ABSTRACT

BELSTRA, BRADLEY AARON. Investigations of postpartum and postweaning factors that may affect subsequent sow reproductive performance (Under the direction of Dr. William L. Flowers and Dr. M. Todd See).

Experiment one examined effects of season, parity, genotype, lactation length, and weaning-to-estrus interval on the postweaning duration of estrus (DE) and onset of estrus-to-ovulation interval (EOI) of sows in three farms. Farm × weaning-to-estrus interval, farm × season, and parity × season interactions for DE and EOI were detected. Weaning-to-estrus interval had an inverse relationship with DE and EOI on each farm, but the weaning-to-estrus interval range that exhibited a stepwise decrease of DE and EOI was narrower on farm 1 (3 to 5 days) than farms 2 and 3 (3 to 6 days). Both DE and EOI were 8 h longer in the summer than the spring on farms 1 and 3, but did not differ between seasons on farm 2. On each farm, parity ≥ 3 sows had a 4.5 h longer DE and EOI than parity 1 and 2 sows in the summer, but there were no differences in DE or EOI among parity classes in the spring. Lactation length and genotype had small but significant effects on DE and EOI, respectively. Results of this experiment indicate that factors other than weaning-to-estrus interval, such as season and parity, can significantly alter DE and EOI. However, the effects of season and weaning-to-estrus interval on DE and EOI are apparently farm dependent.

Experiment two characterized the urinary excretion of two markers of collagen degradation, hydroxyllysyl pyridinoline (HP) and lysyl pyridinoline (LP), by sows during postpartum uterine involution. A postpartum increase of HP excretion occurred that was temporally consistent with the known pattern of uterine weight loss during involution. The mean molar ratio of HP:LP increased postpartum and confirmed that catabolism of a soft,
non-bone tissue dominates this period. Experiment three investigated the use of transabdominal ultrasonography to estimate uterine size. The distance between the abdominal wall and intestines, which sandwich the uterus, decreased 8.7 cm from day 1 to 18 postpartum. Validation of these two techniques to monitor postpartum uterine involution by comparison to postmortem uterine measurements could provide a research tool to test the effects of uterine involution status on subsequent embryo survival and sow reproductive performance.
INVESTIGATIONS OF POSTPARTUM AND POSTWEANING FACTORS THAT MAY AFFECT SUBSEQUENT SOW REPRODUCTIVE PERFORMANCE

by

BRADLEY AARON BELSTRA

A dissertation submitted to the Graduate Faculty of North Carolina State University in partial fulfillment of the requirements for the degree of Doctor of Philosophy

ANIMAL SCIENCE

Raleigh

2004

APPROVED BY:

[Signatures]

Chair of Advisory Committee

Co-Chair of Advisory Committee

Member of Advisory Committee

Member of Advisory Committee
DEDICATION

This dissertation is dedicated to the memory of my father, Max L. Belstra, 1952-2000.
BIOGRAPHY

Bradley A. Belstra was born on October 3, 1973 and grew up near the town of Wheatfield, in a primarily rural and agricultural area of northwest Indiana. From an early age, he was involved in his family’s feed milling and swine finishing operations based in nearby DeMotte, Indiana. With the encouragement of his parents, Brad attended Purdue University and obtained a Bachelor of Science degree in Animal Science in 1996. Through contact with Dr. Wayne Singleton at Purdue, he became interested in swine reproductive physiology and continued his education by pursuing a Master of Science degree in the Department of Animal Science. His research focused on the effects of nutrition and lactation length on sow reproduction. Prior to the completion of his degree, Brad met his future wife Stacey Neuman, a graduate student pursuing a doctorate in avian reproductive physiology in the same department. After completing his master’s degree in the spring of 1999, Brad accepted a position as a swine extension research technician for Dr. Todd See in the Animal Science Department at North Carolina State University. In the fall of 1999, he began coursework and research towards a doctorate degree under the direction of Dr. Billy Flowers and Dr. Todd See. His research continued to examine factors that influence the reproductive efficiency of sows. Brad is currently looking for a position that will allow him to continue to contribute to the scientific and agribusiness communities through research and the dissemination of research findings.
ACKNOWLEDGMENTS

I would like to thank Dr. Billy Flowers and Dr. Todd See for granting me the opportunity to further my investigations in this area of research. Without their advice and support, the experiments described herein would not have been possible. In addition, I would like to thank Dr. Scott Whisnant and Dr. Glen Almond for serving on my committee and for contributing valuable insight. My gratitude is also extended to Curtis Powell and Stephen Beasley at the North Carolina State University Swine Education Unit for their swine husbandry assistance. Many thanks to my parents, Pat and Max, and to my family and friends for all of their love and support. Most of all, I would like to thank my wife Stacey, who supported my efforts with unconditional love, patience, and pragmatic advice.
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POSTWEANING ESTRUS AND OVULATION

Introduction

Reproduction in the female, unlike the male, is characterized by cyclical fertility. Females exhibit a recurrent period of receptive sexual behavior called estrus or “heat” that coincides with ovulation in most mammalian species. As early as 350 B.C., the animal castration experiments of Aristotle demonstrated that sexual behavior and secondary sex characteristics were due to the gonads. However, it was not until the 1900’s that the temporal relationship between ovarian steroid production and estrus was elucidated (review: Beach, 1948). Currently, behavioral estrus represents the only mammalian behavior whose entire neural circuitry has been mapped, at least in rodents (reviews: Pfaff et al., 1994; McCarthy and Becker, 2002). Molecular biology techniques have begun to reveal the details of how estrogen remodels the ventromedial hypothalamus (VMH) and other hypothalamic nuclei that mediate estrus (review: Flanagan-Cato, 2000).

Since the viability of oocytes and spermatozoa is relatively brief once they are released into the female reproductive tract (review: Hunter, 1994), the obvious function of estrus is to attract a male, synchronize mating with ovulation, and maximize the chance of conception. In swine breeding farms, the physical and behavioral signs associated with estrus are used to differentiate cycling, non-pregnant gilts and sows from those that are pregnant in an effort to achieve this same end goal. The receptive behavior of standing immobile in response to back pressure in the presence of a boar is the primary means used to detect estrus in gilts and sows (review: Signoret, 1970). Widespread adoption of artificial insemination (AI) for swine breeding (review: Singleton, 2001) has shifted the responsibility
of detecting estrus from boar to breeding technician. Gilts and sows in estrus need to receive at least one AI service within the 24 h period before ovulation to maximize oocyte fertilization rate and subsequent fertility (review: Kemp and Soede, 1997). Since there is no accurate, prospective indicator of the time of ovulation, repeated detection of estrus must be performed to diagnose the onset of estrus and to provide a cue for the initiation of a schedule of multiple AI services. The task of maintaining synchrony between insemination and ovulation is complicated by the considerable variation of the duration of estrus (DE) and onset of estrus-to-ovulation interval (EOI) among individual sows (reviews: Soede and Kemp, 1997; Flowers, 1998a).

This review will focus on the endocrine regulation of the postweaning estrus and ovulation in sows and on the management, environmental, and sow-specific factors that may contribute to the variation of DE and EOI among individual sows. Advances in the identification of the fundamental sources of this variation could provide pork producers with a means to refine AI protocols and increase sow reproductive efficiency.

**Ovarian Follicular Development and Estrus**

Estrogen produced by developing ovarian follicles triggers estrus and the preovulatory GnRH/LH surge that initiates ovulation. The first estrus typically occurs within 5 to 7 months of age in gilts that have received boar stimulation to induce puberty. In mature sows, estrus usually occurs within 3 to 7 days postweaning. Unlike rodents and sheep, progesterone priming is not required before estrogen administration to effectively induce an intense estrus in ovariectomized female swine (Signoret, 1969). Administration of estradiol
benzoate alone to ovariectomized gilts increased both the proportion of gilts expressing estrus and their DE in a dose-dependent manner (Signoret, 1967; Esbenshade and Huff, 1989). The time at which peripheral estradiol-17β concentrations begin to increase postweaning in sows is related to their weaning-to-estrus interval however neither the amplitude nor the duration of the estradiol-17β peak determines their DE (Rojkittikhun et al., 1992a, b, 1993; Soede et al., 1994b). Thus, estrus does not seem to be regulated simply by the amount of estrogen produced in intact females. However, since estrogen is required to trigger estrus and ovulation, an overview of the basic endocrine regulation of the growth of the ovarian follicles that produce it is warranted.

Postpartum period

Lactation in the sow is generally characterized by a period of ovarian quiescence that results in anestrus and anovulation until weaning (reviews: Edwards, 1982; Varley and Foxcroft, 1990). There is a brief, 3 to 4 day, hypergonadotropic period around parturition due to the sudden removal of the inhibitory feedback of luteal progesterone and feto-placental estrogens on the hypothalamo-hypophysial axis (review: Britt et al., 1985). Most sows exhibit an irregular, high-frequency pattern of LH secretion and an increase in basal LH during this period (De Rensis et al., 1993a). Ovarian follicles as large as 4 to 6 mm in diameter may be present on the ovaries and some estrogen may be produced, however, these follicles generally do not mature and ovulate in lactating sows (Palmer et al., 1965a; Kunavongkrit et al., 1982). An increase in suckling during the first several days postpartum induces a gradual decrease of basal levels of FSH and LH and a decrease in the size and
number of follicles through atresia (Palmer et al., 1965a, b; De Rensis et al., 1993a; Sesti and Britt, 1993a, b, 1994). Activation of an endogenous opioid peptide (EOP) system during lactation, which inhibits GnRH and LH release and stimulates prolactin secretion, is involved in this neuroendocrine feedback loop (reviews: Barb et al., 1991; Foxcroft, 1992). However, factors other than EOP may be more important initially postpartum, since naloxone failed to block the initial suppression of LH secretion at 2 to 3 days postpartum in lactating sows (De Rensis et al., 1993b; 1998). In addition, an ovarian factor (i.e. inhibin) may be more important to the inhibition of FSH secretion than suckling, since ovariectomy during lactation results in an increase in FSH but not LH secretion (Stevenson et al., 1981). Regardless of the specifics of the mechanism, it is clear that sows progress from a hypergonadotropic to a hypogonadotropic state during the first week postpartum.

The second week of lactation can be considered a transitional period. Even though peripheral concentrations of gonadotropins and ovarian steroids are low (Ash and Heap, 1975; Stevenson and Britt, 1980), the concentrations of FSH and LH in the pituitary increase, as does the ability of the hypothalamus to release GnRH (Sesti and Britt, 1993a, b, 1994). The pituitary will release LH in response to exogenous GnRH and the ovaries will develop follicles in response to exogenous gonadotropins during the first two weeks of lactation, however, the hypothalamus often fails to emit a GnRH/LH surge in response to exogenous estradiol benzoate until the third to fourth week of lactation (Cox et al., 1988; Sesti and Britt, 1993c). In contrast, exogenous estradiol benzoate can typically induce a GnRH/LH surge during the second week postpartum in sows if their litters are weaned. Apparently, the hypothalamus is refractory to the positive feedback effects of estrogen during the first two
weeks of lactation. Despite the lack of a GnRH/LH surge response to estradiol benzoate during the first two weeks of lactation, behavioral estrus can be induced throughout most of lactation (Cox et al., 1988; Sesti and Britt, 1993c). This could indicate that these two responses operate by independent hypothalamic mechanisms.

The hypothalamus gradually escapes from the inhibitory effects of the opioid tone established by suckling as lactation progresses, which may be partially due to a decrease in the intensity of suckling (Varley and Foxcroft, 1990). During the third week of lactation, pulsatile GnRH and gonadotropin secretion (Stevenson and Britt, 1980; Stevenson et al., 1981) and the size and number of follicles (Palmer et al., 1965a; Kunavongkrit et al., 1982; Sesti and Britt, 1993a) undergo a gradual increase. The magnitude of the increase of gonadotropin secretion, follicular growth, and estradiol-17β concentration during the third and fourth week of lactation is highly variable among individual sows (Stevenson and Britt, 1980; Shaw and Foxcroft, 1985; De Rensis et al., 1991). Positive correlations between follicle diameter, follicle estradiol concentration, and basal LH levels suggests that insufficient LH support limits follicle growth during this stage of lactation (De Rensis et al., 1991).

Postweaning period

Removal of the suckling stimulus for the sow at weaning causes an immediate decrease of prolactin secretion, followed by an increase of gonadotropin secretion, follicular growth, and estradiol-17β concentrations, that culminates in the induction of estrus, a preovulatory GnRH/LH surge, and ovulation (review: Einarsson et al., 1998). A rapid
increase in the concentration of GnRH in the hypothalamus and in the concentration of LH but not FSH in the anterior pituitary occurs postweaning (Cox and Britt, 1982b). These changes are paralleled by an increase in basal LH concentrations within 8 to 12 h postweaning and an increase in LH pulse frequency within 2 to 3 days postweaning that is highly variable among different sows (Cox and Britt, 1982b; Shaw and Foxcroft, 1985; Foxcroft et al., 1987). Peripheral FSH concentrations increase slightly postweaning, but the increasing follicular growth and estradiol-17β concentrations within 2 to 4 days postweaning are mainly due to the increase in basal LH levels and episodic pulses of LH (Stevenson et al., 1981; Cox and Britt, 1982a; Shaw and Foxcroft, 1985; Sesti and Britt, 1993a).

