INTERACTION OF NUMBER OF BOAR SPERM AND INSEMINATION TIMING ON FERTILITY FOLLOWING INDUCED OVULATION

BY

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THESIS

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ABSTRACT

Variability in estrus and ovulation requires multiple inseminations during estrus to ensure one AI occurs close to ovulation. Induction of ovulation with a GnRH agonist after weaning improves synchrony of ovulation and allows for fixed time AI. However, the interaction between number of sperm in the AI dose and the timing of insemination has not been extensively evaluated. The objective of this study was to determine the effects of sperm numbers used in a single post cervical artificial insemination (PCAI) and the timing of insemination following induced ovulation in weaned sows. The experiment was performed in replicates at a 1000 sow, commercial research farm during summer and fall of 2014. Multiparous sows (n = 641) were allotted by parity (average = 2.8) and lactation length (average = 19.5 d) to receive a single PCAI using 1.5 or 2.5 billion viable sperm at either 22, 26, or 30 h following OvuGel® administration at 96 h post-weaning. Sows received fence-line boar contact once daily 3 to 6 d following weaning. Sub-populations of sows (n = 499) were assessed for follicle size and ovulation utilizing ultrasound at 8 h intervals. Of all sows, 88% expressed estrus within 6 d of weaning. At time of OvuGel® administration, 90% of sows had large (≥ 6.5 mm) follicles; with 89% of those ovulating by 48 h and 92% ovulating by 56 h following OvuGel®. There was no interaction ($P > 0.10$) between number of sperm and timing of insemination for fertility responses. There was a tendency for number of sperm ($P = 0.06$) to affect pregnancy rate with 2.5 billion (87%) inducing a greater pregnancy rate than 1.5 billion sperm (80%). Pregnancy was affected by AI timing ($P < 0.05$) and was greater ($P = 0.002$) following insemination at 22 h (85.1%) than 30 h (75%) whereas AI at 26 h (86%) did not differ. Farrowing rate was affected by the number of sperm with 2.5 billion (85%) increasing farrowing ($P < 0.05$) compared to use of 1.5 billion (75%) and tended ($P = 0.10$) to be affected by AI timing. Pregnancy rate and farrowing rate were not

affected by replicate, lactation length, follicle size, or ovarian cysts, but were affected by parity, estrus expression and ovulation ($P < 0.05$). Total born was affected by the number of sperm ($P < 0.05$) with 2.5 billion ($P = 0.03$) increasing litter size compared to 1.5 billion, and was also influenced by whether ovulation had occurred by 56 h after OvuGel®, but was not affected by AI timing. The results of this study indicate that induction of ovulation in weaned sows resulted in 87% of sows ovulating within a 24 h period and that fertility with a single fixed time AI was improved using 2.5 compared to 1.5 billion sperm and insemination at 22 h to 26 h after OvuGel® compared to 30 h.

"Our deepest fear is not that we are inadequate. Our deepest fear is that we are powerful beyond measure" Marianne Williamson.

This work is dedicated to the memory of William Huskerson "Papa" (1939-2009). Thank you for believing in me and the interval love and memories that we shared.

See you later alligator, after while crocodile!

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AI: Artificial Insemination

AMP: Adenosine monophosphate

ART: Assisted Reproductive Technology

BPT: Back Pressure Test

CL: Corpus luteum

DUI: Deep uterine insemination

(E_2): 17ß-estradiol

eCG/PMSG: Equine chorionic gonadotropin/Pregnant mare's serum gonadotropin

Estrus: Heat

F-T: Frozen- thawed semen

FSH: follicle stimulating hormone

GnRH: Gonadotrophin Releasing Hormone

hCG: Human chorionic gonadotropin

Intra-CAI: Intra-cervical artificial insemination

IUI: Intra-uterine insemination

LH: luteinizing hormone

LHRH: luteinizing hormone releasing hormone

LS: Litter size

LSY: Litter/sow/year

NBA: Number of piglets born alive

PCAI: Post cervical artificial insemination

$PGF_{2\alpha}$: Prostaglandin $F_{2\alpha}$

pLH: Porcine luteinizing hormone

PMN: Polymorph-nuclear neutrophil phagocytosis

PSY: Pigs/sow/year

TB: Total piglets born

UTJ: Uterine Tubal Junction

WEI: Wean-to-estrus interval

CHAPTER 1: LITERATURE REVIEW

INTRODUCTION

Global importance of pork production

The world's population has steadily increased in the last two decades, requiring significant growth in global animal production to meet consumer demand for food (Lamberson and Safranski, 2000). Within food producing animals, the pig's efficiency in feed conversion, carcass yield, and prolificacy qualifies it as a logical choice for production of pork to contribute towards meeting the global need for protein (Gerrits et al., 2005; Knox, 2014; FAO, 2015). Due to the pig's efficiency, pork ranks second in meat produced but first in type of meat consumed according to the Foreign Agricultural Service, (FAS, 2003; Gerrits et al., 2005; FAS, 2015; USDA, 2015). From 2007 to 2015, the global production of pork increased based on total carcass weight in China, the European Union, and the United States (USDA, 2015). Despite the increases, projected demand for pork suggests a need for further increases in production.

Measures of reproductive efficiency

Future growth in the production of pork depends on reproductive efficiency to allow for consistent flow of pigs to market. The total pounds of pork produced annually is dependent on a key indicator of breeding herd performance: the number of piglets produced per sow per year (pigs/sow/year (PSY)). This measure is calculated from the number of piglets weaned per litter multiplied by the number of litters produced per sow per year which depend upon pregnancy rate, litter size, and litters/sow/year (LSY, Figure 1.1).

Figure 1.1: Litters/sow/year (LSY) is calculated as indicated (Stalder, 2002; Abell, 2011).

$$\frac{\#\ of\ litters\ farrowed}{\left(\frac{total\ number\ days\ in\ the\ breeeding\ herd}{gestation\ length + lactation\ length + wean\ to\ estrus\ interval}\right)}$$

In the USA, herd LSY averages 2.3, and is determined using the number of litters farrowed divided by the number of years the sow is in the herd. For calculating the number of years in the herd, the annual number of days in the breeding herd year (365 d) is divided by the sum of the gestation length for the pig (114 d), an average 21 d lactation length, and a typical 5 d wean-to-service interval.

In the female pig, fertility depends upon the expression of estrus, farrowing rate and litter size. The expression of estrus is a state of sexual receptivity linked to timing of ovulation for fertilization and establishment of pregnancy (Nissen et al., 1997) . Fertilization in the reproductive tract requires that the sperm and egg interact in the oviduct within a defined period of time (Hunter, 1981; Waberski et al., 1994) . From a practical breeding perspective, onset of estrus is used to time inseminations. The timing of insemination is critical due to a limited fertile lifespan of the egg following ovulation (Soede and Kemp, 1995). In addition, adequate numbers of capacitated sperm present in the oviduct ahead of ovulation ensures fertilization can occur. However, a major limitation to fertilization is variability in the interval from the onset of estrus to the time of ovulation which is a leading factor resulting in aged gametes which can reduce fertilization, embryo survival, establishment of pregnancy, and litter size (Soede et al., 1995a; Bortolozzo et al., 2005). Methods that can reduce variation in the insemination to ovulation interval will help improve reproductive efficiency in the pig.

Reproductive efficiency for improving pork production

The use of Assisted Reproductive Technologies (ART) that include artificial insemination (AI), and control of reproductive cycles have enabled an increase in pig fertility, reproductive efficiency, and production of pork. These techniques have also had profound effects on the structure of the industry, the size of farms, and the efficiency and profitability of production operations (Knox and Wilson, 2007). Artificial insemination is used in most commercial swine breeding protocols worldwide and serves as the primary tool for genetic improvement, labor efficiency and production consistency (Weitze, 2000; Knox, 2016). However, its impact, although substantial, has been limited by an inability to predict time of ovulation, resulting in an inefficient use of sperm from valuable sires and reduced fertility from less than optimal AI timing.

The problem of variation in ovulation has been recognized for many years, and methods to control its impact have included the use of reproductive hormones to synchronize estrus and control time of ovulation. However, to date, these approaches while successful to some extent, have not optimized efficient use of genetics for a single insemination or reduced numbers of sperm. The development of a practical method to reduce variation in ovulation would allow for more efficient use of semen from genetically superior boars, improve fertility, and facilitate the use of other important technologies such as sexed semen and cryopreserved boar sperm.

REPRODUCTIVE PHYSIOLOGY OF GILTS AND SOWS

The pre-pubertal gilt

The female reproductive system includes the hypothalamic-pituitary axis, and the reproductive tract which are regulated by stimulatory and inhibitory reproductive hormones (Bentley et al., 2010). As the pre-pubertal animal approaches puberty, luteinizing hormone (LH)

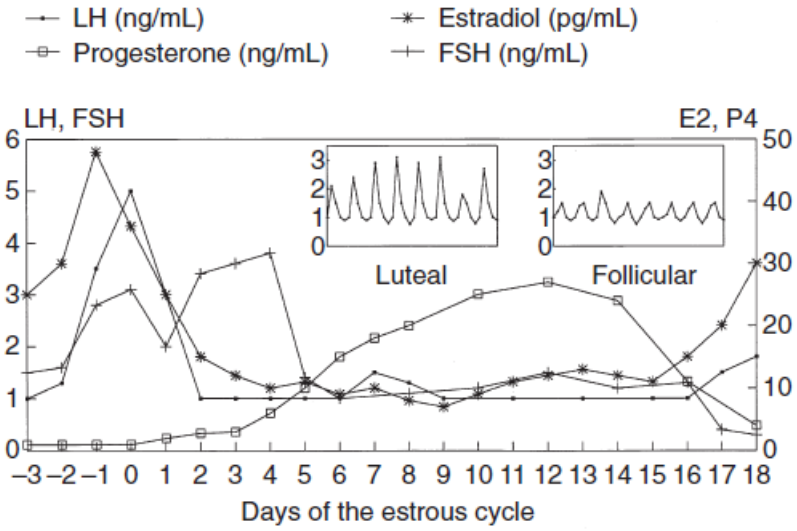
is released with increasing pulsatile intervals to induce follicle growth in the ovaries (Esbenshade et al., 1982; Diekman et al., 1983; Prunier et al., 1993; Foster et al., 2013). Fluctuations in LH concentration in the blood during the pre-pubertal period are also associated with further development of the thecal and granulosa cells. These cells are important for development of follicles (Beltranena et al., 1993; Evans and O'Doherty, 2001) and their growth is responsible for maturation of ova and production of reproductive hormones such as estradiol, progesterone, and inhibin.

Modern genotype gilts reach puberty between 150-220 days (5-7 months) of age and typically weigh at least 180 lbs. depending on genetic and environmental factors (Hughes, 1982; Evans and O'Doherty, 2001; Safranski and Cox, 2007). Studies have shown the age at which a gilt reaches puberty is regulated by the season of birth (Christenson and Ford, 1979), nutrition, boar contact (Brooks and Cole, 1970; Karlbom, 1982), and stress of social environment and translocation (Mavrogenis and Robison, 1976; Rampacek et al., 1981; Einarsson et al., 2008). A combination of these factors and final maturation of the ovarian follicles will eventually result in the occurrence of first estrus and onset of reproductive cycles (Camous et al., 1985; Steverink et al., 1999; Evans and O'Doherty, 2001; Magnabosco et al., 2014).

