transport failures in the reproductive tract of the ewe. Biol. Reprod. 13:322-328.

Hawk, H. W., and B. S. Cooper. 1978. Increased retention of sperm in the reproductive tract and improved ovum fertilization after administration of estradiol to estrus rabbits. Biol. Reprod. 17:850-857.

Hawk, H. W., and B. S. Cooper. 1979. Increased retention of spermatozoa in the reproductive tract of estrus rabbits after administration of prostaglandin F-2∝ immediately before insemination. J. Anim. Sci. 49:154-157.

Hawk, H. W., B. S. Cooper, and V. G. Pursel. 1981. Sperm death and inhibition of sperm transport in the cervix of ewes in estrus after removal of the corpus luteum-bearing ovary. J. Anim. Sci. 52:611-617.

Henricks, D. M., H. D. Guthrie, and D. L. Handlin. 1972. Plasma estrogen, progesterone and luteinizing hormone levels during the estrus cycle in pigs. Biol. Reprod. 6:210-218.

Hertz, R., W. Tullner, and E. Raffelt. 1954. Progestational activity of orally administered 17∝ -ethinyl-19-nortestosterone. Endocrinology 54:228-230.

Hess, E. A., T. M. Ludwick, and H. S. Teague. 1959. Artificial insemination of swine. Ann. Zootech. 8 (Suppl.):31-36.

Horne, H. W., Jr., and J. P. Thibault. 1962. Sperm migration through the human female reproductive tract. Fertil. Steril. 13:444-447.

Hughes, P. E., and M. A. Varley. 1980. Reproduction in the pig. Butterworth and Co. Ltd., London.

Hunter, R. H. F. 1966. Luteal-phase ovulation and fertility in the pig. J. Anim. Sci. 25:925. (Abstr.)

Hunter, R. H. F. 1967a. Polyspermic fertilization in pigs during the luteal phase of the estrus cycle. J. Exp. Zool. 165:451-460.

Hunter, R. H. F. 1967b. Porcine ovulation after injection of human chorionic gonadotrophin. Vet. Rec. 81:21-23.

Hunter, R. H. F. 1967c. The effects of delayed insemination on fertilization and early cleavage in the pig. J. Reprod. Fertil. 13:133-147.

Hunter, R. H. F. 1972a. Fertilization in the pig: Sequence of nuclear and cytoplasmic events. J. Reprod. Fertil. 29:395-406.

Hunter, R. H. F. 1972b. Local action of progesterone leading to polyspermic fertilization in pigs. J. Reprod. Fertil. 31:433-444.

Hunter, R. H. F. 1975a. Physiological aspects of sperm transport in the domestic pig, Sus scrofa, I. Semen deposition and cell transport. Br. Vet. J. 131:565-573.

Hunter, R. H. F. 1975b. Physiological aspects of sperm transport in the domestic pig, Sus scrofa. II. Regulation survival and fate of cells. Br. Vet. J. 131:681-690.

Hunter, R. H. F. 1976. Sperm-egg interactions in the pig: monospermy, extensive polyspermy, and the formation of chromatin aggregates. J. Anat. 122:43-59.

Hunter, R. H. F. 1977. Physiological factors influencing ovulation, fertilization, early embryonic development and establishment of pregnancy in pigs. Br. Vet. J. 133:461-470.

Hunter, R. H. F. 1978. Intraperitoneal insemination, sperm transport and capacitation in the pig. Anim. Reprod. Sci. 1:167-179.

Hunter, R. H. F. 1980a. Physiology and technology of reproduction in female domestic animals. Academic Press, London. 393 pp.

Hunter, R. H. F. 1980b. Transport and storage of spermatozoa in the female tract. Proc. 9th Int. Congr. Anim. Reprod. Artif. Insem. (Madrid) 2:227-233.

Hunter, R. H. F. 1981. Sperm transport and reservoirs in the pig oviduct in relation to the time of ovulation. J. Reprod. Fertil. 63:109-117.

Hunter, R. H. F., and P. J. Dziuk. 1966. Fertilization of pig eggs three hours postinsemination. J. Anim. Sci. 25:1265. (Abstr.)

Hunter, R. H. F., and P. J. Dziuk. 1968. Sperm penetration of pig eggs in relation to the timing of ovulation and insemination. J. Reprod. Fertil. 15:199-208.