Prior to weaning, most follicles are <5 mm in diameter, but the postweaning increase in gonadotropin levels results in development of preovulatory follicles that are 8 to 10 mm in diameter within 2 to 4 days postweaning (Palmer et al., 1965a; Cox and Britt, 1982b; Dyck, 1983; Rojanasthien et al., 1987). The sustained increase of estadiol-17β secreted by the developing follicles induces estrus behavior and eventually triggers ovulation. The increasing concentration of estadiol-17β acts mainly at the hypothalamic level to inhibit GnRH and thus LH release during a negative feedback phase (0 to 54 h) until it triggers an ovulatory GnRH/LH surge during the positive feedback phase (55 to 60 h; Cox and Britt, 1982a; Ziecik et al., 1988; Britt et al., 1991). The concentration of estadiol-17β falls precipitously about 12 to 24 h before the preovulatory LH surge (Rojanansthien, 1988) and progesterone concentration increases rapidly to a peak at about 14 to 16 days post-ovulation (Ash and Heap, 1975). Interestingly, the interval from the postweaning increase of estradiol-17β to the onset of estrus was longer in sows that required ≥6 days versus ≤4 days to return
to estrus (Rojkittikhun et al., 1992a, b). This could suggest a decreased sensitivity to estrogen feedback in sows with extended weaning-to-estrus intervals. However, neither the amplitude nor the duration of the postweaning estradiol-17\(\beta\) peak was related to weaning-to-estrus intervals or DE (Rojkittikhun et al., 1992a, b, 1993; Soede et al., 1994b). In contrast, an increase in prolactin secretion during estrus has been associated with DE in gilts (Van de Wiel et al., 1981). Given the known behavioral effects of prolactin (Malven, 1993), the relationships between estrogen, prolactin, and estrus may be worthy of investigation.

One hypothesis that has been suggested is that the level of follicular development and the amount of estradiol feedback at weaning could affect the postweaning LH secretion pattern and thus, the length of the weaning-to-estrus interval (Shaw and Foxcroft, 1985). This proposal is consistent with the concept of a gradual recovery of both tonic LH secretion and the estradiol-induced GnRH/LH surge mechanism as lactation progresses. Studies that have found a significant relationship between basal LH levels and(or) LH pulse frequency in late lactation and the length of the weaning-to-estrus interval have provided support for this hypothesis (van de Wiel, 1982; Shaw and Foxcroft, 1985; Foxcroft et al., 1987; Rojanansthien, 1988; Paterson and Pearce, 1994). In addition, sows that returned to estrus in \(\leq 4\) days postweaning had a characteristic postweaning increase in basal LH levels and in pulsatile LH secretion whereas sows that returned to estrus in \(\geq 5\) days exhibited an increase in pulsatile LH secretion, but lacked a sustained increase in basal LH levels (Foxcroft, 1985; Shaw and Foxcroft; 1985). However, it should be noted that these experiments have not established causality and have only associated pre- and postweaning changes in LH secretion characteristics with the weaning-to-estrus interval. A functional link between late lactation
LH secretion differences among sows and their postweaning follicular growth and return to estrus has yet to be established (De Rensis et al., 1999).

Ultrasonography-based studies have demonstrated that the average diameter of follicles at day 2 and day 3 postweaning is inversely correlated with sow weaning-to-estrus interval (Soede et al., 1998; Knox and Rodriguez Zas, 2001; Bracken et al., 2003a). However, since there is considerable variation in average follicle diameter among sows with similar weaning-to-estrus intervals, the ability of such measurements to accurately predict the onset of estrus is unlikely. Very few studies have used ultrasonography to examine the development of ovarian follicles both during lactation and postweaning in sows. Lucy et al. (2001) suggested based on their studies that lactating sows have waves of follicular growth and hypothesized that weaning relative to the onset or end of one of these waves has a role in determining sow weaning-to-estrus interval.

In summary, even though the endocrine and follicular changes associated with a quick postweaning return to estrus have been examined, the underlying mechanisms that allow and block these changes remain largely undefined. Interactions between a large number of factors, such as season, genotype, parity, lactation length, litter size suckled, nutrient intake, lactation weight loss, health, and housing, determine sow weaning-to-estrus interval (reviews: Karlberg, 1980; Fahmy, 1981; Tubbs, 1990). Most of these factors alter the weaning-to-estrus interval through effects on gonadotropin secretion and follicular growth.
Physical and Behavioral Signs of Estrus

There are several physical signs that may be observed hours or even days before the onset of standing estrus, during the so-called “proestrus” period (Willemse and Boender, 1966). These signs of impending estrus are not always exhibited and their intensity can vary among different females, genotypes, seasons, and farms. The increasing estrogen levels two to three days prior to onset of estrus (Rojanasthien, 1988; Rojkittikhun et al., 1992a) stimulate increased blood flow and fluid retention in the reproductive tract and cause the vulva and clitoris to swell and turn red. This reddening is commonly observed in gilts (Andersson and Einarsson, 1980; Andersson et al., 1984) but it is less apparent in sows unless the vulva is parted to examine the interior (Stering et al., 1994, 1998; Sterling, 1995). Langendijk et al. (2000b) detected ovulation by transrectal ultrasonography every 4 h and found that postweaning reddening of the inner vulva in sows began and ended on average 52 and 21 h pre-ovulation, respectively. In addition, in those sows that had a reddened vulva after the onset of standing estrus in response to boar exposure, vulva reddening ended within 36 h pre- to –2 h post-ovulation (Langendijk et al., 2000b). Thus, reddening of the vulva is not an accurate predictor of ovulation by itself, though it is more useful when combined with information on standing estrus.

Clear or white mucus may drip and hang from the vulva prior to onset of estrus in sows and especially gilts. The increase in estrogen levels prior to the onset of estrus also alters the amount, consistency, conductivity, pH, and several other characteristics of the vaginal and cervical mucus (Polge, 1960; Betteridge and Raeside, 1962; Haynes, 1971). Several different electronic meters have been developed to measure the conductivity change
of vaginal (Zink and Diehl, 1984; Johnson et al., 1982; Harbison et al., 1987; Ko et al., 1989) or cervical mucus (Foote, 2002). These studies generally found no fertility advantage in sows inseminated based on a change in meter conductivity readings compared to sows inseminated based on onset of estrus as detected by boar exposure. Improvements in measuring conductivity of vaginal mucus have been made and sources of variation have been identified (Dusza et al., 1996; Řezáč et al., 2002). However, Stokhof et al. (1996) found that conductivity changes measured by one meter were not related to the time of ovulation and would have resulted in insemination at > 24 h pre-ovulation in 88% of the sows monitored. It is unlikely that any of these devices can predict the optimum time for insemination.

Changes in body temperature have also been associated with onset of estrus in sows (Godrie et al., 2001). Junge-Wentrup and Holtz (1984) reported that vaginal temperature increased slightly during estrus in sows. Geers et al. (1997) reported that ear base temperature, rectal temperature, and peripheral estradiol-17β concentrations increased two days prior to onset of estrus in sows. However, Henne (1991) failed to detect a consistent change in rectal temperature in relation to estrus and Soede et al. (1997) failed to detect a consistent change in vaginal temperature in relation to estrus or ovulation. Collectively, these experiments indicate that a change in body temperature cannot be consistently associated with estrus or ovulation in sows, even when temperature is monitored continuously.

Beach (1976) suggested that the behavioral and physical characteristics that define estrus could be categorized based on their involvement in female attractiveness, proceptivity, or receptivity towards males. In swine breeding, receptive behavior is currently used to
diagnose estrus and it is measured by applying the back pressure test (BPT) to gilts and sows in the presence of a boar (Willemse and Boender, 1966; Signoret, 1970, 1971). Immobilization in response to the BPT, referred to as the “standing reflex”, confirms that a given female is in estrus. However, in some cases, females can exhibit physical signs of impending estrus and ovulate without exhibiting standing estrus (Eliasson, 1991). The occurrence of such “silent heats” is thought to be rare and it is probably more common for estrus to be missed due to insufficient boar stimulation or some other human error. Even though the BPT is subjective and false-negatives can occur, it is currently the most accurate method of detecting estrus available.

Proceptive behaviors, such as searching for the boar, staying close to the boar, and presenting in front of the boar, tend to reach maximum intensity during the middle of the receptive period (Signoret, 1970; de Jonge et al., 1994). An electronic estrous detection system that continuously records the frequency and duration of visits that individual group housed females make to a “viewing hole” on an adjacent solid-walled pen containing a boar has been developed based on this association between proceptive and receptive behavior (Bressers et al., 1991). An electronic infra-red sensor system has also been used to monitor the increase of activity of individually housed sows during the receptive period in attempt to detect the postweaning return to estrus (Freson et al., 1998). Such systems may someday replace the current labor-intensive process of detection of estrus by the BPT if they can produce more accurate estimates of the onset of estrus.
**Temporal Relationship Between Estrus and Ovulation**

Over 50 year ago, it was recognized that EOI was positively correlated with DE in swine. Burger (1952) noted that in two breeds that had a 15 h difference in average DE (48 vs. 63 h), the breed with the shorter DE also ovulated approximately 20 h earlier than the breed with the longer DE (EOI, 27 vs. 48 h). Estimates of when sows had ovulated had to be made after slaughter (Burger, 1952), surgery (Signoret, 1972), or progesterone assay (Helmond et al., 1986) until transabdominal real-time ultrasonography was applied to more accurately diagnose ovulation in sows (Weitze et al., 1989). Since then, transabdominal and transrectal ultrasonography techniques have been used to study a number of ovarian and uterine processes related to reproduction in swine (reviews: Kähn, 1994; Soede et al., 1994a; Knox and Althouse, 1999; Waberski et al., 1999). Ovulation of the 15 to 25 follicles that develop on the ovaries of swine during estrus can take 2 to 4 h to complete (Soede et al., 1992). However, since the frequency of ovarian examination in most studies is ≥ 4 h, ovulation will be considered to occur at a point in time in this review for the sake of simplicity. Within the last 15 years, ultrasonography-based research has confirmed that DE and EOI are temporally linked, as Burger (1952) had suggested. Sows tend to ovulate at approximately 70% of their estrus, regardless of its duration (Soede and Kemp, 1997). However, these studies also contradicted earlier research that suggested EOI was relatively constant because they revealed considerable variation of DE, EOI, and even the percentage of DE at which ovulation occurs (EOI/DE, %) among individual gilts and sows (Table 1). Weaning-to-estrus interval differences explain a significant portion of the variation of DE and EOI among individual sows (Weitze et al., 1994; Soede and Kemp, 1996).
Table 1. Mean (± Std. Dev.) and range of the duration of estrus (DE), onset of estrus-to-ovulation interval (EOI), and percentage of duration of estrus at which ovulation occurred (EOI/DE, %) in sows and gilts from published literature

<table>
<thead>
<tr>
<th>Reference</th>
<th>DE (h)</th>
<th>EOI (h)</th>
<th>EOI/DE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N Exam intervals</td>
<td>Mean ± Std. Dev.</td>
<td>Range</td>
</tr>
<tr>
<td><strong>Sows</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soede et al., 1992</td>
<td>13</td>
<td>8 h</td>
<td>71.6 ± NR</td>
</tr>
<tr>
<td>Weitze et al., 1994</td>
<td>483</td>
<td>7, 7, 10 h</td>
<td>59.6 ± 14.8</td>
</tr>
<tr>
<td>Soede et al., 1994b</td>
<td>16</td>
<td>4 h</td>
<td>54.0 ± 15.0</td>
</tr>
<tr>
<td>Soede et al., 1995a</td>
<td>151</td>
<td>8 h</td>
<td>50.0 ± 13.0</td>
</tr>
<tr>
<td>Soede et al., 1995b</td>
<td>91</td>
<td>8 h</td>
<td>60.0 ± 11.0</td>
</tr>
<tr>
<td>Mburu et al., 1995</td>
<td>15</td>
<td>4 h</td>
<td>56.0 ± 07.9</td>
</tr>
<tr>
<td>Dalin et al., 1995</td>
<td>15</td>
<td>4 h</td>
<td>47.0 ± 09.2</td>
</tr>
<tr>
<td>Stokhof et al., 1996*</td>
<td>44</td>
<td>8 h</td>
<td>57.0 ± 11.0</td>
</tr>
<tr>
<td>Nissen et al., 1997</td>
<td>91</td>
<td>8 h</td>
<td>60.0 ± 14.0</td>
</tr>
<tr>
<td>Steverink et al., 1997</td>
<td>115</td>
<td>8 h</td>
<td>59.0 ± 12.0</td>
</tr>
<tr>
<td>Chapter 2</td>
<td>501</td>
<td>6 h</td>
<td>55.5 ± 12.0</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
<td></td>
<td>57.2</td>
</tr>
</tbody>
</table>

| **Gilts**          |        |         |            |        |         |         |             |        |
|                    | N Exam intervals | Mean ± Std. Dev. | Range | N Exam intervals | Mean ± Std. Dev. | Range | Mean ± Std. Dev. | Range |
| Weitze et al., 1990| 42     | 10, 14 h | 50.0 ± NR | 36 to 60 | 42     | 10, 14 h | 41.8 ± 08.4 | 23 to 48 | 83.6 ± NR | NR |
| Almeida et al., 2000| 92   | 6 h     | 52.6 ± 08.6 | 30 to 72 | 92     | 6 h      | 43.9 ± 06.2 | 30 to 60 | 85.7 ± 13.8 | 60 to 138 |
| Bracken et al., 2003b| 59   | 12 h    | 58.7 ± NR | NR        | 59     | 6 h      | 33.5 ± 12.3 | NR        | 57.1 ± NR | NR |
| **Average**        |        |         | 53.8       | 39.7     |        |         | 75.5       |        |

Abbreviations: N, number of observations; NR, not reported.
Note: exam intervals refer to the frequency of detection of estrus and ovulation; and in cases where ± NR appears the mean was calculated from other available data in that row.
*Data based on cycling sows, whereas other studies based on postweaning estrus and ovulation of sows.
Factors Affecting Duration of Estrus and Onset of Estrus-to-Ovulation Interval

Intensity and frequency of boar stimuli

A large amount of research was conducted that demonstrates an effect of the intensity and frequency of boar contact on the timing and expression of estrus in gilts and sows (review: Hemsworth and Barnett, 1990). Most of this research ignored the potential effect of boar stimuli on DE and EOI. Even though estrus is measured as a definitive “yes” or “no” at each heat check, the onset and end of estrus is a gradual transition that occurs over several hours. The amount of boar stimuli required to elicit the standing reflex decreases as females approach estrus and increases as estrus ends. For this reason, an increase in the intensity of boar stimulation can lengthen the duration of estrus. For example, Langendijk et al. (2000a) exposed multiparous sows to four increasing levels of postweaning boar stimulation: the BPT without a boar (man-estrus); a boar without the BPT (spontaneous-estrus); the BPT with a boar (boar-estrus); and the BPT with the sow surrounded by four boars (detection-mating area-estrus, DMA-estrus). Exposure to these four increasing levels of boar stimulation every 8 h resulted in DE estimates of 22, 29, 42, and 55 h, respectively. Increased intensity of boar stimulation has also been shown to increase the DE in cycling gilts as well (Hemsworth et al., 1984; Jongman et al., 1996). The olfactory and tactile components of boar stimuli provided to females during close contact are particularly important to inducing expression of estrus (Signoret, 1970).