The mature cyclic gilt

The onset of puberty induces poly-estrus in the cyclic female, with estrus occurring at regular intervals throughout the year. The estrous cycle consists of a follicular phase (proestrus), ovulatory phase (estrus), and luteal phase (diestrus) with the entire cycle lasting approximately 21 days, with a range of 18-24 days (Van de Wiel et al., 1981) (Figure 2.1).

Figure 1.1: Review of average concentrations of reproductive hormones during the estrous cycle in swine (Safranski and Cox, 2007).



The follicular phase of the estrous cycle lasts 5-7 days including follicle recruitment and growth of follicles for ovulation (Soede et al., 2011). Selection of follicles for ovulation occurs during Days 14-16 of the cycle (Miller et al., 1998). Follicles are initially recruited from a pool of small (1-2 mm) and medium (3-5 mm) follicles (Foxcroft and Hunter, 1984). Selection of pre-ovulatory luteinizing hormone (LH) – dependent follicles will allow certain follicles to grow to dominance and progress towards ovulation (Hunter et al., 2004). The growth of ovulatory follicles selected for ovulation will cause rapid atresia of subordinate follicles and block replacement in the proliferating pool (Foxcroft and Hunter, 1984; Driancourt et al., 1986; Guthrie and Cooper, 1996; Miller et al., 1998; Soede et al., 2011).

Follicles develop from primordial to ovulatory size during the process of folliculogenesis which is stimulated by complex interactions of hormones and growth factors from thecal and

granulosa cells (Channing et al., 1979; Hillensjö et al., 1979). As the follicle grows to the medium class size, thecal and granulosa cells proliferate around the oocyte, and the thecal cells differentiate and express LH receptors (Eppig, 2001; Guthrie, 2005). As the thecal cells continue to mature, they change in steroidogenic capability with LH binding to its receptor (Figure 2.2). The binding of the gonadotropin causes a rapid stimulation of adenylate cyclase activity and an increase in cyclic adenosine monophosphate (cAMP) (Channing and Tsafriri, 1977). Interaction with the regulatory subunit of protein kinase results in stimulation of further enzymatic activity (Marsh, 1976; Channing and Tsafriri, 1977).

Induction of the LH receptor will allow granulosa cells to respond to LH, providing an increased ability of follicle stimulating hormone (FSH) to stimulate aromatization of androgen to estrogen for follicle development (Anderson et al., 1979; Tsang et al., 1979; Foxcroft and Hunter, 1984; Osteen et al., 1985). FSH becomes important in accelerating follicular growth and increasing the number of follicles that reach medium-large sizes. Smaller follicles that have insufficient LH-receptors are dependent on FSH and will undergo atresia as FSH decreases (Erickson and Danforth, 1995; Soede et al., 2011). FSH in the granulosa cells of ovarian follicles increase synthesis and secretion of follicular 17ß-estradiol (E_2) that contributes to estrous behavior (Safranski and Cox, 2007). The increase in 17ß-estradiol during the follicular phase will initiate the pre-ovulatory surge of LH and the onset of estrus allowing sexual receptivity displayed by females (Micevych and Kelly, 2012).

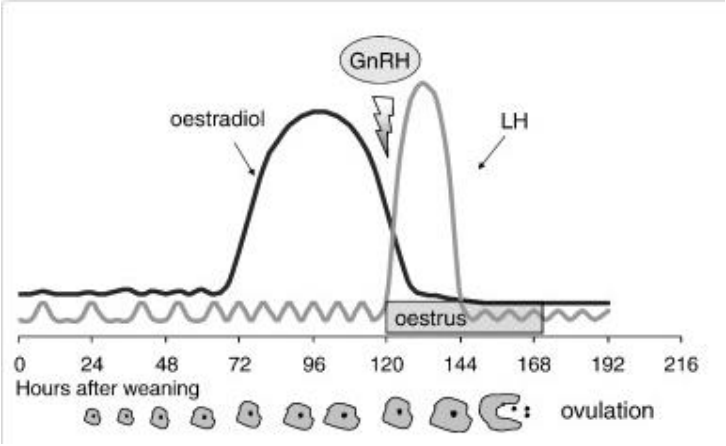
Hypothalamic control of ovulation

As estrogen levels increase, the hypothalamus, located at the base of the brain, releases GnRH (gonadotropin releasing hormone) (Harris and Naftolin, 1970; Van de Wiel et al., 1981). GnRH, a neuropeptide (pyroGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH2) hormone, is produced in the arcuate nuclei to regulate the release of gonadotropins into the blood. GnRH acts on the gonadotrophs of the anterior pituitary to stimulate release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) (Schally et al., 1978). Positive feedback of estrogen from the ovary at the hypothalamus and anterior pituitary allows for LH surge release into the blood to act on the follicles to induce ovulation.

Estradiol triggers the pre-ovulatory LH surge inducing Graafian follicles to shed their eggs and release mature fertilizable oocytes (ovum, Figure 2.3) 36 to 48 hours after the onset of estrus (Soede et al., 1994). After the follicle ovulates and the corpus luteum (CL) forms, negative feedback on LH results from progesterone. Progesterone following immediately after ovulation will stabilize and slightly decline secretion of FSH and promote growth of endometrium for implantation (Foxcroft and Van De Wiel, 1982; Soede et al., 2011). For full inhibition of FSH, inhibin from the porcine follicular fluid will act as a selective inhibitor of FSH synthesis and secretion (Channing et al., 1979; Gregory and Kaiser, 2004). Ovulation will last approximately 1-3 h in spontaneously ovulating sows (Soede et al., 1992; Soede et al., 1998) which may involve rupture of 15-30 follicles.

Figure 1.2: Schematic drawing of the timing of ovulation with the hormonal and behavioral events taking place during the days around estrus (Pedersen, 2007).

Fig. 1.2 (cont.)



Following ovulation, the pig enters the luteal phase of the cycle, which lasts 13-15 days. As ovulation occurs, inhibin and estrogen production decrease, and the negative feedback on FSH is removed (Soede et al., 2011). Concentrations of FSH after ovulation are greater to induce synchronized follicle development and increase in the number of small and medium sized follicles. The follicles will produce inhibin which in turn reduces peripheral concentration of FSH (Soede et al., 2011). The LH surge reduces estradiol production for follicular changes and shifts steroidogenesis to progesterone production for corpora lutea (CL) formation (Catt et al., 1979). The CL formation and function will be influenced by angiogenesis and angiogenic factors such as vascular endothelial growth factor (VEGF) (Neufeld et al., 1999; De Andrea Ribeiro, 2007; Kaczmarek et al., 2007). VEGF acts as a regulator of the CL, stimulating vasculogenesis and angiogenesis to restore oxygen supply in the blood for endothelial cell proliferation. Blood will rapidly fill the central cavity of the follicles and luteinization of follicular remnants will occur in the formation of corpora hemorrhagica (Safranski and Cox, 2007). By day 5-6, CL will have reached a mature diameter of 9-11 mm and the central cavities will be replaced by luteal tissue with identifiable cells of follicular granulosa (large thecal cells) and theca (small luteal

cells) origination (Corner, 1915; Niswender et al., 2000; Safranski and Cox, 2007). The CL produces the steroid hormone progesterone with increasing serum concentrations evident by 2-4 days after estrus. Progesterone levels of developing CL will reach peak concentrations 8-9 days after ovulation that will continue to suppress the secretion of LH. By day 10-12 after ovulation, maintenance of the CL will come from LH stimulating VEGF production. Degeneration of the CL, luteolysis, will begin around day 15 after ovulation with increased concentrations of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) secreted by the uterus if conception and continuance of pregnancy does not occur (Neill and Day, 1964). After luteal regression, functional degradation of the CL occurs, and the cycle enters into the follicular phase. This phase is noted by a decline in circulating progesterone concentration caused by uterine prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$).

In the event of conception, uterine $PGF_{2\alpha}$ secretion is prevented by production of estrogen to allow for maintenance of the corpus luteum (CL) (Ash and Heap, 1975). CL regression is inhibited and results in an establishment of pregnancy with elevated levels of progesterone to prevent uterine contraction throughout gestation. As the female approaches the end of gestation, adrenocorticotropic hormone (ACTH) is released and an elevation of adrenal cortisol causes the uterus to produce and release $PGF_{2\alpha}$. As a female reaches the time to farrow, prostaglandin $PGF_{2\alpha}$ stimulates the release of relaxin from CL to relax and expand the birth canal. Other hormones such as prolactin and oxytocin are secreted for milk production and uterine contractions until the end of farrowing.

The weaned sow

The weaned sow makes up more than 70% of the females in the breeding herd. Following farrowing, sows usually lactate 3-4 weeks prior to weaning for re-breeding. A key indicator used to assess fertility of the weaned sow is estrus. Daily detection for onset of estrus can be used for AI timing in breeding protocols. This approach can be labor intensive, sometimes subjective, and imprecise for predicting time of ovulation. Biological variability in measures of wean to estrus can include the interval to onset, duration, and symptoms. Variability in estrus to ovulation interval can be caused by differences in wean to estrus interval (Kemp and Soede, 1996), lactation length, parity, and season (Foxcroft and Van De Wiel, 1982; Weitze et al., 1994; Brüssow et al., 1996). The impact of these factors limits the overall efficiency of reproductive performance by affecting the time of insemination relative to ovulation (Weitze et al., 1994; Johnson et al., 2000), and increasing the number of inseminations required for fertility.

Wean-to-estrus interval

In the breeding herd, schedules are set by weaning groups. Typically pigs are weaned from a batch of sows on a single day to allow for all-in and all-out disease control technology, efficient use of labor, and uniform production of market pigs. In commercial breeding operations, production farms typically wean in the morning and on specified days of the week for practical aspects related to labor schedules and sow fertility. Wean to estrus interval (WEI) is defined as the number of days between when the piglets are weaned (Day 0) and the first day standing heat is observed. The WEI for most sows occurs on days 3-9 following weaning (Kemp and Soede, 1996), but the majority express estrus on days 4-6 (Kemp and Soede, 2012). The variability in the WEI is important because of its relationship to ovulation. The variability in WEI can be attributed to genetic and management factors such as lactation length (Te Brake, 1978; Xue et al., 1993), energy balance of sows at weaning, parity, number of piglets weaned,

and season (Britt et al., 1983; Clark et al., 1986; Vesseur et al., 1994; Van den Brand et al., 2000; Safranski and Cox, 2007).

Lactation length

The WEI interval may be determined by the physiological events occurring during the lactation period. After farrowing, lactating sows will experience anestrus during lactation and even for a short period after weaning (Kirkwood et al., 1987). The process of lactation or the nursing stimulus from piglets causes the release of endogenous opioid peptides in the brain that suppresses pulsatile luteinizing hormone (LH) secretion (Barb et al., 1986; Armstrong et al., 1988; De Rensis et al., 1999), follicle development, and estrus expression (Gerritsen et al., 2008a). In conventional weaning systems, cyclic activity is inhibited until the piglets are removed with the follicular phase initiated immediately following and estrus occurring 4-7 days after weaning (Clark et al., 1986; Gerritsen et al., 2008a). With extended lactation periods, or with a decrease in suckling frequency, high frequency/low amplitude LH-pulses and increased pituitary response to GnRH is re-established (De Rensis et al., 1993; Soede et al., 2011) and the process of follicle development can occur (King and Williams, 1984; Kemp and Soede, 2012). Fertility problems in sows can occur if there is a reduction in the suckling intensity before weaning which can cause sows to show estrus during lactation, very soon after, or not at all (Stevenson and Britt, 1981; Stevenson and Davis, 1984; Poleze et al., 2006). It is possible, that in these cases, a small proportion of sows may return to estrus during lactation and ovulate prematurely and these females will subsequently be classified as infertile, or with extended wean to estrus intervals.