Hunter, R. H. F., and J. P. Hall. 1974a. Capacitation of boar spermatozoa: The influence of post-coital separation of the uterus and Fallopian tubes. Anat. Rec. 180:597-603.

Hunter, R. H. F., and J. P. Hall. 1974b. Capacitation of boar spermatozoa: Synergism between uterine and tubal environments. J. Exp. Zool. 188:203-214.

Hunter, R. H. F., and P. C. Leglise. 1971. Polyspermic fertilization following tubal surgery in pigs, with particular reference to the role of the isthmus. J. Reprod. Fertil. 24:233-246.

Hunter, R. H. F., J. P. Hall, B. Cook, and P. D. Taylor. 1972. Oestrogens and progesterone in porcine peripheral plasma before and after induced ovulation. J. Reprod. Fertil. 31:499-501.

Hunter, R. H. F., R. Nichol, and S. M. Crabtree. 1980. Transport of spermatozoa in the ewe: Timing of the establishment of a functional population in the oviduct. Reprod. Nutr. Develop. 20:1869-1875.

Hurtgen, J. P. 1979. Seasonal breeding patterns in female swine. Ph.D. Dissertation. Library, University of Minnesota, St. Paul. 151 pp.

Hurtgen, J. P., B. G. Crabo, and A. D. Leman. 1977. Fertility examination of boars. Pages 11-17 in Proc. Ann. Meet. Soc. for Theriogenology, St. Paul, MN. AVSSBS, Hastings, NE.

Ito, S., A. Kudo, and T. Niwa. 1959. Studies on the normal estrus in swine with special reference to proper time for service. Ann. Zootech. 8 (Suppl.):105-107.

Jochle, W., and D. R. Lamond. 1980. Control of reproductive functions in domestic animals. Martinus Nijhoff Publishers, The Hague, The Netherlands. 248 pp.

Johnson, L. A. 1980. Artificial insemination of swine: Fertility with frozen boar semen. Proc. Int. Pig Vet. Soc. Congr. (Copenhagen) 6:37.

Johnson, L. A., J. G. Aalbers, and J. A. M. Arts. 1979. Fertility of fresh and frozen boar semen in gilts inseminated at a fixed time or according to measurements of vaginal mucosa electrical resistance. J. Anim. Sci. 49:307 (Abstr.)

Johnson, L. A., J. G. Aalbers, C. M. T. Willems, and W. Sybesma. 1979. Effectiveness of fresh and frozen semen under practical conditions. J. Anim. Sci. 49:306 (Abstr.)

Johnson, L. A., J. G. Aalbers, C. M. T. Willems, and J. H. M. Rademaker. 1980. Fertility of boar semen stored in BL-1 and Kiev extenders at 18°C for three days. Proc. Int. Pig Vet. Soc. Congr. (Copenhagen) 6:33.

Johnson, L. A., J. G. Aalbers, C. M. T. Willems, and W. Sybesma. 1981. Use of boar spermatozoa for artificial insemination. I. Fertilizing capacity of fresh and frozen spermatozoa in sows on 36 farms. J. Anim. Sci. 52:1130-1136.

Jones, R., and T. Mann. 1977a. Toxicity of exogenous fatty acid peroxides towards spermatozoa. J. Reprod. Fertil. 50:255-260.

Jones, R., and T. Mann. 1977b. Damage to ram spermatozoa by peroxidation of endogenous phospholipids. J. Reprod. Fertil. 50:261-268.

Knight, J. W., D. L. Davis, and B. N. Day. 1976. Estrus synchronization in gilts with a progestagen. J. Anim. Sci. 42:1358-1359. (Abstr.)

Koh, T. J., B. G. Crabo, H. L. Tsou, and E. F. Graham. 1976. Fertility of liquid boar semen as influenced by breed and season. J. Anim. Sci. 42:138-144.

Korenman, S. G., and B. W. O'Malley. 1968. Progesterone action: Regulation of avidin biosynthesis by hen oviduct *in vivo* and *in vitro*. Endocrinology 83:11-17.

Kraeling, R. R., P. J. Dziuk, V. G. Pursel, G. B. Rampacek, and S. K. Webel. 1981. Synchronization of estrus in swine with allyl trenbolone (RU - 2267). J. Anim. Sci. 52:831-835.