An increase of the frequency at which sows received postweaning boar exposure to detect estrus has been reported to increase the DE (Knox et al., 2002). In this experiment, beginning on day 3 postweaning, crate-housed sows received exposure to a boar in the aisle
either once, twice, or three times daily (i.e. every 24, 12, or 8 h) to detect estrus by the BPT. The frequency of boar exposure failed to affect weaning-to-estrus interval or EOI, as determined by transrectal ultrasonography every 8 h. However, the DE was shorter for the once daily compared to the twice and three times daily boar exposure treatments (47 vs. 60 and 67 h, respectively). This difference was most likely due to the greater inaccuracy of estimating DE based on examinations every 24 h as compared to every 12 or 8 h, although Knox et al. (2002) argued that the amount of boar stimuli received may have been responsible.

Even though an increased frequency of postweaning boar exposure did not reduce the weaning to estrus interval (Knox et al., 2002), other experiments have demonstrated that a lack of postweaning boar exposure increases the weaning-to-estrus interval (Walton, 1986; Pearce and Pearce, 1992), and therefore, could decrease DE and EOI. There is also evidence that indicates constant postweaning boar exposure can reduce DE, presumably due to habituation of sows to boar stimuli (Dyck, 1988). These studies have led to the recommendation that boars should be housed away from sows when not in use and that sows should receive at least daily boar stimulation postweaning to reduce the weaning-to-estrus interval. It is also noteworthy that sows exhibit some preference for particular boars (Tanida et al., 1991; de Jonge et al., 1994) and that different individual boars may provide different levels of sexual stimuli (Hughes et al., 1985). Thus, the particular boar used to induce and detect estrus could alter the DE.

In addition, it is clear that the exposure of gilts and sows in estrus to a boar induces oxytocin (Langendijk et al., 2003) and prolactin (Prunier et al., 1987) release, which could
influence their DE and EOI (review: Soede, 1993). Reports that natural mating could advance ovulation (Signoret, 1972) lead to the discovery that components of boar seminal plasma can reduce EOI by 8 to 14 h (review: Waberski, 1997). Thus, the potential exists for DE and EOI to be altered by endocrine changes induced by boar exposure or by local changes induced by exposure of the uterus to boar seminal plasma.

Weaning-to-estrus interval

Weaning-to-estrus interval explains more of the postweaning variation of DE and EOI among sows than any other factor identified to date (Soede and Kemp, 1997). Roj Kittikhun et al. (1992a) reported that as sow weaning-to-estrus interval increased from $\leq 4$ to $\geq 6$ days, the duration of proestrus symptoms increased and the duration of standing estrus decreased based on twice daily observation. Shortly thereafter, more frequent detection of estrus was combined with real-time ultrasonography to examine the temporal relationship between weaning-to-estrus interval and subsequent DE and EOI. Weitze et al. (1994) observed a decrease of 10 h for DE and 7 h for EOI for each day that weaning-to-estrus interval increased from 3 to 5 days and then an increase of 6 h for DE and 5 h for EOI as weaning-to-estrus interval increased from 5 to 6 days. In contrast, Kemp and Soede (1996) reported a consistent decrease of 8 h for DE and 5 h for EOI for each day that weaning-to-estrus interval increased from 3 to 6 days. Steverink et al. (1999) analyzed several months of DE records data based on twice daily detection of estrus in 55 farms and found a decrease of 5 h for DE for each day that weaning-to-estrus interval increased from 4 to 6 days. Collectively, these studies indicate that there is generally a considerable decrease of DE and
EOI for each day that weaning-to-estrus interval increases from 3 to 6 days. However, it is also clear from these reports, and others (Knox and Rodriguez Zas, 2001; Knox et al., 2002), that the range of weaning-to-estrus interval over which a significant stepwise decrease of DE and EOI is maintained is farm dependent. Indeed, there was a farm × weaning-to-estrus interval interaction in the study by Steverink et al. (1999), primarily due to the absence of a significant negative correlation between weaning-to-estrus interval and DE on 11 of the 55 farms (20%) that were surveyed. Apparently, unidentified farm specific factors can influence the range over which weaning-to-estrus interval has a significant inverse relationship with DE and EOI.

**Lactation length**

Due to the gradual recovery of hypothalamo-hypophysial axis from the inhibitory effects of suckling during lactation, there is generally an inverse relationship between lactation length and the subsequent weaning-to-estrus interval, especially at lactation lengths < 21 days (Mabry et al., 1996). Thus, sows that lactate for a short (e.g. < 21 days) versus a long period (e.g. > 21 days) tend to have a shorter DE and EOI due to their longer weaning-to-estrus interval. However, there is some evidence that there is an effect of lactation length on DE and EOI that is not related to weaning-to-estrus interval. Estimates of ovulation time based on twice daily ultrasonography exams of mixed parity sows in the Midwestern US suggested that EOI decreased as lactation length increased from ≤ 16 to ≥ 32 days, though there were no significant differences among the individual lactation length classes (Knox and Rodriguez Zas, 2001). In addition, Willis et al. (2003) recently reported that sows that
lactated for 14 versus 24 days tended to have a longer DE (52 vs. 46 h), despite their greater weaning-to-estrus interval (5.0 vs. 4.7 d), based on twice daily detection of estrus. However, estimates of DE based on detection of estrus twice daily (Corrêa et al., 2002) and three times daily (Lucia et al., 1999) on a Brazilian sow farm were not different across lactation lengths of 8 to 20 days and 14 to 23 days, respectively. In general, the published studies on the effect of lactation length on DE and EOI are contradictory and difficult to compare due to differences in the range of lactation lengths examined.

**Parity**

There is also generally a negative relationship between parity and the subsequent weaning-to-estrus interval (Koketsu and Dial, 1997). Thus, parity 1 and 2 sows should tend to exhibit a shorter DE and EOI compared to parity ≥ 3 sows due to their comparatively longer weaning-to-estrus intervals. However, even after correction for this weaning-to-estrus interval difference, Steverink et al. (1997) found that the DE of parity 1 and 2 sows was shorter than that of parity ≥ 3 sows (55 vs. 62 h) based on detection of estrus every 8 h. Data from the study by Weitze et al. (1994) suggest that parity 1 sows had a shorter DE and EOI compared to parity 2 to 4 sows, especially during the summer as compared to the spring. However, since only parity 1 sows were treated with equine chorionic gonadotropin in their study, the effect of parity and hormone treatment cannot be separated. In contrast, several other recent studies failed to detect an effect of parity on DE or EOI (Knox and Rodriguez Zas, 2001; Knox et al., 2002; Corrêa et al., 2002). Due to these contradictory reports, it is
not possible to determine whether or not there is an effect of parity on DE and EOI irrespective of weaning-to-estrus interval.

The limited available data on successive estrous cycles in gilts indicates that while the duration of proestrus symptoms may decrease, the duration of standing estrus remains relatively constant as the number of estrous cycles increases (Signoret, 1967; Andersson and Einarsson, 1980; Andersson et al., 1984). Information of the repeatability of postweaning DE and EOI across several parities is scarce (Weitze, 1996). However, the DE and EOI of the first postweaning estrus have been found to be quite similar to the DE and EOI of the second postweaning estrus (Sternin et al., 1994; Dalin et al., 1995; Mburu et al., 1995). If the postweaning DE and EOI of a sow across successive parities were similar, and were related to the DE and EOI that the sow exhibited over the first two to three estrous cycle as a gilt, it might be possible to predict the expected lifetime DE and EOI for individual females. However, since weaning-to-estrus interval has such a large impact on DE and EOI, and many factors influence a given sow’s weaning-to-estrus interval, it seems unlikely that DE and EOI would be highly repeatable across successive parities. In accord, Weitze (1996) found that postweaning DE and EOI were identical in approximately 30% or sows and within ± 8 h in approximately 70% of sows when their first and second or second and third parity data were compared. Sternin et al. (1998) reported little correlation between the duration of the pubertal estrus in gilts and their first postweaning estrus (r = -0.03). Therefore, it does not seem likely that the repeatability of DE and EOI across estrous cycles could be used to predict DE and EOI across subsequent parities.
Genotype

Both weaning-to-estrus interval and DE have a genetic component and are lowly heritable \( h^2 = 0.20 \); review: Rydhmer, 2000). There is some evidence that selection for increased growth rate and decreased backfat has the potential to increase the weaning-to-estrus interval and decrease the DE and the ability of gilts to show the standing reflex (Rydhmer, 2000). However, the average DE of gilts and sows from literature published during the early 1900’s (44 and 72 h, respectively; Burger, 1952) is relatively similar to the average DE of gilts and sows from literature published after 1991 (54 and 57 h, respectively; Table 1). Nonetheless, different purebreds have been reported to exhibit substantial differences in their DE: Large Black versus Large White (63 vs. 48 h; Burger 1952), Large White versus Meishan (49 vs. 60 h; Bazer et al., 1988), and Hampshire versus Landrace (42 vs. 53 h; Kopperschmidt, 2000). The negative correlation between growth and reproductive traits, and the different selection focus on these two areas in each of these purebreds is probably responsible for their different average DE. Several experiments on the relationship between insemination-to-ovulation interval and fertilization rate that used different crossbred genotypes did not detect an effect of genotype on DE or EOI (Soede et al., 1995; Kemp and Soede, 1996; Steverink et al., 1997). In theory, heterosis could result in a crossbred sow genotype with a longer DE than either of its’ purebred parents. Whether or not a longer DE would be beneficial in modern swine breeding is somewhat of a paradox. A longer DE might increase the odds that a given female is detected in estrus, but it might also result in longer insemination-to-ovulation intervals and a lower fertilization rate if AI services were not delayed. Genetic nucleus herds would provide the best environment to estimate breed
differences in DE and EOI. However, due to the private nature and strict biosecurity regulations of these commercial farms, it is difficult to gain access to one to conduct a field study.

**Season**

A seasonal reduction of sow reproductive performance during the summer and early fall months is a common occurrence (reviews: Claus and Weiler, 1985; Love et al., 1993). Weaning-to-estrus interval is typically increased during this period, which should tend to reduce DE and EOI. Instead, a longer DE has been observed during the summer versus the winter months in cycling gilts in France (Signoret, 1967) and in weaned sows in Russia (Nauk and Sekrii, 1983) and in the Netherlands (Groenland et al., unpublished, cited by Soede and Kemp, 1997). Conversely, several other studies failed to detect an effect of season on DE or EOI. Weitze et al. (1994) did not detect a significant change of postweaning DE or EOI in mixed parity sows in Germany in the spring (February to May) versus the summer (July to September) based on detection of estrus and transabdominal ultrasonography exams three times daily. Sterning (1995) reported that the DE of primiparous sows in Sweden did not differ among the four quarters of the year based on twice daily detection of estrus. Knox and Rodriguez Zas (2001) found that postweaning EOI was similar among the four quarters of the year in mixed parity sows in the Midwestern US based on twice daily detection of estrus and transrectal ultrasonography exams. Finally, Steverink et al. (1999) calculated a within farm repeatability of 86% for DE based on 3 consecutive months of twice daily detection of estrus records from 55 farms in the
Netherlands (approximately 30 sows/month/farm) collected at different times of the year. Collectively, these four studies suggest season has little affect on postweaning DE or EOI.

Even though the available data on the effect of season on postweaning DE are contradictory, there is experimental evidence that supports a seasonal effect on postweaning DE. Sows exposed to 24 h of light from day 1 postweaning to 1 day post-estrus exhibited an increased DE compared to sows exposed to 12 h of light and 12 h of darkness during the same period (96 vs. 65 h; Perera and Hacker, 1984). Exposure to this constant photoperiod did not affect postweaning serum LH, estrogen, or progesterone concentrations, suggesting that altered sensitivity to similar endocrine signals caused the increased DE (Perera and Hacker, 1984). Seasonal changes in the postweaning sensitivity of the hypothalamo-hypophysial axis to estrogen feedback have been observed in intact primiparous sows (Armstrong et al., 1986) and ovariectomized primiparous sows injected with estradiol benzoate (Cox et al., 1987). If the same hypothalamic nuclei involved in estrogen feedback and GnRH surge generation also mediate behavioral estrus in the sow, as has been demonstrated in the ewe (Blache et al., 1991), a change of DE in response to seasonal cues would be plausible. Unfortunately, the specifics of how swine interpret seasonal cues, such as the gradual 2 h increase of photoperiod that occurs during the spring and summer months, remain controversial (Love et al., 1993; Paterson and Foldes, 1994). Summer temperatures ≥ 27°C can cause heat stress, decrease lactation feed intake, and increase the weaning-to-estrus interval of sows (Armstrong et al., 1986), but these changes would be expected to cause a decrease not an increase in DE. Thus, the seasonal change of photoperiod is a more likely
explanation of the increased DE that has been observed during the summer in some studies (Signoret, 1967; Nauk and Sekrii, 1983).