The interval to return to estrus after weaning can also be affected by the intensity of the nursing stimuli arising from the number of pigs nursed. The number of piglets nursed equates to the size of the litter farrowed. Though litter sizes of 9-12 piglets are desired, small litters averaging or fewer than 7 piglets occur. Small litters can be highly influenced by duration of prior lactation and breeding of sows less than 21 days after farrowing. Sows nursing small litters are associated with WEI due to a reduction in the suckling induced inhibition. Reduction in suckling can reduce the length of lactation and influence sow's return to estrus in breeding herd that requires more time to cycle after weaning. In comparison, sows nursing larger litters place a greater metabolic demand on the sow, which can further compromise hormone release and follicle development and delay estrus, if excessive loss of body condition occurs (Kemp and Soede, 2012). Certain lactation strategies to minimize sow body condition loss can help improve the WEI, such as weaning the larger piglets in the litter several days before the smaller ones, or separation of all piglets in the litter from the sow for several hours each day during the last days of the suckling period (Clark et al., 1986).

Negative energy balance

During lactation, the sow is not able to consume enough nutrients to replace body reserves stored to support milk production. This is more apparent in primiparous sows that are unable to consume sufficient energy and protein to meet requirements and results in a severe negative energy and nitrogen balance (Van den Brand et al., 2000). The inability to eat enough feed to counteract mobilization of body reserves causes a reduction in body weight and back fat thickness that are associated with extended WEI (Mullan and Williams, 1990). Inadequate feed intake during lactation can result in a prolonged WEI (Aherne and Kirkwood, 1984), a decrease

in the duration of estrus, and an increase in the interval from the onset of estrus to ovulation; all associated with an insufficient process for follicle development (Kemp and Soede, 2012).

Parity of sow

First parity sows tend to show longer WEI than observed in multiparous sows (Britt et al., 1983) and may be due to an altered follicle development and LH-pulsatility post-weaning (Shaw and Foxcroft, 1985). Primiparous sows have smaller follicles at weaning and smaller follicles at ovulation (~7 mm) (Langendijk et al., 2000) than multiparous sows (~8 mm) and also have a longer WEI (Gerritsen et al., 2008b). To improve the fertility of young sows, one approach has been to postpone or skip breeding at the first estrus after weaning to limit the negative consequences of lactation on subsequent reproduction. This approach results in sows of parity 1 and 2 having significantly higher litter sizes, pregnancy rates, and improved reproductive performance with high embryo survival rates. The downside of the approach is that there is an increase in the number of non-productive days by 21, and a challenge to fit the sows into a new breeding group. Providing a shorter recovery period than a full cycle length, by using a progesterone analogue post-weaning has been proposed as a method to improve reproductive performance while limiting the effect of non-productive days and preventing the issue of poor detection of second estrus (Kemp and Soede, 2012).

Seasonal effects

Season also affects onset of estrus in the weaned sow (Love et al., 1992). The domestic pig is known to have retained some seasonality of the ancestral wild pig as a short day length

seasonal breeder (Claus and Weiler, 1984; Peltoniemi et al., 2000). This is observed as a reduction in fertility of the sow during the summer-autumn months (Love, 1981; Claus and Weiler, 1984). The impact of seasonality delays puberty in gilts (Ehnvall et al., 1980), reduces the number of weaned sows that are inseminated that farrow (Xue et al., 1994; Love et al., 1995), reduces the proportion of sows pregnant 20-25 days after breeding, increases returns to estrus (Love et al., 1992), and prolongs the weaning to estrus interval (Prunier et al., 1996). As the weaning to estrus interval facilitates the basis for timing of mating and conventional insemination, delays can result in reduced fertility.

ARTIFICIAL INSEMINATION OF SWINE

Since the late 1980s, insemination protocols were developed for use in commercial swine production systems that would enable use of AI to replace natural mating (Crabo, 1990; Johnson et al., 2000; Foote, 2002). In the United States, a substantial growth in pig AI occurred throughout the 1990's (Johnson et al., 2000; Weitze, 2000), and today, more than 90% of all inseminations are performed with liquid-extended semen with 100% AI use on large commercial production farms (Martinez et al., 2002; Knox, 2016). The development of AI has helped advance technologies associated with sire selection, efficient production of semen for AI doses and methods for semen distribution (Reed, 1985). Use of artificial insemination has helped the industry capture opportunities for increased genetic advancement, high fertility, reduced costs, labor efficiency, disease control and new ART technologies.

Breeding in Commercial Swine Production

In commercial swine production, intra-cervical or conventional artificial insemination (CAI), standardized in the 1970s, was the foundation for modern AI (Roca et al., 2006). Conventional insemination involves the deposition of ~ 3.0 billion sperm cells (Watson and Behan, 2002) in 80-100 mL of extender within the posterior portion of the cervical canal by means of a catheter that engages with the folds of the cervix (Hernández-Caravaca et al., 2012). This approach helps insemination of the female, and ensures a minimum number of sperm can establish a functional sperm reservoir.

Artificial insemination (AI) in conventional breeding is most often performed using liquid cooled semen preserved in an extender and stored at 15-18°C. Semen stored at higher temperatures are not inhibited from use of nutrients and energy and will lose viability sooner, while semen stored in colder temperatures, undergo cold shock that results in damaged membranes and acrosomes (Watson, 1995; Johnson et al., 2000; Paulenz et al., 2000). Storage of semen at an average 17°C and in an extender allows for preservation of spermatozoa by reducing metabolic activity to extend survival post collection (Gadea, 2003). Extenders developed to preserve and extend the fertile life of sperm for use in AI can be classified as short, mid, or long-term diluents (White, 1993; Johnson et al., 2000; Paulenz et al., 2000). The extenders are prepared as aqueous solutions used to increase the volume of the ejaculate into numerous insemination doses and functions in conserving characteristics of sperm cells (Harrison et al., 1978; Waberski et al., 1989; Gadea, 2003). In choosing sperm diluents, important factors such as nutrients, pH, ionic strength, and type of ions and osmotic pressure of the medium are accounted for to increase the survival of semen (Johnson et al., 2000). Spermatozoa in the boar are found in seminal plasma that has the capacity to supply nutrients necessary for the high metabolic demands of sperm transport (Gadea, 2003). In semen extenders, diluting the semen to a desired

volume can lower the concentration of nutrients and compounds in the seminal plasma needed for energy, altering the sperm viability (Harrison et al., 1978). To supplement a source of energy, glucose is the main ingredient used for metabolic maintenance of the sperm cell. The use of glucose also affects glycolytic metabolism which reduces intracellular pH and suppresses cell metabolism. At such a low pH, viability and motility can be reduced with loss in concentration of important seminal plasma components. The addition of albumin, specifically bovine serum albumin (BSA) and minimal additions of potassium have been shown to maintain the motility of spermatozoa (Harrison et al., 1978; Waberski et al., 1989). The pH of freshly ejaculated boar sperm tends to vary between 7.2 and 7.5; anything below causes the reduction of motility and further metabolism of the spermatozoa. The addition of ions such as sodium bicarbonate, sodium citrate, and potassium chloride act as buffering agents to maintain the Na^+-K^+ pump of the cells in order to prevent intracellular K^+ exhaustion and controlling pH of the medium (Alvarez and Storey, 1982). More complex buffers are currently used (TES, HEPES, MOPS, TRIS), based on zwitterionic organic buffers which are able to control the pH for a longer period of time (Waberski et al., 1989; Crabo, 1990; Weitze, 1990).

Osmotic pressure of the medium is important in establishing the proper composition of diluents for the storage of semen. Boar spermatozoa tolerate a range of osmolality between 240-380 mOsm. Media that is isotonic or slightly hypertonic have been shown to offer better preservation for fertilizing capacity compared to hypertonic diluents (Weitze, 1990; Johnson et al., 2000). Additives such as ethylene diamine-tetra-acetic acid (EDTA) and antibiotics were also added to extenders to reduce the bacterial growth of sperm cells during storage, prevent initiation of capacitation, and the acrosome reaction (Martin Rillo et al., 1998; Johnson et al., 2000). EDTA, serves as a chelating substance that captures divalent metal ions such as Ca^{2+} and limits

movement across the plasma membrane and prevents initiation of capacitation and the acrosome reaction (Weitze, 1990). For preventing the growth of bacteria in stored semen, certain diluents have been supplemented with gentamycin and neomycinsulfate.

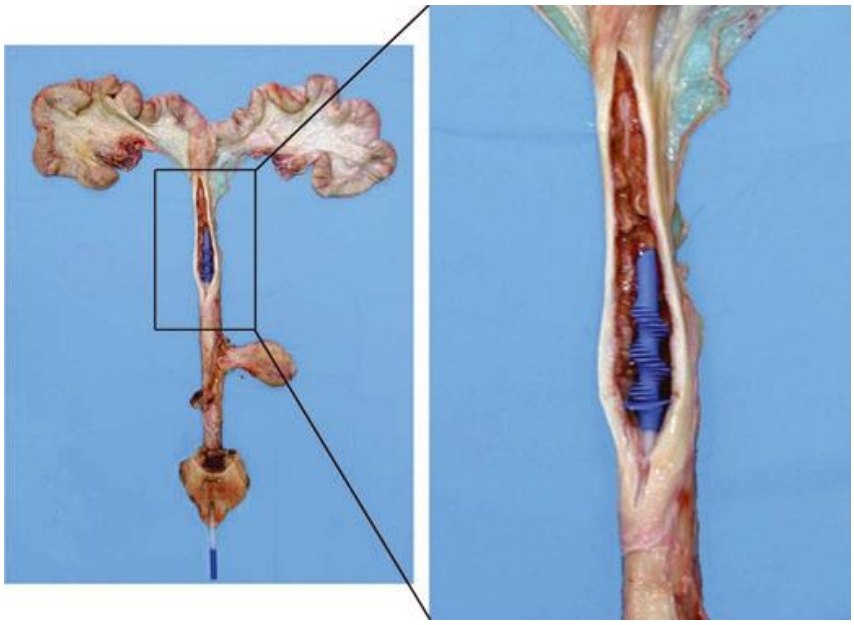
Over time research has focused on combining variations of these aspects into short-term and long-term extenders. Earlier diluents consisted of glucose solutions containing sodium or potassium tartrate, sodium sulfate or peptones to ensure electrolyte levels were low (Foote, 2002). The early glucose extender solutions also included egg yolk and were modified based on diluents used in cattle. These were modified by du Mesnil du Buisson and Dauzier, 1959 and used as a post-diluter in a two-step extension system with the need to be gassed with CO_2 to lower metabolic activity in the sample. Around the 1960s, the Kiev diluent included the addition of EDTA, followed by development of other extenders, such as the Beltsville-TS (BTS) developed by Pursel and Johnson (1975), Zorlesco (Gottardi et al., 1980), and Androhep (Weitze, 1990). Currently, BTS remains the most widely used extender in swine production, but Zorlesco and Androhep are noted for their long-term storage abilities and use with cryopreservation.