Krzanowska, H. 1974. The passage of abnormal spermatozoa through the uterotubal junction of the mouse. J. Reprod. Fertil. 39:81-90.

Kuo, D., H. H. Hodson, and C. L. Hausler. 1976. Induction of ovulation, artificial insemination and conception in lactating sows. Proc. Int. Pig Vet. Soc. Congr. (Ames) 4:D-23.

Lagerlöf, N., and H. Carlquist. 1961. The semen of boars of the Yorkshire breed between the ages of five and nine months. Proc. 4th Int. Congr. Anim. Reprod. Artif. Insem. (The Hague) 4:818-822.

Larsson, K. 1976. Fertility of deep frozen boar spermatozoa at various intervals between insemination and induced ovulation. Influence of boars and thawing diluents. Acta Vet. Scand. 17:63-73.

Larsson, K., and S. Einarsson. 1976. Fertility of deep frozen boar spermatozoa. Influence of thawing diluents and of boars. Acta Vet. Scand. 17:43-62.

Larsson, K., S. Einarsson, and L. Nicander. 1976. Influence of thawing diluents on vitality, acrosome morphology, ultrastructure and enzyme release of deep frozen boar spermatozoa. Acta Vet. Scand. 17:83-100.

Little, T. M., and F. J. Hills. 1978. Agricultural experimentation. John Willey & Sons, New York. 350 pp.

Longenecker, D. E., and B. N. Day. 1968. Fertility level of sows superovulated at post-weaning estrus. J. Anim. Sci. 27:709-711.

Longley, W. J., D. L. Black and G. N. Currie. 1968. Ovarian hormone control of ovum transport in the rabbit as influenced by autonomic drugs. J. Reprod. Fertil. 17:579-581.

Lovell, J. E., and R. Getty. 1968. Fate of semen in the uterus of the sow: Histologic study of endometrium during the 27 hours after natural service. Am. J. Vet. Res. 29:610-625.

Macpherson, J. V. 1970. A system for artificial insemination of swine. Pages 75-78 in Proc. 3rd Tech. Conf. Artif. Insem. Reprod. NAAB, Chicago, IL.

Madden, D. H. L. 1961. A method of obtaining a high fertility level with the artificial insemination of farm pigs. Proc. 4th Int. Congr. Anim. Reprod. Artif. Insem. (The Hague) 4:851-859.

Makepeace, A. W., G. L. Weinstein, and M. H. Friedman. 1937. Effect of progestin and progesterone on ovulation in the rabbit. Am. J. Physiol. 119:512-519.

Mann, T., C. Polge, and L. E. A. Rowson. 1956. Participation of seminal plasma during the passage of spermatozoa in the female reproductive tract of the pig and horse. J. Endocrinol. 13:133-140.

Mastroianni, L. Jr., F. Beer, V. Shah, and T. H. Clewe. 1961. Endocrine regulation of oviduct secretion in the rabbit. Endocrinology 68:92-100.

Mattner, P. E. 1969. Phagocytosis of spermatozoa by leucocytes in bovine cervical mucus in vitro. J. Reprod. Fertil. 20:133-134.

Mattner, P. E. and A. W. H. Braden. 1969. Effect of time of insemination on the distribution of spermatozoa in the genital tract in ewes. Austr. J. Biol. Sci. 22:1283-1286.

Mattner, P. E., K. W. Entwistle, and I. C. A. Martin. 1969. Passage, survival, and fertility of deep-frozen ram semen in the genital tract of the ewe. Austr. J. Biol. Sci. 22:181-187.

Mayer, P., and E. Schutze. 1977a. A new progestin (Sa 45.249) for cycle control in pigs. Communication I: Hormonal activities and dosage. Theriogenology 8:357-366.

Mayer, P., and E. Schutze. 1977b. A new progestin (SA 45.249) for cycle control in pigs. Communication IV: Analysis of hormonal blood levels. Theriogenology 8:389-402.

Mellish, K. S., and R. D. Baker. 1970. Marking boar spermatozoa with fluorochromes for evaluating spermatozoa transport within gilts. J. Anim. Sci. 31:917-922.

Melrose, D. R. 1966. A review of progress and of possible developments in artificial insemination of pigs. Vet. Rec. 78:159-168.