**Stress**

The hormonal responses associated with stressful stimuli can reduce the proportion of gilts and sows that express estrus and the DE. Administration of the endogenous opioid agonist morphine to ovariectomized gilts reduced the duration of an estradiol benzoate induced estrus (Esbensahde and Huff, 1989). Repeated injection of intact gilts with adrenocorticotropic hormone (ACTH) delayed onset of estrus, shortened DE, blocked the LH surge, and ovulation (Liptrap, 1970; Peter and Liptrap, 1985). Injection of ovariectomized gilts with a synthetic glucocorticoid reduced the proportion exhibiting estrus in response to an estradiol benzoate injection (Esbenshade and Day, 1980; Esbenshade et al., 1983; Paterson et al., 1983). Even though ACTH and glucocorticoids can inhibit GnRH and LH secretion, the latter are not required for expression of estrus if estrogen is supplied (Esbenshade and Duff, 1989). These experiments clearly indicate that activation of the hypothalamo-hypophysial-adrenal axis can inhibit estrus, but they do not reveal the details of the mechanism.

Sows that were tethered postweaning exhibited a reduced DE and EOI compared to cohorts that were individually loose-housed postweaning (Soede et al., 1997). Group-housed sows with the lowest social rank exhibited a reduced DE compared to sows with a higher social rank within the group (Pedersen et al., 1993). Housing treatment did not affect peripheral concentrations of cortisol, estradiol-17β, progesterone, or LH in the study by
Soede et al. (1997); and social rank did not affect peripheral concentrations of cortisol, estradiol-17β, or prolactin in the study by Pedersen et al. (1993). Thus, the chronic stressors in these experiments probably acted on hypothalamic and(or) higher brain centers to alter DE. In contrast, an acute stressor, such as a brief electric shock daily (Turner, 1998a) or introduction to a boar and its pen twice daily (Turner, 1998b), stimulated a transient increase of cortisol levels but failed to affect estrus or ovulation in gilts. Therefore, based on the limited literature available, it appears that chronic but not acute stress can reduce the DE in sows.

**Farm**

Based on the factors discussed up to this point, intensity and frequency of boar stimuli, weaning-to-estrus interval, and chronic stress can clearly affect DE and EOI. Contradictory information exists on the effects of lactation length, parity, genotype, and season on DE and EOI. There may be other farm-related factors that influence DE and EOI that have not been identified. Among the 9 different sow studies summarized in table 1, average DE and EOI ranged from 47 to 72 h and 35 to 48 h, respectively. Only the retrospective study of DE records by Steverink et al. (1999) included multiple farms. These researchers reported that average DE ranged from 31 to 64 h among 55 different farms and they estimated that farm accounted for 23% of the total variation of DE.
In the oviducts of the gilt or sow, the fertile lifespan of the oocytes shed at ovulation and spermatozoa deposited at insemination is limited to approximately 8 h and 24 h, respectively (Hunter, 1994). Therefore, it is not surprising that the synchrony between insemination and ovulation can have a substantial effect on the fertilization results achieved (Kemp and Soede, 1997). Ultrasonography-based studies on the effect of the insemination-to-ovulation interval on the percentage of oocytes fertilized indicate that acceptable fertilization rates (≥ 80%) can be achieved if insemination (2 to 3 × 10⁹ spermatozoa) occurs within 24 h prior to ovulation in gilts (Waberski et al., 1994a) and sows (Soede et al., 1995a, b; Steverink et al., 1997). In another study, an even wider insemination-to-ovulation interval range of 28 h pre- to –4 h post-ovulation resulted in optimal fertilization rates, farrowing rates, and litter sizes in sows (Nissen et al., 1997). In contrast, only two studies required insemination to occur within 12 h prior to ovulation to yield acceptable fertilization rates (≥ 80%) in gilts (Waberski et al., 1994b; Bracken et al., 2003b). Taken together, these experiments indicate that at least one insemination must occur within 24 h prior to ovulation to optimize sow reproductive performance. Gilts may require a more synchronous insemination relative to ovulation than sows, but the gilt data currently available are somewhat contradictory. In addition, reduced number of spermatozoa (3.0 × 10⁹ vs. 0.5 × 10⁹; Bracken et al., 2003b), increased age of semen (< 48 h vs. > 48 h; Waberski et al., 1994a), and decreased quality of semen (fresh vs. frozen-thawed; Waberski et al., 1994b) used in the insemination dose have all increased the optimal insemination-to-ovulation interval of gilts. However, it is noteworthy that there was no effect of different insemination
doses containing $1 \times 10^9$, $3 \times 10^9$, or $6 \times 10^9$ spermatozoa on the optimum insemination-to-ovulation interval in sows (Steverink et al., 1997). Thus, the number of spermatozoa in the insemination dose apparently has little effect on the optimal insemination-to-ovulation interval unless the number of spermatozoa administered drops below $1 \times 10^9$.

Even though the study by Nissen et al. (1997) found that insemination could occur 4 h post-ovulation and still yield acceptable fertilization rates, other experiments have suggested that post-ovulation inseminations can be detrimental to sow fertility. Rozeboom et al. (1997) reported a 20% reduction of farrowing rate and 1.0 pig reduction of litter size in sows that received an AI service on the third day after onset of estrus regardless of whether or not they were in estrus compared to sows that only received an AI service each day they were in estrus for two days (1 AI or 2 AI + 1 AI vs. 1 AI or 2 AI). In addition, Kaeoket et al. (2002) found that inseminations performed 15 to 20 h post-ovulation resulted in fewer spermatozoa present in the oviducts and lower fertilization rates compared to insemination performed 20 to 15 h pre-ovulation. Despite these findings, a recent study in two large commercial sow farms in Brazil, observed no effect of one or two post-ovulation inseminations on farrowing rate or litter size (Castagna et al., 2003). It may be that an insemination that occurs post-ovulation and post-estrus, such as in the study by Rozeboom et al. (1997), is more detrimental to fertility than one that only occurs post-ovulation (Castagna et al., 2003). Nonetheless, given the fact that oocytes degenerate and become infertile by 8 h post-ovulation, it is clear that spermatozoa should be present in the oviducts prior to ovulation in the ideal situation to maximize fertilization rate. Since the time of ovulation cannot be
predicted, the only practical way to accomplish this goal is by administering multiple AI services during estrus.

The sole basis for initiation of a schedule of multiple AI services in swine breeding farms is the onset of standing estrus. Detection of estrus is typically only performed once in the morning in swine breeding farms (Singleton, 2001). Since sows can return to estrus at any time, day or night, it is obvious that sows detected in estrus each morning will have been in estrus for various lengths of time. In addition, the EOI can be expected to range from 20 to 60 h among different sows based on relatively accurate estimates obtained by frequent detection of estrus and ovarian ultrasonography (Table 1). Thus, some sows will need an immediate insemination, while in others it may be more appropriate to delay insemination. Swine breeding farms have traditionally accounted for this variation among sows by administering multiple AI services during estrus, one every 12 to 24 h. When Weitze et al. (1994) confirmed that weaning-to-estrus interval was a fairly good predictor of DE and EOI they recommended a specific AI schedule for individual sows based on their weaning-to-estrus interval (early, 3 to 4 days; normal, 5 days; or late, ≥ 6 days). The basic concept was to delay the first AI 24 h after detection of estrus in sows that returned to estrus early or normally to account for their longer EOI and administer the first AI immediately after detection of estrus in sows that returned to estrus late to account for their shorter EOI. In all sows, a second AI service, 24 or 12 h after the first, would also be administered. The extent to which this strategy has been applied in US swine breeding operations is not known, although there is some evidence that it has been applied in sow farms in the Netherlands (Steverink et al., 1999). When a similar weaning-to-estrus interval based AI strategy was
compared to a standard schedule of AI immediately after detection of estrus and every 24 h thereafter on two different farms, the effect on sow fertility and fecundity was farm and weaning-to-estrus interval specific (Flowers, 1998b). The AI schedule that was adjusted based on weaning-to-estrus interval did not improve sow reproductive performance on one farm, and actually reduced reproductive performance on the second farm in sows that returned to estrus late ($\geq 6$ days) compared to the standard AI schedule. These results are not surprising given that the weaning-to-estrus interval range over which DE and EOI exhibit a significant stepwise decrease can vary considerably among different farms. Nonetheless, adjustment of AI schedules based on weaning-to-estrus interval may be beneficial in other farms. However, the study by Flowers (1998b) suggests that estimates of the DE and EOI of sows in different weaning-to-estrus-interval categories are critical to planning appropriate AI timing adjustments.

A number of retrospective records studies have reported an inverse relationship between weaning-to-estrus interval and subsequent sow reproductive performance. Specifically, sows that returned to estrus 7 to 10 days postweaning exhibited lower farrowing rates and litter sizes than sows that returned to estrus in 3 to 6 days (Leman, 1990; Love and Wilson, 1990; Wilson and Dewey, 1993; Dewey et al., 1994; Vesseur et al., 1994; Le Cozler et al., 1997; Xue et al., 1998; Koketsu, 1999; Steverink et al., 1999; Tummaruk et al., 2000). Kemp and Soede (1996) have argued that sows that require $\geq 6$ days to return to estrus are more likely to receive post-ovulation inseminations due to their shorter EOI compared to sows that return to estrus in $\leq 5$ days postweaning. At present, it is not certain if altered insemination-to-ovulation intervals (i.e. fertilization rate), ovulation rate, or prenatal survival
rate is responsible for this relationship between weaning-to-estrus interval and subsequent reproductive performance. There are certainly many other possible explanations. Sows that exhibit a late return to estrus are more likely to be of low parity, low lactation length, and low lactation feed compared to sows that return to estrus normally (Koketsu, 1999). In addition, less detection of estrus attention is applied to sows that fail to return to estrus by day 7 postweaning because the next batch of weaned sows enters the breeding barn on that same day in farms weaning weekly. Clearly, a better understanding of the causes and consequences of different weaning-to-estrus intervals is needed.

**Statement of the Problem**

Significant costs are associated with the production and administration of insemination doses on swine breeding farms. An economically optimal insemination schedule is one that yields a high farrowing rate and litter size, and yet minimizes the number of spermatozoa and insemination doses required. One of the constraints of high reproductive performance is that insemination must occur within 24 h prior to ovulation. Detection of the onset of estrus, even when accurate, does not provide a reliable estimate of the time remaining until ovulation. An increased understanding of the fundamental factors that cause variation of the weaning-to-estrus interval, duration of estrus, and onset of estrus-to-ovulation interval among sows could lead to methods to refine insemination timing and increase sow reproductive efficiency.
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POSTPARTUM UTERINE INVOLUTION

Introduction

Tissues of the postpartum uterus must undergo a degenerative and regenerative process known as involution to return to the non-gravid state and prepare a suitable environment to establish the next pregnancy. Abrupt hormonal and mechanical signals around parturition trigger pathways that remodel the uterus through extensive turnover of the extracellular matrix (ECM) and through apoptosis and proliferation of specific cell types. Expulsion of the fetus(es) and separation of the placenta(e) from the uterine wall induces an inflammatory response. Resident macrophages are joined by neutrophils, which infiltrate the endometrium, become activated, and help mediate ECM and cellular turnover. Continued uterine contractions combined with sloughing of necrotic tissue causes a discharge of fluid and cells from the reproductive tract called “lochia”, which eventually subsides. Regeneration of the innermost layers of the uterine endometrium may begin and be completed in areas where there was no placental attachment first and then gradually in the remainder of the uterus. Degradation of key components of the ECM is an extensive and rapid process that causes a large decrease in uterine size and weight within a few days of parturition, but continued remodeling persists for a much longer period. Completion of the involution process is typically gauged by histological and(or) morphological change, but the renewed ability of the postpartum uterus to support embryonic and fetal development is arguably the ultimate measure. The later half of this review will focus exclusively on postpartum involution in the sow and its potential effects on subsequent reproduction. However, since little molecular research on the signals that stimulate and inhibit postpartum
remodeling of the uterine ECM has been conducted using sows, it was necessary to include data from rats, women, and cows in the initial sections of this review. There are a number of differences in the type of uterus and placentation among these species compared to the sow (Table 2).