Conventional Artificial Insemination

Conventional AI (Figure 3.1) is one of the most widely used and successful reproductive technologies in use today. The technique is relatively simple to perform, inexpensive, and effective with many farms able to achieve high farrowing rates and large litter sizes. With conventional AI the industry utilizes a multiple insemination system on each day of standing heat to compensate for the variation in estrus to ovulation, but variation still reduces the likelihood

that a standard insemination approach will result in optimal fertilization. Standard practice in North America is to make sure the majority of sows in the breeding herd receive multiple inseminations with 2.5-3 billion motile spermatozoa per dose (Kemp and Soede, 1996; Rozeboom et al., 2004). The requirement for a multiple insemination system is most often associated with improved fertility, but can sometime result in postovulatory inseminations which have been shown to negatively affect farrowing rate and litter size (Rozeboom et al., 1997) and perhaps lead to uterine infections (Kaeoket et al., 2005). However, the technology has limitations, in that a large number of spermatozoa are required per female for reproductive success (Roca et al., 2006). This becomes more costly and slower for making genetic advancements in economically important traits, since the number of ejaculates and sperm used from superior sires cannot be distributed to a greater extent.

Figure 1.3: Reproductive tract of a sow showing the position of the tip of a conventional insemination catheter engaging with the posterior folds of the cervix (Roca et al., 2006).



One solution to this problem would be to decrease the number of sperm used in an insemination and extend the use of an ejaculate from a superior boar to serve an increased number of females (Rozeboom et al., 2004). Farm applicable methods such as inseminating the semen further in the reproductive tract allows for a reduction in the number of sperm in an AI dose and could aid in viable sperm selection, alternative methods for semen processing, and inclusion of additives that could improve fertility, transport, and survival of sperm in the female.

Interval from onset of estrus to ovulation

Optimal fertility and prolificacy in sows is dependent on insemination occurring close to the time of ovulation (Soede et al., 1994; Waberski et al., 1994; Soede and Kemp, 1995; Nissen et al., 1997; Steverink et al., 1997; Driancourt et al., 2013). Over the years, research has shown that the time of ovulation can be estimated based on an accurate assessment for the onset of estrus (Steverink et al., 1997). However, as previously described, variation in the relationship of the interval from estrus to ovulation is dependent upon the interval from weaning to estrus (Nissen et al., 1997; Knox et al., 2011). Overcoming this variation has been technically challenging, based on the subjective nature of estrus detection in swine, and the labor issues under intensive farm conditions.

Though limited in its ability to predict time of ovulation, estrus detection remains an important practice in commercial breeding protocols. In natural mating scenarios, a female pig in estrus will stand immobile (lordosis) and allow a boar to mate (Signoret, 1971) which serves as the basis for checking for the standing response in the presence of a boar without allowing boar mating, while a technician applies the back pressure test (BPT). The use of the boar to stimulate

females is important for inducing ovarian activity, advancing estrus, and stimulating estrus behavior (Langendijk et al., 2000; Kemp et al., 2005). Other symptoms associated with estrus may include decreased feed intake, increased vocalization, mucus production, and redness and swelling of the vulva. The boar's stimuli provides important sight, sound and smell while back pressure mimics the physical stimulation of the boar mounting. The intensity and duration of estrus behavior is highly variable and subject to female response to the boar and to human stimuli. For example, estrus duration can vary between 24-72 h in sows (Soede and Kemp, 1997), and gilts (Steverink et al., 1998; Almeida et al., 2000).

The duration of estrus is one of the most important measures for establishing timing and number of inseminations, is related to time of ovulation and can be used to help establish an optimal insemination strategy on farms. A technician's knowledge and experience for detection of estrus is also important for proper breeding (Flowers and Esbenshade, 1992). Based on an average duration of estrus, most gilts and sows are mated two times during estrus to ensure that at least one insemination occurs near the optimum time relative to ovulation. Gilts and sows are often inseminated at onset of estrus with a second insemination administered 18-24 h later if still standing the next day. Since the symptoms and duration of estrus vary considerably in pigs and can be affected by many factors such as intensity and frequency of boar contact (Soede et al., 2011), parity, and weaning-to-estrus interval, an insemination program independent of estrus could result in a more reliable method for AI timing. Previous reports have shown hormonal control of the estrous cycle and ovulation is possible for use with artificial insemination (Brüssow et al., 1996). This approach was adopted to minimize errors associated with detection of estrus, reduce variation in estrus to ovulation interval, and improve insemination timing with use of fixed-time AI in weaned sows. Control of estrus and ovulation could replace AI timing

based on the standing reflex with estrus detection and allow for a fixed time AI. A timed AI could increase genetic improvement by allowing for use of fewer inseminations and spermatozoa per conception and greater use of superior sires.

The use of hormones for synchronization of estrus and ovulation could remove uncertainty concerning time of ovulation and improve timing for successful AI (Brüssow et al., 1996; Stewart et al., 2010). Time AI relative to ovulation is crucial for achieving high fertility (Stewart et al., 2010), because of the short fertile life span of oocytes and survival of a sufficient number of fertile spermatozoa to ensure fertilization (Soede et al., 1995a). Optimal insemination relative to ovulation has been reported to vary between 6-18 h before ovulation with 12 h being optimal (Dziuk, 1970). More recent studies have reported optimal fertilization can occur with AI between 0 and 24 h before ovulation (Waberski et al., 1994; Soede et al., 1995a; Nissen et al., 1997). This is supported by Soede (1995) who reported that the number of normal embryos was significantly higher when insemination occurred in the optimal window rather than insemination before or after the defined period. For sows inseminated outside the optimal window, an increased proportion of unfertilized eggs with fewer accessory sperm cells were present at the site of fertilization, with resulting lower conception rates and litter sizes (Soede et al., 1995a).

CONTROL OF REPRODUCTION

Control of estrus

To facilitate breeding, producers rely on synchronous induction of estrus for introduction breeding of replacement gilts and rebreeding of sows after weaning (Baker and Rajamahendran, 1973; Britt et al., 1989; Estill, 2000).

Managerial methods including relocation and grouping of animals have been shown to induce 5-10% of gilts to express estrus within a defined period of time. To date, boar exposure is one of the most effective methods for inducing puberty whether physical or fence-line exposure is used (Brooks and Cole, 1970; Thompson and Savage, 1978; Christenson, 1981; Pearce and Paterson, 1992; Kingsbury and Rawlings, 1993). Exposure to a male can hasten female maturation as seen in mice (Vandenbergh, 1967), and in pigs can reduce the number of days required for gilts to farrow their first litter (Brooks and Cole, 1970; Estill, 2000). Puberty attainment with boar exposure is credited to an induced stress response from the boar by pheromone stimulation with 16-androsterone from the submaxillary salivary glands along with visual and auditory cues (Pearce and Hughes, 1987).

While boar exposure is the industry standard, it can also be combined with use of gonadotropin drugs for improved responses. For example, an approved exogenous hormone product, PG600, contains 400 IU of pregnant mare's serum gonadotropin (PMSG) and 200 IU of human chorionic gonadotropin (hCG). These hormones exhibit FSH and LH activities to induce follicle growth, estrus and ovulation in gilts and weaned sows and animals that are acyclic (Dziuk and Baker, 1962; Baker and Rajamahendran, 1973; Paterson, 1982; Britt et al., 1989). Following injection, PMSG and hCG diffuse into the blood and act at the ovary, binding to medium sized follicles to stimulate follicle growth and production of estrogen. The hormones are injected intramuscularly or subcutaneously at a low dose to induce synchronous estrus and ovulation in a high proportion (50-80%) of immature gilts (Breen et al., 2005). The dose of PG600 increases the percentage of gilts in estrus and reduces the interval to estrus without negatively affecting reproductive traits compared to higher dose injections (Britt et al., 1989). High dose injections have been shown to elicit variable estrus responses, poor reproductive

performance with low fertility, failure of corpora lutea to persist in pregnant animals, and failure to maintain cyclic activity in non-pregnant gilts (Paterson, 1982). Less satisfactory methods such as the use of estrogen benzoate (EB) have been tested for inducing puberty. EB produced inconsistent responses and poor reproductive performance, limiting its value for inducing puberty.

Approaches to synchronize estrus in gilts and cyclic females have used a synthetic progestin, Altrenogest, labeled as Regumate or Matrix (Merck Animal Health), to inhibit the increased pulsatile release of LH, final stages of follicle development, and expression of estrus (Ulberg et al., 1951; Martinat-Botté et al., 1995). Upon its withdrawal, follicles mature rapidly and ovulate within a predictable time interval (Ulberg et al., 1951; Baker et al., 1954; Day et al., 1959). Treatment with prostaglandins have also been tested and when given between 14 and 35 days of pregnancy, can induce regression of the CL causing abortion of the fetus and return to estrus in 4-5 days. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and estradiol benzoate (EB) have been used together to control the cycle, with EB used extend the luteal lifespan of the CL and $PGF_{2\alpha}$ to induce luteolysis following day 12 of the estrous cycle which has been shown to synchronize return to estrus (Guthrie and Rexroad, 1981; Guthrie and Bolt, 1983).

CONTROL OF OVULATION

Control of ovulation

The control of ovulation by exogenous hormones has been shown to be effective for induction and synchronization of the time of ovulation (Brüssow et al., 1996; Brüssow et al., 2009; Knox et al., 2011). Controlling the process of induced follicle maturation and ovulation would eliminate breeding based on symptoms of estrus, allowing for a more precise timed AI to

minimize insemination to ovulation intervals (Knox et al., 2011; Driancourt et al., 2013). Furthermore, this would allow for a single insemination at a fixed time while maintaining reproductive performance similar to that of sows bred twice during estrus (Driancourt et al., 2013). Overall, benefits for controlling ovulation could permit more uniform breeding and farrowing in large groups with more efficient use of labor.