Melrose, D. R., and C. O'Hagan. 1959. Some observations on the collection of boar semen and its use for artificial insemination. Ann. Zootech. 8 (Suppl.):69-79.

Melrose, D. R., and C. O'Hagan. 1961. Investigations into the techniques of insemination in the pig. Proc. 4th Int. Congr. Anim. Reprod. Artif. Insem. (The Hague) 4:855-859.

Morton, D. B., and T. D. Glover. 1974a. Sperm transport in the female rabbit: The role of the cervix. J. Reprod. Fertil. 38:131-138.

Morton, D. B., and T. D. Glover. 1974b. Sperm transport in the female rabbit: The effect of inseminate volume and sperm density. J. Reprod. Fertil. 38:139-146.

Neill, J. E., and B. N. Day. 1964. Relationship of development stage to regression of the corpus luteum in swine. Endocrinology 74:355-360.

Nellor, J. E., J. E. Ahrenhold, N. L. First, and J. A. Hoefer. 1961. Estrus, ovulation and fertility in gilts subsequent to the oral administration of 6-methyl-17-acetoxyprogesterone. J. Anim. Sci. 20:22-30.

Nelson, L. 1978. Chemistry and neurochemistry of sperm motility control. Fed. Proc. 37:2543-2547.

Nishikawa, Y. 1964. History and development of artificial insemination in the world. Proc. 5th Int. Congr. Anim. Reprod. Artif. Insem. (Trento) 6:162-259.

Niwa, T. 1961. Researches and practices in the artificial insemination of pigs. Proc. 4th Int. Congr. Anim. Reprod. Artif. Insem. (The Hague) 1:83-102.

Niwa, T., and T. Hashizume. 1978. Studies on the concentrations of prostaglandin $F_2\alpha$ in seminal plasma and on the effect of prostaglandin $F_2\alpha$ upon semen properties. Proc. Int. Pig Vet. Soc. Congr. (Zagreb) 5:KB-5.

O'Malley, B. W., and C. A. Strott. 1973. The mechanism of action of progesterone. In R. O. Greep and E. W. Astwood eds. Handbook of Physiology. Section 7. Endocrinology. American Physiological Society, Washington, D.C.

O'Reilly, P. J., C. N. Graves, and P. J. Dziuk. 1972. Heterospermic insemination of rabbit semen as a means of evaluating techniques of semen handling. J. Reprod. Fertil. 29:49-56.

O'Reilly, P. J., R. McCormack, K. O'Mahony, and C. Murphy. 1979. Estrus synchronization and fertility in gilts using a synthetic progestagen (allyl trenbolone) and inseminated with fresh stored or frozen semen. Theriogenology 12:131-137.

Overstreet, J. W. 1970. Sperm numbers and fertilization in the rabbit. J. Reprod. Fertil. 21:279-288.

Overstreet, J. W., and G. W. Cooper. 1978a. Sperm transport in the reproductive tract of the female rabbit: I. The rapid transit phase of transport. Biol. Reprod. 19:101-114.

Overstreet, J. W., and G. W. Cooper. 1978b. Sperm transport in the reproductive tract of the female rabbit: II. The sustained phase of transport. Biol. Reprod. 19:115-132.

Paredis, F., and M. Vandeplassche. 1961. Effect of initial motility, number and age of spermatozoa on farrowing rate and litter size. Proc. 4th Int. Congr. Anim. Reprod. Artif. Insem. (The Hague) 4:828-831.

Perez, L., K. Rajkumar, and C. A. Eddy. 1981. Fertility and ovum transport after microsurgical removal of the uterotubal junction in rabbits. Fertil. Steril. 36:803-813.

Polge, C. 1956. Artificial insemination in pigs. Vet. Rec. 68:62-76.

Polge, C. 1959. Some experiments on the preservation of boar semen. Ann. Zootech. 8 (Suppl.):113-120.

Polge, C. 1978. Fertilization in the pig and horse. J. Reprod. Fertil. 54:461-470.

Polge, C., and P. Dziuk. 1965. Recovery of immature eggs penetrated by spermatozoa following induced ovulation in the pig. J. Reprod. Fertil. 9:357-358.

Polge, C., B. N. Day, and T. W. Groves. 1968. Synchronization of ovulation and artificial insemination in pigs. Vet. Rec. 83:136-142.