Table 2. Characteristics of the uterus, placenta, postpartum uterine involution, and postpartum fertility intervals of the rat, woman, cow, and sow

<table>
<thead>
<tr>
<th>Item</th>
<th>Rat¹</th>
<th>Woman²</th>
<th>Cow³, ⁴</th>
<th>Sow³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterus</td>
<td>Duplex</td>
<td>Simplex</td>
<td>Bipartite</td>
<td>Bicornuate</td>
</tr>
<tr>
<td>Placenta</td>
<td>Hemochorial</td>
<td>Hemochorial</td>
<td>Epitheliochorial</td>
<td>Epitheliochorial</td>
</tr>
<tr>
<td>Chorioallantoic attachment</td>
<td>Discoid</td>
<td>Discoid</td>
<td>Cotyledonary</td>
<td>Diffuse</td>
</tr>
<tr>
<td>Chorioallantoic attachment</td>
<td>Deciduate</td>
<td>Deciduate</td>
<td>Semi-deciduate</td>
<td>Adeciduate</td>
</tr>
<tr>
<td>Tissue sloughing completed</td>
<td>&lt; 01 d</td>
<td>15 to 20 d</td>
<td>15 to 20 d</td>
<td>01 to 03 d</td>
</tr>
<tr>
<td>Endometrial regen. completed</td>
<td>02 to 03 d</td>
<td>20 to 25 d</td>
<td>25 to 30 d</td>
<td>10 to 15 d</td>
</tr>
<tr>
<td>Morphological change completed</td>
<td>04 to 06 d</td>
<td>30 to 35 d</td>
<td>35 to 40 d</td>
<td>20 to 25 d</td>
</tr>
<tr>
<td>Postpartum implantation blocked</td>
<td></td>
<td></td>
<td>20 d</td>
<td>05 d</td>
</tr>
<tr>
<td>Postpartum fertility impaired</td>
<td>30 d</td>
<td>03 d</td>
<td></td>
<td>20 d</td>
</tr>
</tbody>
</table>

¹ Based on data from Harkness and Harkness, 1954; Harkness and Moralee, 1956; Takamoto et al., 1998
² Based on data reviewed by Salamonsen, 2003
³ Based on data reviewed by Kiracofe, 1980
⁴ Based on completion of involution in the previously gravid horn, which occurs more slowly than in the previously non-gravid horn.

The porcine trophoblast does not invade the uterine endometrium and compromise its integrity during placentation, so only the surface epithelium and a portion of the underlying endometrium are shed postpartum and the tissue sloughing period in the sow is brief. The bovine trophoblast also does not invade the endometrium but it does form specialized cotyledons that couple with maternal caruncles that grow out of the uterine wall. Bovine placentation is normally classified as adeciduate, but it could be considered semi-deciduate since postpartum sloughing of the caruncles (i.e. parts of the endometrium) does take place. Placentation in the rat and woman is invasive and deciduate, and several layers of the endometrial stroma, beneath the surface epithelium, must be shed postpartum. Despite this
invasive attachment, uterine involution is completed in less than a week in the rat, which has a much shorter generation interval than the woman, cow, and sow, which require 4, 5, and 3 weeks, respectively, to complete involution.

An obvious question is whether or not any of these species is likely to return to estrus, ovulate, and be mated or inseminated before uterine involution is complete. Fertility data during the period of incomplete involution could not be located for rats or humans. The rat is a model of reproductive efficiency because it can ovulate and conceive during lactation and then delay the development and implantation of its embryos (i.e. embryonic diapause) until the suckling litter is weaned. In humans, a quick resumption of postpartum reproduction is not usually a priority and lactating women do not typically ovulate before 6 weeks postpartum. In contrast, dairy cows often ovulate within 20 to 30 days postpartum and sows typically ovulate within 4 to 7 days postweaning after a 14 to 22 day lactation (18 to 29 days postpartum). Thus, the current management of dairy cows and sows has created a situation where insemination could occur and embryos could enter the uterus before involution is complete. Fertility is impaired for approximately 30 days postpartum in cows and 20 days postpartum in sows. The contribution of incomplete involution to reduced reproductive performance during these periods has been suggested, but has not been verified (review: Kiracofe, 1980). A number of factors, such as nutrient intake and energy balance, parity, season, suckling, and resumption of follicular growth have been reported to affect the rate of uterine involution in cows (review: Hussain and Daniel, 1991). Parturition complications, such as dystocia and retained fetal membranes, and the development of uterine infection, can significantly retard uterine involution and decrease postpartum fertility in cows (review:
Lewis, 1997). Information on the incidence of dystocia and retained fetal membranes in the sow is scarce (Jones, 1966; van Rens and van der Lende, 2004). Though, urogenital tract infections have been associated with reduced reproductive performance in sows (Muirhead, 1986). Additional research on postpartum uterine involution is warranted given its potential impact on the subsequent reproduction of these two economically important food animal species.

**Composition of Cervical and Uterine Extracellular Matrix**

In few other mammalian tissues is the remodeling of the ECM more obviously requisite to physiological function than in the cervix and uterus. Consequently, connective tissue turnover has been studied extensively in the uterus during the menstrual cycle, estrous cycle, and gestation and in the cervix around parturition (reviews: Jeffrey, 1991; Kaidi et al., 1991b; Salamonsen, 2003). Postpartum remodeling of the ECM has received less attention even though incomplete uterine involution could be an important determinant of fertility in situations where ovulation and mating take place soon after parturition. A general overview of the different tissues and ECM components that makeup the reproductive tract is necessary before their involution can be discussed.

The uterine wall consists of three basic layers of tissue from the lumen to the outside: endometrium, a serous-type mucosa; myometrium, comprised of an inner circular layer and outer longitudinal layer of smooth muscle; and perimetrium, which is the serous peritoneal outer covering of the uterus (Bartol, 1999). A layer of columnar or cuboidal epithelial cells covers the endometrium, contacts the lumen, and can form tubular glands that branch into the
stroma. The endometrium can be further divided into three or four zones of stromal cells depending on the species. In general, there is a compact, a spongy, and a basal layer of stromal cells that contacts the myometrium (stratum compactum, stratum spongiosum, and stratum basale, respectively). The mesometrium, a portion of the broad ligament, attaches to and supports the uterus and its smooth muscle is continuous with the longitudinal layer of smooth muscle of the myometrium. The mesometrium also provides a bridge for blood vessels, lymphatics, and nerves to branch into the uterus.

The ECM of the uterus is comprised of fibril-forming molecules such as collagens type I, III, V, VI, and elastin; a network-forming collagen, type IV; and several other components including proteoglycans, and the multi-domain glycoproteins fibronectin and laminin. Collagen is the most abundant protein in mammals, constituting 25 to 30% of total body protein mass. In the uterus, collagen represents 30 to 40% of total tissue protein and in the cervix as much as 80% of total tissue protein (Jeffrey, 1991). Each collagen molecule is composed of three proline and glycine-rich, left-handed helix, polypeptide chains (α chains), which wind around each other to form a rope-like superhelix. Twenty-five distinct collagen α chains, each encoded by a separate gene, have been identified. Given that each collagen molecule is comprised of 3 α chains, in theory greater than 10,000 types of collagen could be assembled, yet only around 20 types have been identified. The bulk of uterine collagen is type I (80%) and type III (20%) and it is concentrated mainly in the myometrium (woman: Stenbäck, 1989; Borel, 1991; Kucharz, 1992; cow: Boos, 2000; Boos and Stelljes, 2000). These types of collagen are called fibrillar-forming because individual collagen molecules assemble into fibrils (10 to 300 nm diameter), which can then aggregate to form even larger,
cable-like bundles called collagen fibers (0.5 to 3.0 µm diameter). Inter- and intra-molecular crosslinks that form between lysine and hydroxylsine residues at the non-helical ends of some adjacent collagen molecules increase the tensile strength of collagen type I and type III (review: Eyre, 1984). A network-forming collagen, type IV, which is typically associated with basement membranes, is found beneath the endometrial epithelium in the basal lamina and it surrounds blood vessels and the smooth muscles cells of the myometrium, as does a fine meshwork of the fibrillar collagen type VI (Boos, 2000; Boos and Stelljes, 2000). The collagen components of the cervical and uterine ECM provide the structure to contain and support the fetal load and are extremely insoluble, stable, and resistant to cleavage by most proteolytic enzymes (Kucharz, 1992).

There is an 8 to 12-fold increase in the wet weight of the uterus during gestation that is closely paralleled by a 6 to 10-fold increase in uterine collagen content in several different mammals (Table 3). In contrast, the magnitude of the increase in the wet weight and collagen content of the cervix is much smaller and less consistent across species as compared to the uterus. Mechanical distention (i.e. stretch) causes large amounts of collagen to be deposited in the gravid uterus, particularly during late gestation, to support the rapid increase in fetal weight that occurs (rat: Harkness and Harkness 1954; Harkness and Harkness, 1956; woman: Montfort and Pérez-Tamayo, 1961; Morrione and Seifter, 1962; cow: Kaidi et al 1995; ewe: Regassa and Noakes, 2001). In fact, the number of fetuses in a uterine horn is positively correlated with the amount of collagen that has accumulated at term in rats, and only a slight increase in collagen content occurs in a horn that contains no fetuses compared to an ipsilateral horn that contains several (Harkness and Harkness, 1954; Harkness and
Harkness, 1956; Shimizu et al., 1985). Myometrial smooth muscle cells synthesize collagen (Ross and Klebanoff, 1971) and the myometrium increases in mass during gestation due to the hypertrophy and hyperplasia of these cells (Jeffrey, 1991; Kucharz, 1992). Recently, mechanical distention of rat myometrial smooth muscle cells in vitro was shown to up-regulate mRNA of the immediate early gene c-fos in a time- and strain intensity-dependent manner (Shynlova et al., 2002). Thus, the distention of pregnancy seems to be a primary signal to the cells of the uterine myometrium to proliferate and strengthen the ECM.

Table 3. Gestation and postpartum changes in the wet weight and total collagen content of the uterus and cervix in the rat, woman, cow, and sow

<table>
<thead>
<tr>
<th>Item</th>
<th>Rat 1</th>
<th>Woman 2</th>
<th>Cow 3</th>
<th>Sow 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterus wet wt.</td>
<td>0.39-2.61 g (+2.22)</td>
<td>0.1-1.3 kg (+1.2)</td>
<td>1.1-12.1 kg (+11.0)</td>
<td>0.4-2.7 kg (+2.3)</td>
</tr>
<tr>
<td>Uterus collagen</td>
<td>10.4-57.9 mg (+47.5)</td>
<td>7.0-60.0 g (+53.0)</td>
<td>40.0-525.0 g (+485.0)</td>
<td></td>
</tr>
<tr>
<td>Cervix wet wt.</td>
<td>0.08-0.15 g (+0.07)</td>
<td>0.4-1.1 kg (+0.7)</td>
<td>0.1-0.3 kg (+0.2)</td>
<td></td>
</tr>
<tr>
<td>Cervix collagen</td>
<td>3.0-5.8 mg (+2.8)</td>
<td>45.0-75.0 g (+30.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postpartum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uterus wet wt.</td>
<td>2.61-0.16 g (-2.45)</td>
<td>1.3-0.1 kg (-1.2)</td>
<td>12.1-0.9 kg (-11.2)</td>
<td>2.7-0.4 kg (-2.3)</td>
</tr>
<tr>
<td>Uterus collagen</td>
<td>57.9-6.3 mg (-51.6)</td>
<td>60.0-4.0 g (-57.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cervix wet wt.</td>
<td>0.15-0.03 g (-0.12)</td>
<td>1.1-0.4 kg (-0.7)</td>
<td>0.3-0.1 kg (-0.2)</td>
<td></td>
</tr>
<tr>
<td>Cervix collagen</td>
<td>5.8-2.1 mg (-3.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Based on data from Harkness and Harkness, 1954
2 Based on data from Pomeroy, 1960; Palmer et al., 1965a
3 Based on data from Kaidi et al., 1995; uterine wet weight and collagen content includes caruncles
4 Based on data from Morrione and Seifter, 1962

**Postpartum Remodeling of Cervical and Uterine Extracellular Matrix**

Rapid and extensive degradation of uterine collagen is the hallmark of postpartum involution in mammals (rat: Harkness and Moralee, 1956; Woessner, 1962; Shimizu and Hokano, 1988; Gunja-Smith and Woessner, 1989; woman: Morrione and Seifter, 1962; Woessner and Brewer, 1963; cow: Kaidi et al. 1991a). In fact, postpartum degradation of uterine collagen represents the fastest rate of non-pathological collagen catabolism known
(review: Harkness, 1964). The breakdown and removal of this primary component of the ECM results in a corresponding decrease in the wet weight of the uterus (Figure 1). Within 1 to 2 days postpartum in the rat, and within 4 to 6 days postpartum in the woman, cow, and sow, the wet weight of the uterus has decreased to approximately 40% of its wet weight at term. The rate of catabolism slows but continues and within 5 days postpartum in the rat, and within 25 to 35 days postpartum in the woman, cow, and sow, approximately 90% of the uterine collagen present at term has been removed (Kucharz, 1992). The wet weight of the uterus and the amount of collagen and elastin left at the completion of involution increases with each successive parity in women (Woessner and Brewer, 1963; Gunja-Smith and Woessner, 1985; Schultka et al., 1993). Though the hypothesis has not been tested, it is tempting to speculate that this accumulation could alter the uterine ECM architecture and could be related to the decreased fertility that is associated with old age and high parity in mammals.

Figure 1. Uterine wet weight by day postpartum in the rat (Harkness and Harkness, 1954), woman (Morrione and Seifter, 1962), cow (Kaidi et al., 1991b), and sow (Palmer et al., 1965a) as a percentage of uterine wet weight at term.
Phagocytosis and intracellular degradation of collagen in the endometrial stroma and myometrium was evident in early histological work on the postpartum rat uterus (Montfort and Pérez-Tamayo, 1961; Luse and Hutton, 1964; Schwarz and Güldner, 1967; Brandes and Anton, 1969; Parakkal, 1969), but the identity of the cells involved was inconsistent. Collagen fibrils in various stages of degradation had been visualized within cytoplasmic lysosomes or vacuoles in macrophages (Parakkal, 1969; Dessouky, 1971; Kaidi et al., 1991a), or in myofibroblastic interstitial cells (Luse and Hutton, 1964; Schwarz and Güldner, 1967; Nishinaka and Fukuda, 1991), and in both cell types (Inouye et al., 1983). These findings, combined with the in vitro identification of several enzymes with collagenolytic activity at acidic but not neutral pH during early involution (Woessner, 1962; Woessner and Brewer, 1963; Woessner, 1965), lead to the hypothesis that intracellular lysosomal degradation was the most likely route of collagen removal in the postpartum uterus.