Throughout the years, ovulation induction has been used in many species that can respond to exogenous hormones including the pig (Brüssow et al., 2009; Knox, 2015). Researchers have used hormones with the intent of directly and indirectly mimicking the endogenous pre-ovulatory LH peak (Von Kaufmann and Holtz, 1982; Brüssow et al., 1996; Stewart et al., 2010; Knox et al., 2011; Fontana et al., 2014). For an efficient ovulation induction response, development and triggered oocyte release from follicles depends upon LH binding to its receptors (Esbenshade et al., 1989; Knox, 2015). As follicles reach maturity, the LH pulse changes from a luteal to follicular pattern with increased production of estradiol. The positive estrogen feedback induces the LH surge, which starts the process of ovulation and start of luteinization for restructuring of the follicle wall (Knox, 2015). Hormones shown to be effective in activating the pre-ovulatory surge, for inducing ovulation in the pig are highly purified human chorionic gonadotropin (hCG), porcine luteinizing hormone (pLH) (Cassar et al., 2005), and gonadotropin-releasing hormone (GnRH) and its analogs. Human chorionic gonadotropin is the most widely applied hormone for ovulation induction. The use of the drug was reported in Eastern Europe around the 1970s (Hühn et al., 1996), and it has continued to be used in breeding protocols for inducing ovulation and production of an ova for in vitro fertilization with minimal problems associated with fertility (Dziuk and Gehlbach, 1966; Baker and Coggins, 1968; Knox, 2015). Studies have reported that hCG was effective for inducing ovulation in gilts (Estienne et

al., 2001) and sows (De Rensis et al., 2003) 40-42 h after treatment while also shortening weaning-to-estrus intervals (Estienne and Hartsock, 1998; Kauffold et al., 2007). Though effective alone, hCG is often paired with eCG in order to stimulate follicle development (Tanabe et al., 1949; Hunter, 1967b; Estienne et al., 2001; Brüssow et al., 2009; Knox, 2015). The combined drugs have been shown to allow treated animals to ovulate more uniformly compared to non-treated controls with ovulation occurring 42-53 h after hCG (Brüssow et al., 2009). As highly effective as hCG is for inducing ovulation, it remains only FDA approved in the USA swine industry in combination with eCG as the product named P.G. 600. This may continue due to issues involving product sourcing, availability, and isoforms.

Shown to be as effective as hCG in inducing and synchronizing ovulation, porcine LH (pLH, Lutropin-V, Bioniche Animal Health, Belleville, Canada) is reported to induce ovulation approximately 38 hours after treatment in gilts and sows (Candini et al., 2004; Cassar et al., 2005; Degenstein et al., 2008). Used in studies after treatment with eCG, sows treated with pLH 80 h later, ovulated between 34-42 h later (Cassar et al., 2005; Fontana et al., 2014). Compared to use of hCG and GnRH, the use of pLH in research studies and production farms in the US is minimal with concerns of product sourcing, availability, and use in food production. The discovery of GnRH and production of its analogues appeared as a good alternative to the gonadotropin hormones. GnRH is a decapeptide that acts at the pituitary to stimulate the release of endogenous LH (Brüssow et al., 2009). Studies incorporating GnRH agonists, buserelin (Martinat-Botté et al., 2010; Driancourt et al., 2013), deslorelin, gonadorelin, goserelin (Brüssow et al., 2007), peforelin, and triptorelin (Taibl et al., 2008; Stewart et al., 2010; Knox et al., 2011; Knox et al., 2014; Gesing, 2015) reported their effectiveness at stimulating the pre-ovulatory surge and ovulation among gilts and sows. The GnRH analogues used independently or in

combination with estrus induction drugs resulted in improved ovulation among females (Guthrie, 1977; Hühn et al., 1996; Kirkwood and Kauffold, 2015; Knox, 2015). Comparing hCG to GnRH used alone or in combination with other drugs, showed similar responses and in certain cases was more effective at synchronizing ovulation in swine (Brüssow et al., 2009). For example, a study tested the GnRH analog buserelin to hCG when given at the onset of estrus in weaned sows for effectiveness to induce ovulation (Wongkaweewit et al., 2012). While both hormones could control the time of ovulation, some of the animals treated with the analog developed cysts. In a study using D-Phe^{6} LHRH, gilts were found to ovulate with a range of 24-40 hours with an average ovulation time around 35-37 hours after treatment (Brüssow et al., 1996). In the same study, GnRH used in conjunction with eCG yielded improved fertility results compared to hCG (Brüssow et al., 1996). Another study comparing hCG to GnRH when given to sows 72 hours after weaning reported that hCG induced smaller follicles to develop and reduced estrus expression while GnRH induced follicle growth, but resulted in greater cyst formation, similar to other studies (Nissen et al., 1994).

More recently, research with GnRH analogs has included use of triptorelin (Taibl et al., 2008; Stewart et al., 2010; Knox et al., 2011; Gesing, 2015). Intravaginal administration of the GnRH analog, triptorelin, has been shown to induce an LH surge within 4-16 hours and synchronize ovulation within 44 hours following treatment which could be altered by changing the viscosity of the carrier gel and the dose of triptorelin (Stewart et al., 2010; Knox et al., 2011). Sows treated with triptorelin intravaginally exhibited preovulatory LH surges with magnitudes comparable to those that occur spontaneously in control sows (Stewart et al., 2010). Studies also have evaluated the administration of GnRH at 96 hours after weaning or at the onset of estrus to advance ovulation. They concluded that ovulation tended to advance when given at 96 hours

after weaning (Knox et al., 2011; Esparza-Harris et al., 2015). Triptorelin acetate, (pGlu-His-Trp_Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-NH_2), has been developed into the FDA approved drug OvuGel® (JBS United Animal Health, Indiana). OvuGel® contains triptorelin acetate, in a 1.2% methylcellulose gel and phosphate buffered saline. The hormone treatment has been shown to induce an ovulation rate similar to that observed during spontaneous follicular development (Knox et al., 2011; Gesing, 2015). The OvuGel® system uses an intravaginal gel delivery that requires no needle for administration and transports GnRH across the vaginal membrane which is optimized when the viscosity of the vehicle matches the viscosity of the cell membrane. OvuGel® is approved for the use in food animals in the US with global development for approval being sought by Elanco animal health.

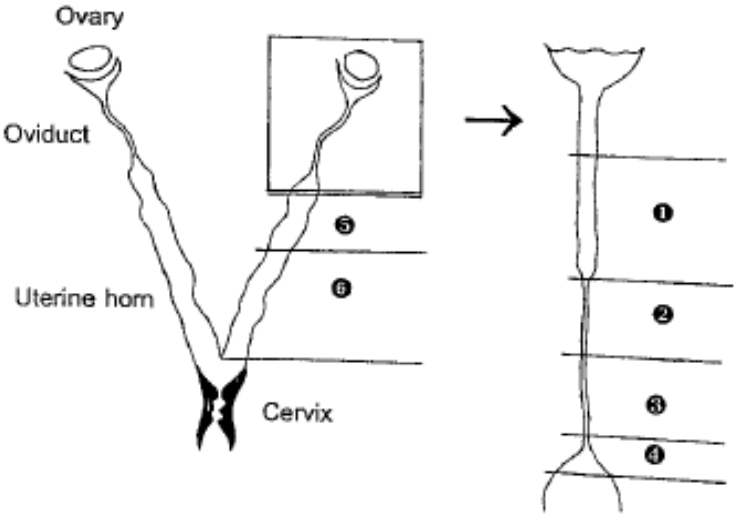
TECHNOLOGIES FOR REDUCING THE NUMBER OF SPERM

Reducing the number of sperm used per insemination can allow production companies to access top indexing sires to a greater extent for increasing herd genetic performance (Watson and Behan, 2002). Reduction of the sperm dose can improve semen distribution by supplying more doses produced per boar, with annual semen production producing 1500 doses per boar and able to inseminate approximately 700 sows. In a conventional system, the 1500 doses would be used to inseminate each sow 2-3 times using ~3 billion sperm per dose (Levis et al., 2001). The use of an alternative insemination technique that would result in comparable fertility to conventional AI would allow reduction of sperm in the insemination dose (Bortolozzo et al., 2015).

The goal of insemination is to guarantee that a sufficient number of spermatozoa reach the utero-tubal junction (UTJ) to establish an adequate sperm reservoir capable of fertilizing all

ovulated oocytes. During natural mating the boar deposits more than 50 x 10^9 spermatozoa in the cervical canal. This overabundance of spermatozoa helps overcome the female's hostile uterine environment (Roca et al., 2006). This becomes difficult with artificial insemination because the industry inseminates only 3-4 x 10^9 sperm in the same location. In conventional AI, inseminating sperm in the cervix has resulted in consistent fertility rates of 80-90%, but is inefficient in use of semen while insemination deeper into the reproductive tract can also result in successful farrowing rates and litter sizes with less sperm (Watson and Behan, 2002; Rozeboom et al., 2004). The unique nature of the pig is that is has two large uterine horns that the sperm must traverse to the site of insemination (Rath, 2002). Spermatozoa must transverse difficult barriers such as the mucus-filled folds of the cervical canal into the body of the uterus before they can reach the UTJ. Once semen is deposited into the cervix (Figure 6.1), some of the sperm will be trapped and die in the cervical barriers. Survivors are transported into the uterus towards the tubal ends of the uterine horns. Within the uterus, the local immune system perceives the spermatozoa as foreign bodies. Past 30 minutes and up to 3 h following insemination, there is a massive invasion of leukocytes that enters the uterine lumen and the polymorph-nuclear neutrophil (PMN) phagocytosis decreases the sperm population (Lovell and Getty, 1968; Hadjisavas et al., 1994), as reviewed by Rath (2002). The immune reaction is a consequence of an inflammatory response provoked by both spermatozoa and seminal plasma (Robertson, 2007). As a result of this response, ≤100,000 of spermatozoa will reach the oviduct after the deposition of billions during insemination (Matthijs et al., 2003; Roca et al., 2006). By 4 h after insemination, 60-90% of the inseminated spermatozoa have been eliminated from the uterus with 25-30% loss attributed to leakage during and backflow after insemination (Matthijs et al., 2003).

Figure 1.4: The parts of the uterine horn and oviduct: 1) Ampulla, 2) Cranial isthmus, 3) Caudal isthmus, 4) UTJ, 5) Cranial uterine horn, 6) Caudal uterine horn (Kunavongkrit et al., 2003).



Backflow restricts the number of spermatozoa able to reach the UTJ, and with 3×10^9 sperm inseminated, only approximately 1×10^5 will successfully migrate to the UTJ, and 1×10^3 reach the sperm reservoir in the isthmus of the oviduct (Martinez et al., 2001). Excessive loss of sperm or reduced numbers of sperm can interfere with fertilization and reduce fertility for pig production. However, new insemination procedures have been developed based on a deeper deposition site for insemination in the reproductive tract. This approach could allow for a greater proportion of sperm to survive and colonize the oviduct and therefore fewer sperm would be necessary to achieve the same probability of fertilization achieved with a standard AI (Vazquez et al., 2008b). The deeper insemination methods are intended to bypass or limit sperm transit through some of the uterus, thus decreasing sperm losses by phagocytosis and avoiding backflow of spermatozoa (Vazquez et al., 2008b).

Intra-uterine insemination/post-cervical artificial insemination

When semen is deposited in the cervix, as with cervical artificial insemination (CAI), it is necessary to use a large volume to ensure an adequate sperm transport to the fertilization site (Mezalira et al., 2005). Early studies showed that intrauterine insemination could permit a reduction in the number of spermatozoa and volume of inseminating dose required (Hancock, 1959; Mezalira et al., 2005). Recently, there have been attempts to obtain satisfactory farrowing rates and litter sizes using low numbers of cooled spermatozoa per dose using DUI (deep uterine insemination) and PCAI (post-cervical insemination), both of which involve placing semen directly into the uterus of the sow.