Polge, C., S. Salamon and I. Wilmut. 1970. Fertilizing capacity of frozen boar semen following surgical insemination. Vet. Rec. 87:424-428.

Pond, W. G., W. Hansel, J. A. Dunn, R. W. Bratton, and R. H. Foote. 1965. Estrous cycle synchronization and fertility of gilts fed progestational and estrogenic compounds. J. Anim. Sci. 24:536-540.

Poyser, N. L. 1974. Some aspects of prostaglandins in reproduction. Biochem. Soc. Trans. 2:1196-1200.

Pursel, V. G. 1979a. Advances in preservation of swine spermatozoa. Pages 145-158 in Beltsville symposia in agricultural research. Animal reproduction. Allenheld, Osmun and Co., Montclair, New Jersey.

Pursel, V. G. 1979b. Effect of thawing solution on fertilizing capacity of frozen boar semen. J. Anim. Sci. 49:129. (Abstr.)

Pursel, V. G. 1980a. Capacitation time of fresh and frozen boar spermatozoa. Proc. 9th Int. Congr. Anim. Reprod. Artif. Insem. (Madrid) 8:32.

Pursel, V. G. 1980b. Fertilizing capacity of frozen boar semen: Time of insemination relative to ovulation. J. Anim. Sci. 51:316-317 (Abstr.)

Pursel, V. G., and L. A. Johnson. 1971a. Fertility with frozen boar spermatozoa. J. Anim. Sci. 33:265. (Abstr.)

Pursel, V. G., and L. A. Johnson. 1971b. Fertilizing capacity of frozen boar spermatozoa. J. Anim. Sci. 33:1162. (Abstr.)

Pursel, V. G., and L. A. Johnson. 1972. Fertility comparison of boar semen frozen in two extenders. J. Anim. Sci. 35:1123. (Abstr.)

Pursel, V. G., and L. A. Johnson. 1974. Glutaraldehyde fixation of boar spermatozoa for acrosome evaluation. Theriogenology 1:63-68.

Pursel, V. G., and L. A. Johnson. 1975a. Freezing of boar spermatozoa: Fertilizing capacity with concentrated semen and a new thawing procedure. J. Anim. Sci. 40:99-102.

Pursel, V. G., and L. A. Johnson. 1975b. Effect of extender components on fertilizing capacity of frozen boar spermatozoa. J. Anim. Sci. 41:374. (Abstr.)

Pursel, V. G., and L. A. Johnson. 1975c. Effect of time of insemination on fertilizing capacity of frozen boar semen. J. Anim. Sci. 41: 375. (Abstr.)

Pursel, V. G., L. A. Johnson, and G. B. Rampacek. 1972. Acrosome morphology of boar spermatozoa incubated before cold shock. J. Anim. Sci. 34:278-283.

Pursel, V. G., L. A. Johnson, and L. L. Schulman. 1973a. Effect of dilution, seminal plasma and incubation period on cold shock susceptibility of boar spermatozoa. J. Anim. Sci. 37:528-531.

Pursel, V. G., L. A. Johnson, and L. L. Schulman. 1973b. Fertilizing capacity of boar semen stored at 15°C. J. Anim. Sci. 37:532-535.

Pursel, V. G., L. A. Johnson, and L. L. Schulman. 1974. Acrosome morphology of boar spermatozoa during in vitro aging. J. Anim. Sci. 38:113-116.

Pursel, V. G., L. L. Schulman, and L. A. Johnson. 1978a. Distribution and morphology of fresh and frozen-thawed sperm in the reproductive tract of gilts after artificial insemination. Biol. Reprod. 19: 69-76.

Pursel, V. G., L. L. Schulman, and L. A. Johnson. 1978b. Effect of Orvus paste on acrosome morphology, motility and fertilizing capacity of frozen-thawed boar sperm. J. Anim. Sci. 47:198.

Pursel, V. G., D. O. Elliott, C. W. Newman, and R. B. Staigmiller. 1981. Synchronization of estrus in gilts with allyl trenbolone: Fecundity after natural service and insemination with frozen semen. J. Anim. Sci. 52:130-133.

Quinlivan, T. D., and T. J. Robinson. 1969. Numbers of spermatozoa in the genital tract after artificial insemination of progestagen-treated ewes. J. Reprod. Fertil. 19:73-86.