However, a specific collagenase was soon isolated from rat uterine tissue explants in culture that was active under physiological conditions (i.e. near neutral pH) and was produced in synchrony with postpartum collagen degradation (Jeffrey and Gross, 1970; Jeffrey et al., 1971b; Ryan and Woessner, 1971; Woessner and Ryan, 1973). Subsequent studies focused on determining whether or not myometrial cells actually synthesized and secreted this collagenase, if it was stored intra- or extra-cellularly in a latent form, how the latent form was activated, and which collagen types it could cleave (Weeks et al., 1976; Woessner, 1977; Woessner, 1979; Afting et al., 1979; Sellers and Woessner, 1980; Halme et al., 1980; Milwidsky et al., 1982; Welgus et al., 1983; Roswit et al., 1983; Elce et al., 1984; Shimada et al., 1985; Welgus et al., 1985). Blair et al. (1986) used an antibody to rat uterine
collagenase to confirm that it was in fact present in the perinuclear region of myometrial 
smooth muscle cells for a short period postpartum but was not present in these cells during 
gestation or in non-gravid uteri (i.e. no storage). Interstitial collagenase is an extremely 
important and efficient enzyme in that it makes a single cleavage of all three collagen α 
chains at a specific locus on the collagen molecule that renders it unstable, causes it to 
denature, and leaves it susceptible to further degradation (Roswit et al., 1988; Woessner, 
1991). The current consensus is that extracellular cleavage of collagen is an essential first 
step to its rapid removal during uterine involution but, during steady state conditions of soft 
tissue turnover, intracellular degradation alone may be sufficient (Shimizu and Maekawa, 
1983; Everts et al., 1996). Even though phagocytosis and intracellular degradation of 
collagen is not the primary route of removal during involution, subsequent sections of this 
review will reveal that the myofibroblasts and macrophages involved play a role in secreting 
and activating the enzymes that degrade collagen extracellularly.

**Matrix Metalloproteinases**

Since Gross and Lapière (1962) first discovered that an enzyme in skin from a tadpole 
undergoing metamorphosis (i.e. tail resorption) could digest collagen in vitro under 
physiological conditions, the family of vertebrate matrix metalloproteinases (MMPs) has 
grown to at least 23 different members with diverse but often overlapping substrate 
specificities (Table 4). Several proteolytic enzyme classes, including the aspartate and 
cysteine proteinases (e.g. cathepsins), and the serine proteinases (e.g. plasminogen activators, 
plasmin) are involved in degradation of ECM components. However, MMPs (e.g.
collagenases, gelatinases, stromelysins) have emerged as the key mediators of ECM turnover (reviews: Woessner, 1991; Nagase, 1996; Woessner, 1998; Nagase and Woessner, 1999).

The MMPs, also called matrixins, are a family of Ca\(^{2+}\) and Zn\(^{2+}\)-dependent endopeptidases that are active near neutral pH, consist of a single polypeptide chain, and range in mass from approximately 28 to 92 kDa. From their N-terminus, all MMPs contain: a short signal or pre domain for cellular export; followed by a pro domain (≈ 80 a.a.) that contains a cysteine in a conserved PRCG(V/N)PD sequence that interacts with and blocks the active site (i.e. cysteine switch); and then a catalytic domain (≈ 170 a.a.) that contains the active site and the characteristic zinc binding motif HEXXHXXGXXH. Most MMPs are secreted locally into extracellular space as inactive zymogens that can be activated by tissue or plasma proteinases (e.g. plasminogen-activator/plasmin system) or denaturing agents, all of which destabilize the Cys-Zn\(^{2+}\) interaction in the active site. This destabilization leads to further cleavage of the pro domain by activated MMPs and often, autocatalytic cleavage that completely removes the pro domain (review: Woessner and Nagase, 2000). Both MMP-7 and MMP-26 are known as minimal domain MMPs because they are transcribed with only these three basic domains and once activated consists of only the catalytic domain. Table 3 groups the members of the MMP family based on the domains that they contain in addition to these three fundamental ones. Most of the remaining MMPs have a C-terminal hemopexin/vitronectin-like domain (≈ 210 a.a.), which is folded into a four-blade, propeller-like structure that is connected to the catalytic domain by a short hinge. This domain interacts with inhibitors and ECM components and is an absolute requisite to cleave fibrillar collagens. The gelatinases, MMP-2 and MMP-9, contain a fibronectin-like domain that
allows them to bind and cleave gelatin (i.e. denatured collagen). The transmembrane domain MMPs contain a domain at their C-terminus that anchors them in the cell membrane and a furin cleavage site on the amino-terminus end of the catalytic domain that allows the protein-processing enzymes called furins to activate them before they are secreted in some cases. Connective tissue cells and immune cells that reside in or infiltrate connective tissue can often synthesize and secrete MMPs. Substrates for many of the most recently discovered MMPs have not been defined. Overall, the ECM substrate specificity of MMPs is broad and several non-ECM substrates, such as inactive MMPs, urokinase-type plasminogen activator (uPA), and tumor necrosis factor α (TNF-α) have been identified. It is not surprising that the secretion, activation, and inhibition of MMPs are tightly regulated given their destructive potential. Both plasma (e.g. α-macroglobulins, α1-inhibitor-3) and locally produced tissue inhibitors of metalloproteinases (TIMPs) inhibit MMPs by binding and altering the catalytic domain. There are four known two-domain TIMPs (TIMP-1, 2, 3, and 4) and in addition to inhibiting MMP activation, some have the ability to stimulate cellular proliferation or apoptosis independent of their ability to block MMP activation (reviews: Woessner, 1999; Brew et al., 2000). Even though the main function of the MMPs is to simply breakdown ECM components, it is important to remember that in doing so, protein signals trapped in the ECM can be released and cryptic sites on the ECM that can affect the differentiation, proliferation, or apoptosis of surrounding cells can be exposed (review: Vu and Werb, 2000). In short, the complex and diverse architecture and functions of the ECM necessitate a large family of MMPs and TIMPs to regulate ECM turnover.
Table 4. Matrix metalloproteinase (MMP) family grouped by common domains and their known substrates

<table>
<thead>
<tr>
<th>MMP Names</th>
<th>Additional domains</th>
<th>ECM substrates</th>
<th>Enzyme precursor substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Minimal domain MMPs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP-7 Matrilysin, Pump-1</td>
<td>None</td>
<td>Col IV, X, Agg, Ela, Ent, Fib, Gel, Lam, Prg</td>
<td>MMP-1, -2, -9, uPA, TNF-α</td>
</tr>
<tr>
<td>MMP-26 Matrilysin-2, Endometase</td>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hemopexin domain MMPs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP-1 Collagenase-1, Interstitial collagenase</td>
<td>H, HP/V</td>
<td>Col I, II, III, VII, VIII, X, Agg, Gel</td>
<td>MMP-2, -9, TNF-α</td>
</tr>
<tr>
<td>MMP-3 Stromelysin-1, Transin-1</td>
<td>H, HP/V</td>
<td>Col I, II, III, IV, V, IX, X, XI, Agg, Ela, Fib, Gel, Lam, Prg</td>
<td>MMP-1, -7, -8, -9, -13, TNF-α</td>
</tr>
<tr>
<td>MMP-8 Collagenase-2, Neutrophil collagenase</td>
<td>H, HP/V</td>
<td>Col I, II, III, V, VII, VIII, X, Agg, Ela, Fib, Gel, Lam</td>
<td>MMP-2, -9, TNF-α</td>
</tr>
<tr>
<td>MMP-10 Stromelysin-2, Transin-2</td>
<td>H, HP/V</td>
<td>Col III, IV, V, Agg, Ela, Fib, Gel, Lam, Prg</td>
<td>MMP-1, -8</td>
</tr>
<tr>
<td>MMP-11 Stromelysin-3</td>
<td>H, HP/V, F</td>
<td>Agg, Fib, Lam</td>
<td></td>
</tr>
<tr>
<td>MMP-12 Metalloelastase, Macrophage elastase</td>
<td>H, HP/V</td>
<td>Col IV, Ela, Fib, Gel, Lam</td>
<td></td>
</tr>
<tr>
<td>MMP-13 Collagenase-3</td>
<td>H, HP/V</td>
<td>Col I, II, III, IV, Agg, Gel</td>
<td></td>
</tr>
<tr>
<td>MMP-18 Collagenase-4 (Xenopus)</td>
<td>H, HP/V</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP-19 RASI-1</td>
<td>H, HP/V</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP-20 Enamelysin</td>
<td>H, HP/V</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fibronectin domain MMPs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP-9 Gelatinase B, 92-kDa Gelatinase</td>
<td>H, HP, FN, CO</td>
<td>Col I, II, III, IV, V, VII, X, XIV, Agg, Ela, Fib, Gel, Lam</td>
<td>TNF-α</td>
</tr>
<tr>
<td><strong>Transmembrane Domain MMPs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP-14 MT1-MMP, MT-MMP-1</td>
<td>H, HP/V, TM, F</td>
<td>Col I, II, III, IV, Agg, Ela, Fib, Gel, Lam, Ten</td>
<td>MMP-2, -13</td>
</tr>
<tr>
<td>MMP-15 MT2-MMP, MT-MMP-2</td>
<td>H, HP/V, TM, F</td>
<td>Fib, Gel, Lam</td>
<td>MMP-2</td>
</tr>
<tr>
<td>MMP-16 MT3-MMP, MT-MMP-3</td>
<td>H, HP/V, TM, F</td>
<td></td>
<td>MMP-2</td>
</tr>
<tr>
<td>MMP-17 MT4-MMP, MT-MMP-4</td>
<td>H, HP/V, TM, F</td>
<td></td>
<td>MMP-2</td>
</tr>
<tr>
<td>MMP-21 X-MMP (Xenopus)</td>
<td>TM, F</td>
<td></td>
<td>MMP-2</td>
</tr>
<tr>
<td>MMP-22 C-MMP (Chicken)</td>
<td>TM, F</td>
<td></td>
<td>MMP-2</td>
</tr>
<tr>
<td>MMP-23 CA-MMP (Cysteine Array)</td>
<td></td>
<td></td>
<td>MMP-2</td>
</tr>
<tr>
<td>MMP-24 MT5-MMP, MT-MMP-5</td>
<td>H, HP/V, TM, F</td>
<td></td>
<td>MMP-2</td>
</tr>
<tr>
<td>MMP-25 MT6-MMP, MT-MMP-6, Leukolysin</td>
<td>H, HP/V, TM, F</td>
<td></td>
<td>MMP-2</td>
</tr>
</tbody>
</table>

Adapted from: Hulboy et al., 1997; Nagase and Woessner, 1999; Vu and Werb, 2000; Curry and Osteen, 2001.

Abbreviations: MMP = Matrix Metalloproteinase; ECM = Extracellular Matrix

Domain Abbreviations: H = Hinge; HP = Hemopexin; V = Vitronectin-like domain; F = Furin cleavage site; FN = Fibronectin type II domain; CO = Collagen-like; TM = Transmembrane-like (Note that all MMPs have Pre-, Pro-peptide, and Catalytic Domains and that only MMP-7 and MMP-26 have no additional domains).

Substrate Abbreviations: Col = Collagen; Agg = Aggrecan; Ela = Elastin; Ent = Entactin; Fib = Fibronectin; Gel = Gelatins; Lam = Laminin; Prg = Proteoglycans; Ten = Tenascin; uPA = Urokinase-type Plasminogen Activator; TNF-α = Tumor Necrosis Factor-α
Activation and Inhibition of Matrix Metalloproteinases

Female reproductive tissues are structurally dynamic by nature and the role of MMPs and TIMPs in the ovary, uterus, and mammary gland has recently been the subject of several reviews (Hulboy et al., 1997; Fata et al., 2000; Vu and Werb, 2000; Curry and Osteen, 2001). Collagenase activity was first identified in the postpartum uterus over 30 years ago (Jeffrey and Gross, 1970) but only within the last 10 years have some of the details of MMP regulation in the uterus been revealed. Since MMPs are usually secreted in an inactive form, controlling the balance between the expression of the genes for MMPs and the genes for the TIMPs that block their activation is paramount to regulation of ECM turnover. Connective tissue and immune cells that synthesize and secrete MMPs often have the ability to synthesize and secrete TIMPs as well. Steroid hormones, protein growth factors, and cytokines all seem to have significant roles in transcriptional and post-translational regulation of MMP activity, though the pathways through which their effects are mediated have only begun to be defined.