Post-cervical artificial insemination (PCAI) deposits the insemination dose into the uterine body and can allow up to a three-fold reduction in the number of sperm (Vazquez et al., 2008b). PCAI uses a thin and semi rigid inner (cannula) catheter that passes through a conventional catheter. The inner rod extends past the tip of the catheter that has been previously inserted in the cervical folds, allowing semen deposition directly into the uterine body (Watson and Behan, 2002; Rozeboom et al., 2004). Use of PCAI is a targeted tool for minimizing the number of sperm (Driancourt et al., 2013). Studies show that high fertility is achievable compared to CAI using 3×10^9 sperm, with PCAI use of 1.0×10^9- 1.5×10^9 or DUI with 0.6×10^6 spermatozoa per dose. PCAI (Figure 6.2) allows for a substantial reduction in the number of cooled spermatozoa required per dose in comparison with intra-CAI, but can also result in a reduction in the number of piglets born per litter depending on technician skill level and presence of blood (Rozeboom et al., 2004; Roca et al., 2006).

The PCAI technique has been reported to have a substantial reduction in semen backflow to less than 20% of the inseminated spermatozoa. Backflow after insemination is a normal

process in swine, independent of the site of deposition and the volume of the insemination dose (Steverink et al., 1998; Mezalira et al., 2005). Research on backflow volume in traditional AI ranged from 20-120% (Steverink et al., 1998) or 0-118% (Wentz et al., 2001). Mezalira (2005) observed the percentage of backflow volume in the intrauterine insemination in close to two-thirds of the infused volume (Mezalira et al., 2005). If backflow occurred not at insemination but 0.5-2.5 h later, no affects were observed on the establishment of a sufficient sperm reservoir at the UTJ (Steverink et al., 1998). Semen backflow was not observed at the moment of IUI, but up to 2 h after insemination, ranging from 12.1-17.1% (Mezalira et al., 2005). Hernández-Caravaca (Hernández-Caravaca et al., 2012) hypothesized that the decrease in backflow with PCAI compared to CAI could be attributed to fewer estrogens present in the sperm and seminal plasma from the ejaculate of the boar that functions to produce prostaglandin release by the endometrium (Claus, 1989; Langendijk et al., 2005). Prostaglandin release by the endometrium increases myometrial activity in the uterus stimulating contractility. While it improves semen transport, it can also increase the timing of semen uptake in reproductive tract during insemination. Post cervical artificial insemination has been shown to decrease backflow and facilitate inseminating a reduced number of sperm in the female. PCAI should be considered as a practical insemination approach under field conditions to lower sperm numbers and achieve fertility in pigs (Roca et al., 2006; Vazquez et al., 2008b).

FINAL THOUGHTS

The use of artificial insemination (AI) continues to allow for efficient genetic advancement. Further success of modern AI relies on a selection program for use of high indexing sires and production of high-quality sperm (Knox, 2016). Methods that enable more

efficient use of high-quality sperm from superior sires involve low-dose insemination, ovulation induction, and fixed timed AI (Brüssow et al., 1996; Knox et al., 2011; Driancourt et al., 2013). Though these technologies have been used individually or in combination with promising fertility, the effectiveness of a combination of all with regard to farrowing rates and litter size needs further evaluation.

CHAPTER 2: EFFECT OF NUMBERS OF SPERM AND TIMING OF A SINGLE, POST-CERVICAL INSEMINATION ON THE FERTILITY OF WEANED SOWS TREATED WITH OVUGEL®

INTRODUCTION

The success of the swine industry in production efficiency and product quality can be linked to improvements in genetic selection for economically important traits (Notter, 1999). Genetic advancement for the modern pork production system is primarily accomplished through the use of artificial insemination (AI) and the distribution of those genes from highly selected sires (Reed, 1985; Knox, 2016). Sire selection for use in AI programs is focused on production traits, such as feed conversion, growth performance, and carcass measures (Kanis et al., 2005) and relies on the production and distribution of high numbers of quality sperm (Alm et al., 2006). Limitations to more extensive gene distribution is the number of sperm produced by a sire each week, and the numbers of sperm required in the insemination dose to produce a litter. At the present time, the standard breeding protocol used in North America relies on once daily estrus detection, with an insemination performed on each day the female is detected standing. AI is performed using 3 billion sperm in 80 mL of extender (Lamberson and Safranski, 2000; Roca et al., 2006) with an industry average of an 84% farrowing rate and 12 total born pigs (PigChamp, 2015). In the standard breeding protocols, the time of insemination is determined by onset of estrus (Langendijk et al., 2000) but the duration of estrus (Soede and Kemp, 1997) and interval from onset of estrus to ovulation can be variable and influenced by external factors (Weitze et al., 1994; Soede and Kemp, 1995; Knox and Rodriguez-Zas, 2001; Belstra et al., 2004). High fertility in swine requires one insemination within the 24 h period before ovulation in order to maximize fertilization rate, normal embryos, farrowing rate and litter size (Soede et al., 1995b;

Nissen et al., 1997). The optimal fertilization window is influenced by the relatively short lifespan of the pig oocyte after ovulation (Hunter, 1967a) and the lifespan of spermatozoa in the female genital tract (Hunter, 1981; Matthijs et al., 2000; Rodríguez-Martínez et al., 2005). Because of the observed variability in estrus and time of ovulation, especially in weaned sows, an insemination is performed on each day of standing estrus, to improve the likelihood that at least one AI will occur within the 24 h before ovulation.

Approaches to extend the use of superior sires include reducing the number of sperm in the AI dose (Vazquez et al., 2008a), use of a post-cervical artificial insemination (PCAI) (Watson and Behan, 2002), and reducing the need for multiple inseminations by control of ovulation and single, fixed time AI (Brüssow et al., 2009; Driancourt, 2013; Fontana et al., 2014; Knox, 2015). With lower sperm used with PCAI, there appears to be a minimum number of sperm needed to achieve similar fertility to controls, but these values may be influenced by other factors including whether single sire or pooled semen is used, number of sperm calculated based on total or motile cells, and current herd fertility (Watson and Behan, 2002; Rozeboom et al., 2004; Mezalira et al., 2005; Vazquez et al., 2008b). The impact of lower sperm used for mated females has been modelled for increased economic value as a result of greater genetic gains through access to higher indexing sires (Rath, 2002; Fontana et al., 2014).

Hormonal control of ovulation is a technology that has been effective for reducing variation in time of ovulation in swine with use of hCG (Dziuk and Baker, 1962), pLH (Bennett-Steward et al., 2007) and GnRH (Brüssow et al., 1996) for use of fixed time insemination (Brüssow et al., 2009). In contrast to the other hormones, GnRH or its analogues can be synthesized with high purity, and can be modified to increase its bioactivity and half-life, and delivered by different routes for more practical delivery (Knox, 2015). In pigs, most GnRH

analogues have been developed for use by I.M. injection, while one product, contains the GnRH agonist triptorelin, has been formulated for intravaginal delivery in gel form (Stewart et al., 2010). It has been reported to be effective at advancing and inducing ovulation in weaned sows with similar fertility responses to controls when used in a multiple AI system (Stewart et al., 2010; Knox et al., 2011). While the approved product OvuGel®, which contains a higher dose of triptorelin, has available fertility data for use with a single insemination (Knox et al., 2014), limited research is available on utilization of number of sperm used in a single PCAI and the effect of time of insemination following triptorelin administration. Therefore the objectives of the present study were to induce ovulation in weaned sows at 96 h after weaning, and test for the effects of a single PCAI at 22, 26, or 30 h following OvuGel® treatment with AI doses containing 1.5 or 2.5 billion sperm.

MATERIALS AND METHODS

The use of animals for this experiment was approved by the University of Illinois Institutional Animal Care and Use Committee (#14130).

2.1. Animals and housing

The study was performed at a 1,400 sow commercial research farm in Indiana (JBS United, Inc., Frankfort, IN) in nine replicates during July through October 2014. Multiparous PIC 1050 sows (n = 641; Hendersonville, TN), were allocated to treatment from parities 1 to 6 (average = 2.8 ± 0.1) following a 19 d lactation (average = 19.5 ± 0.1 d). Sows were weaned and relocated from farrowing into a breeding and gestation barn and housed in stalls (0.55 m x 2.13 m) throughout the study. A sub-population of the sows (n = 24) used in replicate 2 of the study, had been previously weaned and were fed the synthetic progestogen, altrenogest (Matrix, Merck

Animal Health, Kenilworth, NJ, USA) as a top dress on the daily feed for 14 d to synchronize return to estrus with the next breeding group. The last day of altrenogest feeding coincided with day of weaning, and sows were randomly assigned by parity to treatment and administered OvuGel® 96 h after last altrenogest feeding.

2.2. Experimental design:

Sows were weaned at 0900 h and randomly assigned by parity and lactation length to receive a single, PCAI using 1.5 or 2.5 billion viable sperm at 22, 26, or 30 h following OvuGel® administration at 96 h after weaning. All sows were inseminated with a commercially prepared dose of 1.5 billion sperm in 45 mL or 2.5 billion sperm in 75 mL volume. The effect of insemination volume has been previously reported to have no effect on fertility when using PCAI (Hernández-Caravaca et al., 2012; Sbardella et al., 2014), with semen suppliers concerned about over dilution of low sperm numbers in extender. The insemination doses for each treatment were created from a pool of boars collected on the same day at a commercial boar stud (Birchwood Advanced Genetics Solutions, West Manchester, OH, USA). Semen was collected and evaluated for quality and concentration, and then pooled and aliquoted into the small or large semen tubes. Semen was shipped from the boar stud to arrive at the sow farm the next day and was stored at 16° C and used within 3 days from day of collection.

2.3. Administration of OvuGel®

All sows were treated with 200 µg of triptorelin acetate (OvuGel®, JBS United Animal Health LLC, Sheridan, IN USA) at 96 h after weaning as previously described (Knox et al., 2011). The 2 mL dose was deposited approximately 1 to 2 cm posterior to the cervix using a multi-dose applicator and new disposable outer sheath for each sow.

2.4. Detection of ovulation

Trans-rectal real-time ultrasound was performed using an Aloka 500V (Hitachi Aloka Medical America, Inc. Wallingford, CT) with a 7.5 MHz linear transducer attached to a PVC stabilizing rod (Knox et al., 2011). Ultrasound assessment was performed in a sub-population of sows (n = 499) to determine the presence and size of ovarian follicles and other ovarian structures starting at 8 h following OvuGel® administration and continuing at 8 h intervals until 56 h. Of all sows scanned, some observations could not be performed at subsequent time intervals due to rectal irritation or ability to find the ovaries. Data for ovulation status at each time interval following OvuGel® included 499 to 411 sows. Time of ovulation was defined as the time when < 4 large follicles were counted in total from both left and right ovaries and following a noticeable (>50%) reduction in the counts for the number of large follicles from the previous scans. Scanning of sows also identified the presence of corpora lutea, small (< 3 mm) and medium follicles (3.0 to 6.49 mm), or follicle cysts (>12.9 mm). Regardless of follicle size, the presence of cysts or corpora lutea, all sows were treated with OvuGel® and inseminated based on the experimental protocol.

2.5. Detection of estrus and insemination

Fence-line boar contact was performed once daily starting on Day 3 after weaning and continuing until Day 6 to determine expression, onset and duration of estrus. A mature boar that was housed 12 m downwind was placed into an electric boar cart (Contact-O-Max, MOFA, Verona, WI USA) that was moved in the alleyway in front of the gestation stalls to provide 2 min of fence-line exposure. One of three boars were chosen each day and rotated for exposure. The time of onset of estrus was defined as the first time a show showed a standing response, sow stood rigidly to back pressure, with no vocalization and a positive indication of an ear reflex. Sows received a single insemination at 22, 26, or 30 h after the administration of OvuGel® by

PCAI in the absence of a boar. The PCAI device included a standard AI foam tip catheter that was inserted through the vagina and fixed into the cervix and then a flexible inner catheter with a diameter of 4 mm and a length of 750 mm was inserted up to 200 mm beyond the tip of the standard foam catheter (Magapor, Zaragoza Spain).