Rampacek, G. B., F. L. Schwartz, R. E. Fellows, O. W. Robison, and L. C. Ulberg. 1976. Initiation of reproductive function and subsequent activity of the corpora lutea in prepuberal gilts. J. Anim. Sci. 42:881-887.

Ray, D. E., and R. W. Seerley. 1966. Oestrus and ovarian morphology in gilts following treatment with orally effective steroids. Nature (London) 211:1102-1103.

Rigby, J. P. 1964. The fate of spermatozoa in the genital tract of the sow following artificial insemination. Proc. 5th Int. Congr. Anim. Reprod. Artif. Insem. (Trento) 4:421-425.

Rigby, J. P. 1966. The persistence of spermatozoa at the uterotubal junction of the sow. J. Reprod. Fertil. 11:153-155.

Robinson, T. J. 1973. Factors involved in the failure of sperm transport and survival in the female reproductive tract. J. Reprod. Fertil. Suppl. 18:103-109.

Rodolfo, A. 1934a. The physiology of reproduction in swine. I. The semen of boars under different intensivenesses of mating. Phillip. J. Sci. 53:183-203.

Rodolfo, A. 1934b. The physiology of reproduction in swine. II. Some observations on mating. Phillip. J. Sci. 55:13-18.

Rowson, L. E. A. 1955. The movement of radio opaque material in the bovine uterine tract. Br. Vet. J. 111:334-342.

Saacke, R. G., W. E. Vinson, M. L. O'Conner, J. E. Chandler, J. Mullins, and R. P. Amann, C. E. Marshall, R. A. Wallace, W. N. Vincel, and H. C. Kellgren. 1980. The relationship of semen quality and fertility: A heterospermic study. Pages 77-78 in Proc. 8th Tech. Conf. on Artif. Insem. and Reprod. NAAB, Milwaukee, WI.

Schilling, E., and F. Cerne. 1972. Induction and synchronization of oestrus in prepuberal gilts and anoestrus sows by a PMS-HCG compound. Vet. Rec. 91:471-474.

Schutze, E., and P. Mayer. 1977. A new progestin (Sa 45.249) for cycle control in pigs. Communication II: Duration of treatment and effectiveness as an estrus cycle synchronizer. Theriogenology 8:367-377.

Self, H. L. 1961. Factors affecting results in swine artificial insemination research and field tests. Proc. 4th Int. Congr. Anim. Reprod. Artif. Insem. (The Hague) 4:860-863.

Serdiuk, S. I. 1968. Boar sperm storage: medium and method. Proc. 6th Int. Congr. Anim. Reprod. Artif. Insem. (Paris) 2:1155-1157.

Sergeev, N. I. 1964. Neuro-humoral factors in artificial insemination of swine. Proc. 5th Int. Congr. Anim. Reprod. Artif. Insem. (Trento) 6:181.

Short, R. V. 1962. Steroids in the follicular fluid and the corpus luteum of the mare. A 'two-cell type' theory of ovarian steroid synthesis. J. Endocrinol. 24:59-63.

Short, R. V. 1964. Ovarian steroid synthesis and secretion in vivo. Recent Prog. Horm. Res. 20:303-333.

Signoret, J. P. 1972. The mating behavior of the sow. Pages 295-314 in E. D. J. Cole, eds. Pig production. Butterworths, London.

Signoret, J. P., and F. DuMesnil du Buisson. 1961. Studies on the behavior of the sow in estrus (in French). Proc. 4th Int. Congr. Anim. Reprod. Artif. Insem. (The Hague) 2:171-175.

Singleton, W. L., and D. R. Shelby. 1974. Variation among boars in semen characteristics and fertility. J. Anim. Sci. 34:762-766.

Smidt, W. J. 1961. The practical application of artificial insemination in pigs. Proc. 4th Int. Congr. Anim. Reprod. Artif. Insem. (The Hague) 4:864-867.

Snedecor, G. W., and W. G. Cochran. 1967. Statistical methods. 6th ed. The Iowa State University Press, Ames, Iowa. 593 pp.

Steel, R. G. D., and J. H. Torrie. 1960. Principles and procedures of statistics with special references to the biological science. McGraw-Hill Book Company, New York. 481 pp.