The mechanical separation of the placenta from the uterus is one of the key signals that initiate the involution process, which is inflammatory and in several ways similar to tissue repair during wound healing (Salamonsen et al., 2003). Postpartum retention of placentae has been shown to block uterine involution and the degradation of uterine collagen in the rat (Yamey, 1977; Shimizu et al., 1995). Detachment of the placenta from the uterine endometrium may stimulate activation of MMPs by releasing plasminogen activators and plasminogen into the uterine lumen which can then be activated to plasmin and attack the pro domain of inactive MMPs (Shimizu et al., 1985; Shimada et al., 1985).
Circulating levels of progesterone and estrogens in the maternal system drop abruptly around parturition due to the demise of the corpus luteum (or corpora lutea) and the expulsion of the placenta(e), which can produce these steroids in some species. Several lines of evidence suggest that progesterone and estrogen can inhibit the activity of uterine MMPs postpartum. The postpartum administration of exogenous progesterone to rats tends to cause a significant decrease in uterine collagen degradation (Halme and Woessner, 1975; Bieńkiewicz et al., 1996). The production of uterine collagenase by postpartum rat myometrial cells in vitro was inhibited by physiological concentrations of progesterone and its ability to do so was enhanced by the addition of estradiol to the culture media (Jeffrey et al., 1971a; Koob and Jeffrey, 1974; Tyree et al., 1980; Jeffrey, 1981; Jeffrey et al., 1990). In addition, physiological concentrations of progesterone have been shown to inhibit the secretion of several MMPs and plasminogen activators and stimulate the secretion of some TIMPs and a plasminogen activator inhibitor-1 (PAI-1) in cultured human endometrial explants (Marbaix et al., 1992). Putative hormone-response elements have been identified in the promoter regions of several MMP genes but there has been no evidence that progesterone acts directly at these sites (review: Fini et al., 1998). Collectively, these data suggest that the ability of progesterone to inhibit MMP activity and expression is most likely through indirect mechanisms that may even be mediated by other hormones such as cytokines and growth factors. Regardless of the mechanism, it is clear that progesterone functions to inhibit the untimely activation of MMPs in the uterus during gestation.

Estrogens are a well-known requisite for pubertal uterine growth and development. Based on the anabolic role of estrogens in the uterus, one might expect that they would
inhibit the catabolic portion of the involution process. Indeed, the postpartum administration of 100 µg to as little as 1 µg of estradiol per day to rats significantly retards uterine collagen degradation (Woessner, 1969; Ryan and Woessner, 1972, 1974) and somehow inhibits collagenase activity (Woessner, 1979; Woessner and Ryan, 1980). The inhibitory effect of progesterone on postpartum collagen catabolism is enhanced by the addition of estradiol (Jeffrey et al., 1971a; Takamoto, 1998), which is known to up-regulate the progesterone receptor. Glucocorticoids also tend to inhibit uterine collagenase production (Koob et al., 1980; Jeffrey et al., 1990). Ovariectomy can activate apoptotic pathways in uterine cells (Sato et al., 2003) and there is evidence that apoptosis contributes significantly to postpartum involution in rats (Afting and Elce, 1978; Takamoto et al., 1998) but not ewes (Gray et al., 2003). However, ovariectomy just before or just after parturition did not significantly affect the rate of collagen removal during involution in the rat (Wray, 1982; Shimizu and Hokano, 1992). Nonetheless, there is sufficient evidence to suggest that estradiol, like progesterone, can inhibit MMP activity but, there is little support for a direct effect on transcription of MMP genes.

The promoter region of all the MMP genes, with the exception of MMP-2, contain recognition sites for the AP-1 transcription factor (TRE = TPA (tetradecanoylphorbol acetate) Response Element), which consists of dimeric complexes of the *fos* and *jun* gene families (part of the larger family of bZip transcription factors). Estrogen has been shown to induce the transcription of the immediate early genes *c-jun*, *junB*, and *junD* in the rat uterus (Nephew et al., 1994). Thus, the failure of estrogen to affect the transcription of MMPs is perplexing and underscores the complexity of the hormonal regulation of MMP gene
expression. It is important to note that the \textit{jun} proteins can form heterodimers with \textit{fos} proteins or homodimers within their own family, resulting in a number of \textit{jun:fos} and \textit{jun:jun} combinations that may have different affinities for the TRE, different interactions with other transcription factors, and thus, different transcriptional activities. The complexity of regulation is further increased when you consider that there are often several other response elements present in the promoter regions of MMPs, in addition to AP-1 (Fini et al., 1998).

A number of experiments involving the culture of rat myometrial smooth muscle cells (SMC) by Jeffrey and colleagues lead to the discovery of the requirement of a positive signal for these cells to produce collagenase (MMP-13). Rat and human SMC failed to produce collagenase when the indoleamine serotonin (5-hydroxytryptamine, 5-HT) was not present in the culture media and then regained the ability to produce collagenase when serotonin was supplemented to the media (Jeffrey et al., 1991). This effect was specific to myometrial SMC, blocked by progesterone, and was mediated by the serotonin 5HT\textsubscript{2A} receptor since agonists and antagonists to this specific receptor could mimic and block the effect, respectively (Wilcox et al., 1992; Rydelek-Fitzgerald et al., 1993a, b). This serotonin-induced stimulation of collagenase expression may be mediated via protein kinase C (PKC) and the TRE-site since phorbol 12-myristate 13-acetate (PMA) could also induce serotonin depleted cells to up-regulate collagenase. As mentioned above, progesterone blocks the serotonin-induced up-regulation of the collagenase gene. In addition, the progesterone antagonist, RU-486, removes the ability of progesterone to inhibit serotonin-induced collagenase expression, which suggests that the effects of progestins in this pathway are receptor-mediated (Wilcox et al., 1994a). Serotonin has also been shown to down-regulate
the genes for fibronectin, type I collagen, type III collagen, and the proteinase inhibitor α2-macroglobulin (α2M), albeit by a pathway downstream of the serotonin 5HT2 receptor different from the one it uses to up-regulate collagenase (Passaretti et al., 1996; Huang and Jeffrey, 1998). Collectively, these experiments uncovered a portion of the signal pathway that allows uterine myometrial SMC to sense the reproductive status of the animal and regulate the turnover of the ECM around them in an appropriate manner.

While the steroids progesterone and estradiol seem to inhibit MMP activity and expression, cytokines like TNF-α, interleukin-1α (IL-1α), and IL-1β, produced by local connective tissue cells or immune cells that infiltrate the uterus stimulate the expression and activity of MMPs (Hulboy et al., 1997). The serotonin-induced induction of the collagenase gene also stimulates rat uterine myometrial SMC to transcribe the genes for IL-1α and IL-1β, but only IL-1α is required in the serotonin-induced up-regulation of collagenase (Wilcox et al., 1994b; Dumin et al., 1998). Addition of progesterone to serotonin stimulated SMC increases the rate of decay of IL-1α mRNA (Lan et al., 1999). However, the levels of IL-1α mRNA and translated IL-1α increase from day 15 to 22 of gestation in rats despite the high levels of progesterone (Melendez et al., 2001). These data suggest that serotonin-induced IL-1α mediates collagenase expression during gestation via an autocrine loop and sets up the uterus to produce collagenase postpartum. Dr. Jeffrey’s laboratory has also demonstrated that even though the AP-1 site and extended palindrome in the promoter region of the rat collagenase gene is bound by fosB, fra-2, c-jun, junB, and junD, only the binding and expression of fra-1 is serotonin-dependent (Wilcox et al., 2000). Most recently, these researchers have produced evidence that serotonin binding through its 5-HT2A receptor
activates phospholipase C (PLC) via a G-protein and the diacylglycerol (DAG) produced subsequently activates PKC (probably δ isoform), which activates the extracellular signal-regulated kinase 1/2 (ERK1/2) pathway that phosphorylates transcription proteins that then bind the AP-1 site in the promoter region of MMP-13 (Shum et al., 2002). Even though the pathways through which progesterone inhibits the serotonin and IL-1α-induced increase in postpartum MMP-13 production are currently incomplete, these experiments provide important insight into the central role of myometrial SMC in regulation of the uterine ECM and involution.

Another MMP that likely has an important role in involution is the minimal domain MMP, MMP-7 (i.e. Matrilysin), which was first discovered in early postpartum rat uterine tissue (Sellers and Woessner, 1980). Matrilysin was subsequently purified and characterized and found to cleave a wide variety of gelatins and proteoglycans (Woessner and Taplin, 1988; Woessner, 1992; Abramson et al., 1995; Woessner, 1996). The mouse homologue of MMP-7 was cloned and mRNA levels were found to be high in the uterus soon after parturition and to then fall precipitously to levels below detection by 4.5 days postpartum, which is consistent with the pattern of involution (Wilson et al., 1995, Wolf et al., 1996; Woessner, 1996). When a null mutation in the gene encoding MMP-7 or MMP-3 (i.e. Stromelysin-1) was induced in two different groups of mice, they exhibited normal estrous cycles, gestation, parturition, and uterine involution (Rudolph-Owen et al., 1997). Compared to wild-type mice however, the levels of MMP-3 and MMP-10 (i.e. Stromelysin-2) were up-regulated in the endometrial stroma of the MMP-7 deficient group and the levels of MMP-7 and MMP-10 were up-regulated in the endometrial epithelium and stroma, respectively, of
the MMP-3 deficient group. This study highlights the redundancy in important biological systems such as those involved in reproduction, which have evolved through natural selection. These results suggest that several MMPs probably mediate uterine involution and that a system is in place that can compensate for the loss of an individual MMP by up-regulating other MMPs. Matrilysin (i.e. MMP-7) is unique in that it seems to be synthesized and secreted specifically by epithelial cells, were it is localized on their apical surface in a complex with a heparan sulfate proteoglycan (i.e. CD44) and heparin-binding epidermal growth factor (HB-EGF). Activated MMP-7 is recruited by CD44 and pro-HB-EGF to from a complex in which MMP-7 cleaves and activates HB-EGF, which then binds and activates its receptor, ErbB4, leading to promotion of cell survival, among other things (Yu and Woessner, 2000; Yu et al., 2001). Interestingly, postpartum involution is accelerated in CD44 null mice and MMP-7 is found in the basal stroma of the endometrium instead of the epithelium (Yu et al., 2001). This study suggests that the docking of secreted MMPs by adhesion receptors can mediate ECM remodeling and growth factor activity.

In addition to MMP-13 and MMP-7, the expression of MMP-8 (i.e. Collagenase-2) and MMP-10 is normally at high levels in the mouse uterus soon after parturition and then falls to low or undetectable levels by 4.5 postpartum (Rudolph-Owen et al., 1997; Balbin et al., 1998). In contrast, MMP-1, MMP-2, MMP-3, MMP-11, TIMP-1, TIMP-2, and TIMP-3 remain at relatively constant levels of expression throughout postpartum uterine involution (Rudolph-Owen et al., 1997). However, this does not mean that TIMPs are not important to reproductive function. Indeed, TIMP-1 deficient female mice have been shown to exhibit reduced fertility (Nothnick, 2001). Further investigation of the role of MMP-8 and MMP-10
in involution may be warranted given their pattern of expression. However, simply knocking out each of these MMPs would likely only up-regulate others, as in the previous experiment by Rudolph-Owen et al. (1997). Even though the ECM substrate specificity of many MMPs overlaps, an alternate approach would be to target mutations to specific ECM components. Collagenases specifically cleave the peptide bonds between the residues Gly$_{775}$ and Ile$_{776}$ on the $\alpha$-1(I) chain of type I collagen, which is a major component of the uterine ECM. When Liu et al. (1995) mutated the $\alpha$-1(I) chain gene at this site in mice, the mutants developed normally, but their skin became thickened as they matured and uterine involution and fertility were impaired. Large persistent collagenous nodules developed in the uteri of these mice. Thus, Liu et al. (1995) clearly demonstrated that cleavage of type I collagen is essential to normal uterine involution and fertility.

In summary, the underlying role of multiple MMPs in the remodeling of the uterine ECM during postpartum involution is clear. Ovarian steroids and retained placenta(e) inhibit the initiation of this process. Interestingly, there is evidence that a positive signal is required for uterine myometrial SMC to produce collagenase. Since uterine involution is initially an inflammatory reaction, the cells of the immune system have an obvious role that has only begun to be investigated. Though some research on the role of the gelatinases (i.e. MMP-2, MMP-9) in prepartum remodeling of the cervix and uterus in gilts has been completed (Lenhart et al., 2001, 2002), no published work on MMP activity in the postpartum uterus of sows could be located.
Measures of Postpartum Involution in the Sow

Physical measurements of uterine weight and length and(or) histological measurements of different uterine tissues from excised reproductive tracts at different postpartum intervals have traditionally been used to assess the state of the involution process in sows (Table 5). In general, during the first 3 to 4 days postpartum, the epithelial, compact, and spongy layers of the endometrium degenerate and are sloughed off (Palmer et al., 1965b; Geissinger et al., 1980). By the 7th to 8th day postpartum, regeneration of the epithelium has begun and by 14 to 21 days postpartum it appears to be complete (Rumjancev, 1954; Palmer et al., 1965b). Even though the weight of the uterus continues to decrease until 21 to 28 days postpartum (Palmer et al., 1965a; Smidt et al., 1969), histological samples suggest the endometrium may be receptive to embryonic attachment within 7 to 14 days postpartum (Geissinger et al., 1980; Rhodes et al., 1983). The rate of uterine involution was slower in non-suckled than in suckled sows (Graves et al., 1967; Smidt et al., 1969). However, since weaning allows follicle size and estrogen levels to increase, the anabolic effects of estrogen on the uterus probably contributed to this difference.