2.6. Statistical analysis

Continuous response variables were analyzed using MIXED and binary response variables analyzed using the GENMOD procedures of SAS (SAS Institute Inc., Cary, NC). Least squares means were computed for all response variables. The continuous response variables included: duration of estrus, wean-to-estrus interval, average follicle size 8 h following OvuGel®, length of gestation, total piglets born alive, number of piglets born alive, mummified fetuses, and number of stillborn pigs. The binary response variables included: estrus expression within 6 d of weaning, sows ovulating within 6 d of weaning, sows ovulating from time of OvuGel®, and sows that were pregnant and those that farrowed. All models included the main effects of dose of sperm and time of insemination and their interaction, as well as replicate, and parity. Lactation length and synchronization with Matrix were tested and removed from all final models when determined to be non-significant. In separate analyses, the effects of estrus, ovulation (yes or no), and ovarian classification for abnormalities were also tested for effects on fertility. The effect of time of ovulation following OvuGel® and its interaction with treatment was also tested for effects on farrowing rate and total born. To evaluate the effect of ovarian activity on ovulation and litter size, the average follicle size at 8 h after administration of OvuGel® post-weaning (< 6.5 or ≥ 6.5 mm) was tested using one-way ANOVA. Significant differences were identified at $P \leq 0.05$ and trends at $P > 0.05$ and $P \leq 0.10$.

RESULTS

3.1. Estrus responses and induced ovulation

Among weaned sows, estrus was not used as a predictor for timing of insemination, but is reported as an indicator of sow fertility (Table 1). Wean to estrus interval (4.2 d), duration of estrus (1.7 d), and expression of estrus within 6 d of weaning (88%) were not different among treatments ($P > 0.10$). However, wean to estrus interval and duration of estrus were affected by parity ($P < 0.01$) and were shorter in primiparous sows than the higher parities. Of the 88% of sows that expressed estrus, very few (0.3%) were detected on Day 3, but 27% were detected standing for the first time on Day 4, 54.2% on Day 5, and 6.0% on Day 6. Estrus expression was associated with a greater proportion of sows ovulating ($P < 0.001$). For the sub-population of sows scanned using ultrasound at 8 h following OvuGel®, 87.9% had large follicles, 12.0% had small to medium sized follicles or ovarian cysts, and 0.8% had corpora lutea. Of the sows not expressing estrus within 6 days of weaning and that were scanned, 76% had ovaries with large sized follicles, with 18% having small to medium follicles, 3% polycystic, and 3% with corpora lutea. Ovaries having corpora lutea, small to medium follicles and cysts reduced the expression of estrus and reduced ovulation ($P < 0.005$). Of sows scanned with ultrasound, 89% ovulated by 48 h following OvuGel® (Figure 1) and was not influenced by treatment ($P > 0.10$) with the majority of sows determined to have ovulated at the 32, 40 and 48 h treatment to ovulation intervals (Figure 2). The average interval from OvuGel® to ovulation was 42.2 ± 0.4 h with 94% confirmed to have ovulated by 56 h.

3.2 Pregnancy, farrowing and litter size

There was no interaction of number of sperm and timing of insemination ($P > 0.10$) and therefore only main effects are reported. Pregnancy rate tended to be affected by numbers of sperm ($P = 0.09$) and time of insemination ($P < 0.05$, Table 2). Farrowing rate tended to be influenced by number of sperm ($P = 0.06$) and tended to be affected by time of insemination ($P = 0.10$). Sows inseminated further from the average time of ovulation showed higher pregnancy and farrowing rates with sows inseminated 22 and 26 h after administration of OvuGel® showing greater farrowing rates than sows inseminated at 30 h (Table 2). In addition to treatment, other factors such as parity, estrus expression, and ovulation also influenced both pregnancy and farrowing rates ($P < 0.05$). Of the 12% of weaned sows that did not exhibit estrus, approximately half ($n = 28$) farrowed a litter. There was no effect of treatment on gestation length which averaged 114.8 ± 0.1 d. The total number of piglets born and number of piglets born alive were affected by number of sperm ($P < 0.05$) but not by the timing of insemination ($P > 0.10$). Litter size was increased with 2.5 compared to 1.5 billion sperm ($P < 0.05$, Table 2.) and was also affected by other factors such as parity and whether the sows had ovulated by 56 h. The time of ovulation did not interact with number of sperm or time of insemination for effects on farrowing rate ($P > 0.10$) or litter size ($P > 0.10$). Though, as the insemination to ovulation interval increased so did the percentage of sows that farrowed and litter size. There was no effect of treatment ($P > 0.10$) on the number of stillborn pigs (1.0 ± 0.1) or mummified fetuses (0.3 ± 0.1). A piglet index was calculated based on the farrowing rate x the number of piglets born alive for each treatment. Though not statistically tested, the index was lower with 1.5 compared to 2.5 billion motile sperm and increased as time of insemination was further from the average time of ovulation (Table 2).

DISCUSSION

The available data for swine indicates that a single insemination occurring within the 16 to 24 h before or 0 to 8 h period after ovulation, will result in high fertilization and pregnancy rates and increased litter size when compared to earlier or later inseminations (Soede et al., 1995a; Nissen et al., 1997; Bortolozzo et al., 2005). Research has also shown that numbers and quality of sperm can affect fertility (Johnson et al., 2000). Due to the reported variation in the interval from onset of estrus to ovulation in weaned sows (Weitze et al., 1994; Steverink et al., 1997), multiple inseminations are required and used in >90% of sows to optimize breeding herd fertility (PigChamp, 2015). However, the use of multiple inseminations is inefficient from the standpoint that greater numbers of sperm are required per conception and this limits distribution of semen from high indexing sires, as well as increases labor and costs of production (Safranski, 2008; Knox, 2016). In an attempt to address some of these limitations, the present study tested the fertility effects of lower numbers of sperm used in a single insemination when given at various time intervals following synchronization of ovulation in weaned sows. The use of the GnRH agonist, OvuGel®, in this study, effectively synchronized ovulation in the majority (~88%) of weaned sows to a 24 h period (24 to 48 h following OvuGel®) with very few sows ovulating outside of this time frame. There was a significant fertility advantage when using 2.5 compared to 1.5 billion sperm with intra-uterine insemination (IUI) on pregnancy and farrowing rate but not on litter size. Also, fertility was greater with a single insemination given at 22 and 26 h following OvuGel® treatment compared to an AI at 30 h. This was unexpected, as previous studies had suggested some fertility advantage as the interval from AI to ovulation was reduced, especially with lower numbers of fertile sperm (Waberski et al., 1994; Steverink et al., 1997; Bortolozzo et al., 2005). In other scientific studies, lowered fertility from a single AI occurring ≥

24 h ahead of ovulation occurred due to an insufficient number of viable spermatozoa in the oviducts and when occurring too far after ovulation resulted from problems with aged oocytes (Soede et al., 1995a; Steverink et al., 1997; Bortolozzo et al., 2005). Yet in the present study, with the spread in time of ovulation of only 24 h among sows, there was no indication of an interaction of number of sperm with interval from insemination to ovulation on farrowing rate or litter size. The results lead us to conclude that fertility was affected by number of sperm and timing of a single intrauterine insemination following ovulation synchronization. The effects of numbers of sperm on fertility might reflect the efficiency of the insemination technique (Steverink et al., 1998) or changes in sperm fertility with time in storage. Further, when using ovulation induction, optimal insemination to ovulation interval appeared to be further from time ovulation rather than closer, and should be investigated further for on farm application.

The timing of insemination relative to ovulation is important for ensuring pregnancy establishment and increased litter size. Optimal AI timing has been reported to be 6 to 18 h before ovulation with natural mating (Dziuk, 1970), and 0 to 12 h (Waberski et al., 1994), or 0 to 24 h before ovulation (Soede et al., 1995a) when using fresh semen. The discrepancy in the estimates for optimal timing may reflect differences in sire or sperm fertility, number of sperm deposited, the effectiveness of the AI technique (Steverink et al., 1998), the intervals used for insemination, and the experimental variability in determining when ovulation has occurred. The effects of insemination time with fresh semen are evident on fertilization rate, percent normal embryos, and conception rate (Hunter, 1967a). While the role of accessory sperm are unclear, numbers of sperm tend to increase with insemination at 0 to 8 h before compared to insemination 16 h before ovulation (Soede et al., 1995b). Fertilization rates are greatest among sows inseminated 0 to 24 h before ovulation and are lower and much more variable 24 to 48 h before

ovulation (Steverink et al., 1997). Insemination within various intervals 0 to 24 h before ovulation result in similar percentages of normal embryos while abnormalities increase when performed before or after this time (Kemp and Soede, 1996). In competitive fertilization tests, a boar used at 16 h before ovulation sired fewer offspring within the litter than the one used 14 to 6 h before ovulation (Dziuk, 1970). The critical components for fertility when considering AI timing involves the establishment and maintenance of a critical the number of fertile spermatozoa in the sperm reservoir and the lifespan of the oocytes following ovulation (Hunter, 1967a; Soede et al., 1995a).

The number of sperm used in the insemination dose is also a critical component to achieving high fertility in the pig. Much of the research that has tested the effects of number of sperm have used a double insemination system during estrus or deposited sperm deeper into the reproductive tract using intrauterine insemination (IUI). The fertility advantages with use of IUI arise from placement of semen closer to the site of fertilization (Watson and Behan, 2002; Fontana et al., 2014). This technology provides a means for achieving high fertility at a lower sperm dose as a result of reducing the time and distance sperm require in transport to the utero-tubal junction (Rath, 2002; Watson and Behan, 2002) and reducing leakage during and back-flow following insemination (Hernández-Caravaca et al., 2012). The limits for number of sperm in the insemination dose on fertility have been studied using IUI in a double insemination system. In the publications available in weaned sows, it appears that depending upon the study and what levels were tested, as the number of sperm falls below 2.0 to 1.5 billion, measures of farrowing rate or litter size start to decline (Watson and Behan, 2002; Rozeboom et al., 2004; Hernández-Caravaca et al., 2012). Other data evaluating sperm numbers, showed that a single conventional insemination of gilts with 0.5 billion sperm resulted in low numbers of embryos

compared to 3 billion sperm (Bracken et al., 2003) while others observed that overall fertilization rates in sows were similar for 1 to 3 billion sperm, but that the percentage of normal embryos and accessory sperm declined with sperm numbers (Steverink et al., 1997).