Stratman, F. W., and H. L. Self. 1960. Effect of semen volume and number of sperm on fertility and enbryo survival in artificially inseminated gilts. J. Anim. Sci. 19:1081-1088.

Stratman, F. W. and H. L. Self. 1962. Fertility studies with naturally mated or artificially inseminated sows. J. Anim. Sci. 21:647-650.

Stratman, F. W., H. L. Self, and V. R. Smith. 1958. Fertilization rates obtained with boar semen stored for zero and twelve hours. J. Anim. Sci. 17:598-603.

Stratman, F. W., H. L. Self, and V. R. Smith. 1959. The effect of oxytocin on fertility in gilts artificially inseminated with a low sperm concentration and semen volume. J. Anim. Sci. 18:634-640.

Sumption, L. V. 1961. Multiple sire mating in swine; evidence of natural selection for mating efficiency. J. Agric. Sci. 56:31-37.

Sweat, M. L., D. L. Berliner, M. J. Bryson, C. Nabors, Jr., J. Haskell, and E. G. Holmstrom. 1960. Synthesis and metabolism of progesterone in the human and bovine ovary. Biochim. Biophys. Acta 40:289-296.

Swierstra, E. E. 1970. The effect of low ambient temperatures on sperm production, epididymal sperm reserves, and semen characteristics of boars. Biol. Reprod. 2:23-28.

Szollosi, D., and R. H. F. Hunter. 1973. Ultrastructural aspects of fertilization in the domestic pig: sperm penetration and pronucleus formation. J. Anat. 116:181-206.

Szollosi, D., and R. H. F. Hunter. 1978. The nature and occurrence of the acrosome reaction in spermatozoa of the domestic pig, Sus scrofa. J. Anat. 127:33-41.

Tanabe, T. Y., A. C. Warnick, L. E. Casida, and R. H. Grummer. 1949. The effects of gonadotrophins administered to sows and gilts during different stages of the estrual cycle. J. Anim. Sci. 8:550-557.

Tausk, M. 1971. Historical introduction. Pages 1-13 in G. Peters, ed. Pharmacology of the endocrine system and related drugs: progesterone, progestational drugs and antifertility agents. International Encyclopedia of pharmacology and therapeutics. Vol. 1. Pergamon Press, Oxford.

Thibault, C. 1973. Sperm transport and storage in vertebrates. J. Reprod. Fertil., Suppl. 18:39-53.

Thibault, C., and S. Winterberger-Torres. 1967. Oxytocin and sperm transport in the ewe. Int. J. Fertil. 12:410-415.

Ulberg, L. C., R. H. Grummer, and L. E. Casida. 1951. The effects of progesterone upon ovarian function in gilts. J. Anim. Sci. 10: 665-671.

Van Demark, N. L., and R. L. Hays, 1951a. The effect of oxytocin, adrenalin, breeding techniques and milking on uterine motility in the cow. J. Anim. Sci. 10:1083. (Abstr.)

Van Demark, N. L., and R. L. Hays. 1951b. Uterine motility responses to motility. Am. J. Physiol. 170:518-521.

Veenhuizen, E. L., J. F. Wagner, W. P. Waite, and L. Tonkinson. 1965. Estrus control in gilts treated sequentially with DES and CAP. J. Anim. Sci. 24:931. (Abstr.)

Viring, S. 1980. Distribution of live and dead spermatozoa in the genital tract of gilts at different times after insemination. Acta Vet. Scand. 21:587-597.

Viring, S., and S. Einarsson. 1980a. Influence of boar seminal plasma on the distribution of spermatozoa in the genital tract of gilts. Acta Vet. Scand. 21:598-606.

Viring, S., and S. Einarsson. 1980b. Effect of boar seminal plasma on uterine and oviductal motility in oestrus gilts. Acta Vet. Scand. 21:607-616.

Viring, S., S. Einarsson, B. Jones, and K. Larsson. 1980a. Transuterine transport of small- and medium-sized molecules deposited in the uterus in gilts. J. Reprod. Fertil. 59:459-462.

Viring, S., S. Einarsson, L. Nicander and K. Larsson. 1980b. Localization of the sperm "reservoir" at the uterotubal junction of the pig. Proc. 9th Int. Congr. Anim. Reprod. Artif. Insem. (Madrid) 2:331. (Abstr.)

Wagner, J. F., and R. W. Seerley. 1961. Synchronization of estrus in gilts with an orally active progestin. J. Anim. Sci. 20:980-981. (Abstr.)

Warnick, A. C., R. H. Grummer, and L. E. Casida. 1949. The nature of reproductive failures in repeat breeder sows. J. Anim. Sci. 8:569-577.

Webel, S. K. 1977. Ovulation control in the pig. Pages 421-434 in D. B. Crighton, N. B. Haynes, G. R. Foxcroft, and G. E. Lamming, eds. Control of ovulation. Butterworths, London.

Webel, S. K., and R. H. Rippel. 1975. Ovulation in the pig with releasing hormones. J. Anim. Sci. 41:385. (Abstr.)

Wiggins, E. L., R. H. Grummer, L. E. Casida. 1951. Minimal volume of semen and number of sperm for fertility in artificial insemination of swine. J. Anim. Sci. 10:138-143.

Wilmut, I., and C. Polge. 1971. Recent progress in the deep-freeze preservation of boar semen. J. Reprod. Fertil. 25:301-311.

Winer, B. J. 1971. Statistical principles in experimental design. 2nd ed. McGraw-Hill, New York. 907 pp.

Zander, J. 1958. Steroids in the human ovary. J. Biol. Chem. 232:117-122.

Zerobin, K. 1977. A new progestin (Sa 45.249) for cycle control in pigs. Communication III: Confirmation of effectiveness in field trials. Theriogenology 8:379-388.

Zimmerman, D. R., I. Kabashima, and B. Lantz. 1974. Influence of short-term liquid storage of boar semen on fertility. J. Anim. Sci. 39:996. (Abstr.)

VIII. ACKNOWLEDGEMENTS

I wish to thank Dr. L. E. Evans for serving as my major professor and for his continuous support, assistance and guidance throughout the course of this thesis and graduate program.

I would like to express my gratitude to Drs. T. L. Clark, W. M. Wass, P. A. Martin, and N. G. Ghoshal, for their contributions, encouragement and patience during the graduate studies and for serving on my committee.

I would also like to acknowledge to all the Faculty and Staff of the Department of Veterinary Clinical Sciences, and the section of Theriogenology for their counsel and assistance.

I appreciate the statistical assistance of P. McGovern, J. De Dios Garza, and J. Sahagun.

The excellence of Mrs. Linda Genetzky in typing this manuscript is very much appreciated.

This thesis is dedicated to my parents, brother, and sister.

Finally, I would like to acknowledge the support provided by the National University of Mexico and the Faculty of Veterinary Medicine.

To the memory of Yolanda.

IX. APPENDIX

Table A1. Random breeding schedule of animals inseminated with 2 X 10^8 sperm cells diluted in a liquid semen extender

	Gilts			
	Single Service		Double Service	
	Gilt number	Date of breeding	Gilt number	Date of breeding
Liquid semen extender with 5 mg of progesterone	111(182)	7/21/80	121(187)	9/11,12/80
	112(185)	11/11/80	122(407)	11/11,12/80
	113(188)	12/9/80	123(99)	12/9,10/80
	114(184)	4/9/81	124(97)	4/16,17/81
	115(98)	6/1/81	125(89)	6/1,2/81
Liquid semen extender without 5 mg of progesterone	211(21-1)	10/22/80	221(186)	10/23,24/80
	212(96)	11/17/80	222(222)	11/19,20/80
	213(21-3)	1/28/81	223(183)	1/28,29/81
	214(20-8)	4/16/81	224(181)	4/26,27/81
	215(21-2)	6/22/81	225(189)	6/22,23/81

Sows			
Single Service		Double Service	
Sow number	Date of breeding	Sow number	Date of breeding
131(100)	10/21/80	141(188g)	11/4,5/80
132(170)	11/21/80	142(169)	11/21,22/80
133(167)	1/31/81	143(166)	2/9,10/81
134(184g)	4/29/81	144(179)	4/29,30/81
135(194)	6/25/81	145(180)	6/29,30/81
231(171)	11/11/80	241(197)	11/11,12/80
232(198)	11/28/80	242(89)	11/29,30/80
233(173)	3/27/81	243(165)	4/9,10/81
234(168g)	5/26/81	244(182g)	5/29,30/81
235(185g)	6/29/81	245(187g)	7/1,2/81