The approach in the present study to synchronize ovulation in weaned sows, allowed us to test for the fertility effects when reducing numbers of sperm used in a single, intrauterine insemination. The synchronization approach induced ~90% of sows to ovulate within a 24 h period, which in comparison to other reports in untreated weaned sows, greatly reduced the spread in ovulation time of 60 h or more (Kemp and Soede, 1996; Knox and Rodriguez-Zas, 2001). Ovulation of mature follicles can be induced through administration of GnRH or its analogues, or through administration of pLH or hCG (Knox, 2015). However, for practical use, regulatory approval is required in most countries, and OvuGel® was chosen for the current study because it is approved for use in weaned sows in the USA. Some previous studies have reported fertility following synchronization of ovulation with multiple inseminations with use of GnRH analogues such as triptorelin (Stewart et al., 2010; Knox et al., 2011), buserelin (Martinat-Botté et al., 2010) or GnRH-A (Brüssow et al., 1996) or pLH (Bennet-Steward et al., 2008; Zak et al., 2009). Others have induced ovulation and used only a single, fixed time insemination following administration of pLH (Cassar et al., 2005; Cassar et al., 2010), buserelin (Driancourt et al., 2013), and triptorelin (Knox et al., 2014). In the present ovulation induction study, fertility was greatest when insemination occurred at 22 or 26 h following OvuGel® compared to 30 h and when using 2.5 billion compared to 1.5 billion with a PCAI. Others also have noted a fertility advantage of 2.5 versus 1.25 billion sperm, but that effect was with more sperm inseminated closer to ovulation when following pLH administration (Garcia et al., 2007). The time of ovulation in the present study averaged ~40 h from the time of GnRH agonist administration, with a range for ovulation of

24 to 48 hours. There was no effect of interval from insemination to ovulation, and this might be expected since from the earliest to the last ovulators, the insemination interval ranged from +6 h after to -26 h before ovulation, and these times have not been reported to have large detrimental effects on fertility with fresh semen. However, one possible difference among studies is the exclusion of sows based on failure to express estrus at time of insemination which may improve ovulation synchrony and fertility (Driancourt, 2013). In the present study, 12% of the sows failed to express estrus, and in the sub-population that was assessed by ultrasound, both estrus and farrowing were reduced in those with ovarian abnormalities (27%) and with corpora lutea (10%). Yet of those that failed to express estrus, nearly half farrowed a litter and abnormalities were reduced in half with no evidence of corpora lutea. Even in those sows that expressed estrus, there was some association of ovarian abnormalities with failure to farrow, but not the presence of corpora lutea. In the present study, parity also affected fertility, and others have also noted that primiparous sows are less responsive to ovulation induction and less fertile when inseminated in fixed time AI programs (Cassar et al., 2010; Driancourt, 2013). In primiparous sows, this is most likely associated with delayed follicle development or with ovarian abnormalities (Knox and Rodriguez-Zas, 2001). In regard to the effects in the present study of lower numbers of sperm in the single timed PCAI reducing both farrowing rate and total born, a previous study reported that semen backflow and insemination occurring after the time of ovulation were associated with reduced fertility (Ulguim et al., 2016).

CONCLUSIONS

The ability to synchronize ovulation will allow for the use of a single timed insemination with reduced number of sperm. This would facilitate use of genetically superior boars for

economically valuable traits. The use of ovulation induction synchronized ovulation to a 24 h period in 90% of the weaned sows. The success of the ovulation induction and the ability to produce a litter are related to the parity of sow, the follicle status of the ovaries, the expression of estrus, the number of sperm and the timing of the post-cervical insemination. Methods to improve or diagnose the fertility of weaned sows assigned for SFTAI could improve the technique. Further, as sperm numbers are reduced, PCAI technique becomes more important, and timing of insemination even with ovulation induction, may still affect fertility especially in sows that ovulate earlier than predicted.

TABLES AND FIGURES

Table 2.1 Least squares means for weaned sows expressing estrus from Days 3 to 6 and percentage of sows ovulating in response to number of sperm and time of a single post-cervical insemination following OvuGel administration.

	Number of Sperm								
	1.5×10^9			2.5×10^9					
	Time Following OvuGel (h)								
Measure	22	26	30	22	26	30	SEM	P-Value Dose	P-Value Time
Sows (n)	107	107	106	106	107	108		.	.
Wean to estrus (d)	4.1	4.2	4.4	4.2	3.9	4.3	0.2	0.40	0.14
Duration of estrus (d)	1.6	1.6	1.7	1.7	1.7	1.7	0.1	0.44	0.96
Expression of Estrus (%)	85.9	89.3	93.1	88.9	90.0	90.0	3.3	0.47	0.22
Number of piglets born alive	91.6	94.5	100	91.3	95.0	95.0	3.5	0.65	0.05

[1]Sows scanned at all intervals for determination of time of ovulation (n = 411)

Table 2.2 Least squares means for reproductive measures of weaned sows in response to number of sperm and time of a single post-cervical insemination following OvuGel administration.

	Number of Sperm								
	1.5×10^9			2.5×10^9					
	Time Following OvuGel (h)								
Measure	22	26	30	22	26	30	SEM	P-Value Dose	P-Value Time
Sows (n)	107	107	106	106	107	108		.	.
Pregnancy (%)	84.2	75.4	71.1	85.8	85.5	75.8	4.2	0.09	0.01
Farrowing (%)	78.4	75.4	70.9	84.1	84.0	75.0	5.5	0.06	0.10
Total number of piglets born	12.8	13.0	12.8	14.1	13.3	13.6	0.5	0.03	0.76
Number of piglets born alive	11.5	10.7	11.8	12.7	12.1	12.3	0.5	0.004	0.27
Piglet index[1]	902	807	837	1,068	1,016	923			

[1]Index = 100 x farrowing rate x number of piglets born alive

Figure 2.1 Cumulative percentage of weaned sows (n = 411) ovulating following OvuGel® administration at 96 h after weaning.

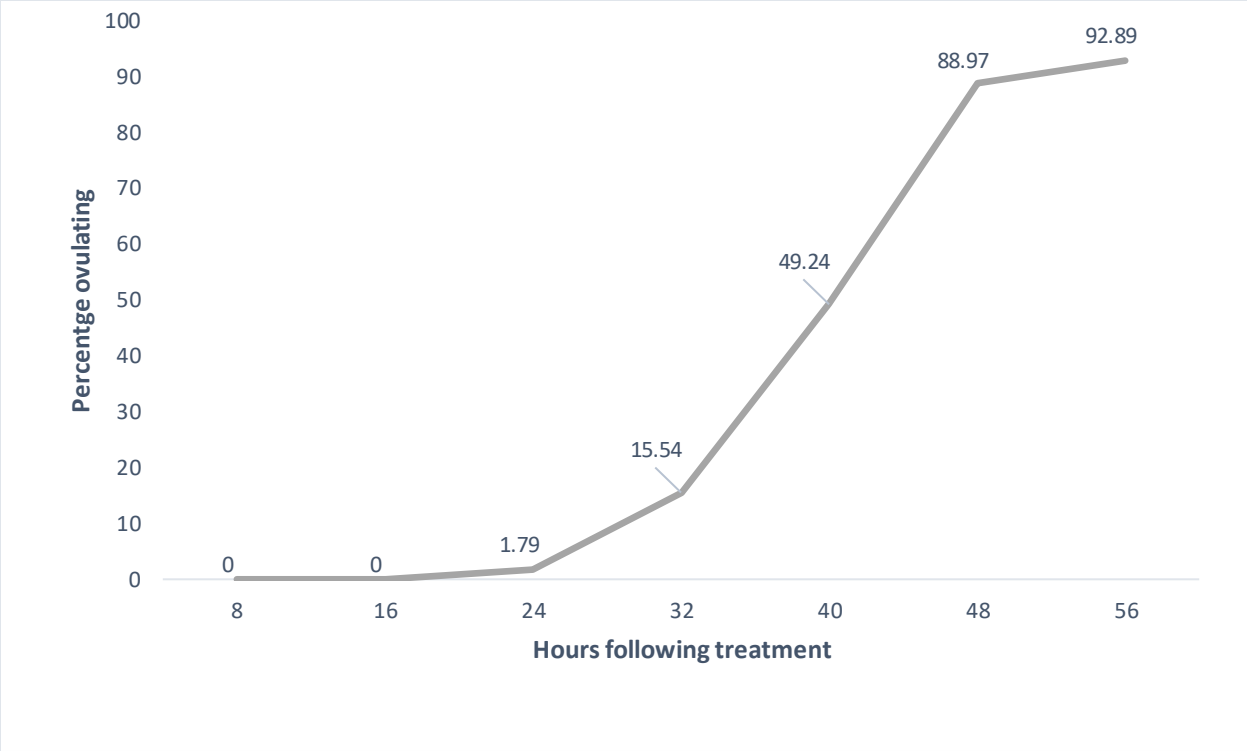
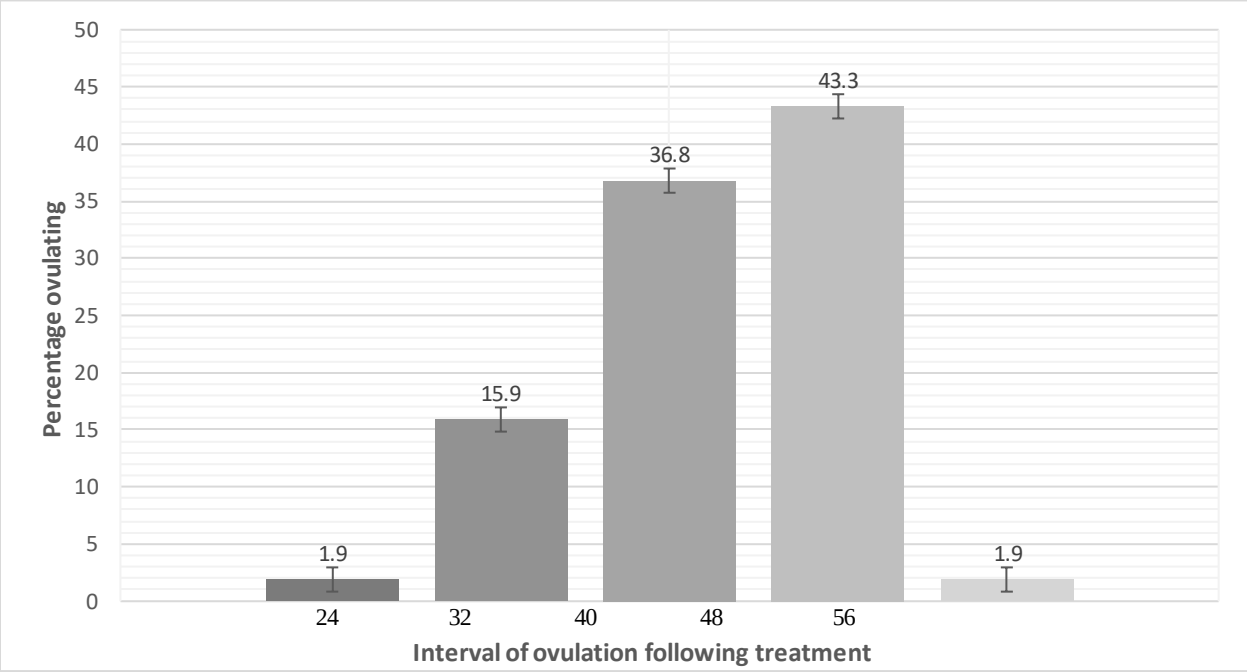


Figure 2.2 Percentage of weaned sows (n = 411) ovulating at discrete intervals determined by ultrasound following OvuGel® given 96 h after weaning.



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