Table 5. Postpartum intervals to complete uterine epithelial regeneration and uterine morphological reduction in sows from published literature

<table>
<thead>
<tr>
<th>Reference</th>
<th>Epithelial regeneration</th>
<th>Additional findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rumjancev, 1954</td>
<td>07 to 21 days postpartum</td>
<td>Glandular epithelial cells developed postweaning</td>
</tr>
<tr>
<td>Palmer et al., 1965b</td>
<td>07 to 21 days postpartum</td>
<td>Considerable heterogeneity among different sows</td>
</tr>
<tr>
<td>Geissinger et al., 1980</td>
<td>07 to ?? days postpartum</td>
<td>Examined location of laminin and type IV collagen</td>
</tr>
<tr>
<td>Ogawa et al., 2001</td>
<td>14 to 21 days postpartum</td>
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</table>

<table>
<thead>
<tr>
<th>Reference</th>
<th>Morphological reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmer et al., 1965a</td>
<td>Uterine weight and length increased postweaning</td>
</tr>
<tr>
<td>Graves et al., 1967</td>
<td>Involution delayed in non-suckled vs. suckled sows; weight lost by endometrium and myometrium day 0 to 5; weight lost mainly by myometrium after day 5</td>
</tr>
<tr>
<td>Smidt, et al., 1969</td>
<td>Involution delayed in non-suckled vs. suckled sows</td>
</tr>
</tbody>
</table>
Most of these conventional measures of uterine involution were not repeatable within an individual sow since they required excision of the reproductive tract. Indirect methods to monitor postpartum involution would be repeatable and would have the advantage of allowing effects of involution status on subsequent reproductive function to be examined. Transabdominal real-time ultrasonography has been used to monitor postpartum changes in uterine echotexture (Irie, 1987), uterine wall thickness (Lee et al., 2000), and uterine size (Martinat-Botté et al., 2000) in the sow. Transverse sectional images of the uterus have been obtained using ultrasonography in gilts and sows (Lee et al., 2000; Toriumi et al., 2003; Kauffold et al., 2004). However, it is difficult to obtain transverse sectional images of the uterus in sows, as compared to women and cows, because their uterus is comprised almost entirely of long, winding uterine horns (reviews: Kähn, 1999; Knox and Althouse, 1999). Martinat-Botté et al. (2000) simply measured the distance between the abdominal wall and “gut loops” (i.e. intestines) to estimate uterine size since the uterus occupies the area between these two boundaries. They reported that mean abdomen-to-gut loop distance decreased from approximately 9.5 to 4.5 cm from day 3 to 18 postpartum. Even though this technique is theoretically sound, comparison of abdomen-to-gut loop distance to postmortem uterine weight will be necessary to confirm that it is a valid measure of postpartum involution. Interestingly, abdomen-to-gut loop distance was highly correlated with postmortem uterine weight in peripubertal gilts (r = 0.88, Martinat-Botté et al., 2003). Therefore, this simple technique may be a useful postpartum involution research tool.

The cervix also undergoes postpartum involution, although at a slower rate than the uterus (cows: Morrow et al., 1969). Rectal palpation of the postpartum decrease of cervical
diameter and/or increase of cervical tone is commonly used to gauge involution status in cows (Hussain and Daniel, 1991). The cervix, a portion of the uterus, and the ovaries can also be palpated per rectum in sows. There are several reports on changes of cervical tone during the estrous cycle and postweaning (Bollwahn, 1972; Cameron, 1977; Kunavongkirt et al., 1983), but data on postpartum cervical changes in sows are scarce (Meredith, 1977). Meredith (1977) reported that the cervix was enlarged and soft immediately postpartum and that it became firmer as lactation progressed. It may be possible to make transrectal measurements of cervical diameter in sows with a small, hand-worn, electronic caliper that has been used in cows (Hindson et al., 1984; Watson, 1985; Tian and Noakes, 1991). Transrectal ultrasonography may also be useful since portions of the cervix have been visualized with this technique in sows (Kähn, 1999; Knox and Althouse, 1999). Another approach would be to attempt transvaginal measurement of intra-cervical diameter. Several research groups have used a graduated series of different diameter conical rods, called cervical sounds, to measure dilation of the cervix in gilts and sows (Zarrow et al., 1956; Smith and Nalbandov, 1958; Kertiles and Anderson; 1979). One or more of these three techniques could prove useful for measurement of postpartum cervical changes in sows, which may reflect postpartum uterine involution.

Since catabolism of the collagen that is deposited in the reproductive tract during gestation is the underlying process that allows the postpartum reduction of cervical and uterine size to occur, a biochemical marker of this process might be an ideal, indirect means to monitor involution. The non-reducible, 3-hydroxypyridinium crosslinks, hydroxylysyl pyridinoline (HP) and lysyl pyridinoline (LP), form on mature, fibrillar collagens and are
released during collagen degradation (reviews: Eyre, 1984, 1996). Current evidence indicates that pyridinium crosslinks are not metabolized and measurement of their excretion has been developed as a clinical tool to study bone catabolism in humans (review: Fraser, 1998). The fibrillar collagens that make up the uterus (i.e. type I and type III) contain significant amounts of HP and only trace amounts of LP (rat: Gunja-Smith and Woessner, 1989; woman: Gunja-Smith and Woessner, 1985; cow: Kaidi et al., 1991a). Urinary excretion of HP increased substantially during the period of postpartum uterine involution in dairy cows (Kaidi et al., 1991a; Liesegang et al., 2000) and women (Stone and Franzblau, 1995; Naylor et al., 2000). Even though the HP crosslink is not uterine-specific and is present in many different tissues (Eyre, 1996), this postpartum increase of HP excretion is temporally consistent with the rapid and extensive catabolism of uterine collagen that is known to occur (Harkness, 1964). Kaidi et al. (1991a) found that increased postpartum HP excretion coincided with a reduction of cervical diameter from approximately 5 to 2 cm in dairy cows. Postpartum excretion of HP may be an ideal means to monitor involution, but it needs to be related to postpartum uterine measures and confirmed by postmortem uterine measures in future studies to be validated.

**Postpartum Involution and Subsequent Reproduction in the Sow**

Given the inflammatory and degenerative nature of early postpartum involution, there is no doubt that some degree of involution is requisite for subsequent reproductive success (Kiracofe, 1980). The question that remains unanswered is whether or not incomplete involution is limiting sow reproductive performance under current management practices. As discussed in the previous section, histological evidence indicates that the uterus should be
receptive to embryonic attachment within 14 days postpartum and morphological evidence indicates that involution is nearly complete by 21 days postpartum. Lactation lengths among US swine breeding farms currently average approximately 18 days (USDA, 2001; PigCHAMP® Inc., 2002), so a range of 14 to 22 day lactation lengths within a farm is probably common. Based on this information, one might conclude that postpartum involution is not complete when litters are weaned in a significant percentage of sows. However, it should be noted that since sows typically do not ovulate until 4 to 7 days postweaning, embryos will not enter the uterus until 6 to 10 days postweaning, and their trophoblasts will not begin to attach to the uterine lumen until 16 to 20 days postweaning. Even if uterine involution is inhibited by the postweaning increase of follicle size and estrogen levels, sows have at least an additional 2 to 5 days postweaning to complete the involution process. Therefore, one could argue that current lactation lengths should allow enough time to complete uterine involution in most cases.

Nonetheless, a number of studies based on retrospective analysis of farm records indicate that litter size and farrowing rate are reduced substantially following lactations < 21 and < 14 days, respectively, compared to levels achieved after longer lactations (Clark and Leman, 1984; Dewey et al., 1994; Mabry et al., 1996; Koketsu and Dial, 1997; Gaustad-Aas et al., 2004). Reduction of lactation length below 21 days does increase the length and variability of the weaning-to-estrus interval, but it does not decrease ovulation or fertilization rates (Sväjgr et al., 1974; Hays et al., 1978; Marsteller et al., 1997). Increased embryo mortality, particularly between 9 and 20 days postmating, is apparently responsible for the reduced reproductive performance of sows following short lactations (Varley and Cole, 1976,
1978). Since maternal recognition of pregnancy and attachment to the uterine lumen are established during this period of embryonic development, these studies may suggest that the uterus is not completely functional after lactation lengths < 21 days. However, it should be noted that sows can in some cases reproduce successfully after lactations of only a few days (reviews: Varley 1982; Varley and Foxcroft, 1990), even though lactation lengths < 21 days clearly reduce embryo survival and sow reproductive performance.

Two other examples of increased fertility and fecundity in sows inseminated after long postweaning intervals may also suggest that uterine involution is not complete by the end of current lactation lengths. Sows that returned to estrus in ≤ 6 days postweaning had a greater farrowing rate and litter size than sows that returned to estrus 7 to 10 days postweaning in a number of retrospective records studies (Dewey et al., 1994; Vesseur et al., 1994; Le Cozler et al., 1997; Xue et al., 1998; Steverink et al., 1999; Tummaruk et al., 1999). However, it was also noted that farrowing rate and litter size were greater for sows that returned to estrus ≥ 11 days postweaning compared to those that returned 7 to 10 days postweaning in these studies. In addition, it has been reported that subsequent litter size is approximately 1.5 to 2.5 pigs greater in sows inseminated or mated at the second versus the first postweaning estrus (Love, 1979; Morrow et al., 1989; Sterning and Lundeheim, 1995). It is not clear if an increase in ovulation rate, embryo survival, or both is responsible for these phenomena (Dyck, 1974; Clowes et al., 1994; Deckert et al., 1997). If increased embryo survival is involved, these studies may suggest that a long postweaning interval to first service results in increased farrowing rate and litter size because it gives the uterus additional time to complete involution.
Statement of the Problem

Even though incomplete postpartum uterine involution has been associated with decreased embryo survival and decreased sow reproductive performance, a causal link has not been established. This is largely because most traditional measures of involution required excision of the reproductive tract and precluded any further observation of reproductive function. New indirect and repeatable techniques to monitor involution have been developed, but in most cases their validity has not been confirmed by relating them to postmortem uterine measurements. Since remodeling of the extracellular matrix is central to postpartum involution, it may be possible to use biochemical markers of this process to gauge uterine involution status. Given the lactation lengths less than 21 days currently in use, and the potential impact of incomplete involution on subsequent sow reproductive performance, research in this area is warranted. A repeatable, non-invasive technique to measure postpartum involution must be developed and validated before the effect of involution status on subsequent reproductive function can be tested.
Literature Cited


CHAPTER 2

FACTORS AFFECTING TEMPORAL RELATIONSHIPS
BETWEEN ESTRUS AND OVULATION IN COMMERCIAL SOW FARMS
Abstract

The main objective was to examine effects of season, parity, genotype, lactation length, and weaning-to-estrus interval on duration of estrus (DE) and onset of estrus-to-ovulation interval (EOI) in three sow farms. Detection of estrus and ovulation by the back pressure test and transabdominal ultrasonography, respectively, were performed every 6 h from day 2 to 10 postweaning in 535 sows (approximately 89/farm/season). The average weaning-to-estrus interval, DE, and EOI of the 501 sows that returned to estrus by day 10 postweaning were 4.6 ± 0.1 days, 55.2 ± 0.5 h, and 41.8 ± 0.5 h, respectively. Farm × season (P < 0.01), parity × season (P < 0.05), and farm × weaning-to-estrus interval (P < 0.05) interactions for DE and EOI were detected. Sows weaned in the summer had an 8 h longer (P < 0.001) DE and EOI than those weaned in the spring on farms 1 and 3. On farm 2 however, DE and EOI did not differ (P = 0.09) in sows weaned in summer versus spring. On each farm, parity 3 and ≥ 4 sows had a 4.5 h longer (P < 0.05) DE and EOI than parity 1 and 2 sows in the summer, but there were no differences (P > 0.11) in DE or EOI among parity classes in the spring. There was a linear decrease of DE (P < 0.001) and EOI (P < 0.05) as weaning-to-estrus interval increased from the 3 to the ≥ 7 day class on each farm. However, the range of weaning-to-estrus interval that exhibited a stepwise decrease of DE and EOI was narrower on farm 1 (3 to 5 days) than farms 2 and 3 (3 to 6 days). Only farms 1 and 3 had multiple genotypes. Genotype did not affect (P > 0.14) DE on either farm, but the EOI of genotype B was 4 h shorter (P < 0.05) than genotype C on farm 1. On each farm, DE decreased linearly (P < 0.01) as lactation length increased from ≤ 13 to ≥ 20 days. In general, factors that affected EOI also affected (P < 0.05) insemination-to-ovulation intervals
and the percentage of inseminations that occurred within 24 h pre- to -3 h post-ovulation. These data indicate that factors other than weaning-to-estrus interval, such as season and parity, can significantly alter DE and EOI. However, the effects of season and weaning-to-estrus interval on DE and EOI can be inconsistent among different farms.

**Introduction**

The strategy of administering 2 to 3 inseminations, one every 12 to 24 h after the detection of estrus, is standard practice in most swine breeding programs. Such multiple insemination regimens are necessary because the viability of oocytes and spermatozoa in the sow reproductive tract is relatively short (Hunter, 1994) and time of ovulation during estrus is unpredictable (Flowers, 1998). Real-time ultrasonography has revealed that sows tend to ovulate at approximately 70% of their estrus, regardless of its duration (Weitze et al., 1994; Kemp and Soede, 1996). Thus, variation in the duration of estrus is directly related to variation in the onset of estrus-to-ovulation interval. Duration of estrus has a significant inverse relationship with weaning-to-estrus interval on most sow farms (Steverink et al., 1999). However, other factors known to affect reproductive performance, such as season (Love et al., 1993), parity (Tantasuparuk et al., 2000), genotype (Rydhmer, 2000), and lactation length (Varley, 1982), may also contribute to variation in the duration of estrus among weaned sows (Soede and Kemp, 1997). An improved understanding of factors that influence the temporal relationships between estrus and ovulation could allow producers to refine insemination protocols and increase reproductive efficiency. Therefore, the objective of this study was to examine the effects of season, parity, genotype, lactation length, and
weaning-to-estrus interval on estrus, ovulation, and subsequent reproductive characteristics of weaned sows in three different commercial farms.

**Materials and Methods**

*Animals and farms*

The study was conducted on three different commercial farrow-to-wean sow farms in southeastern North Carolina. Data were collected on farms 1, 2, and 3 in March and August 2000, March and July 2002, and April and August 2002, respectively. Farm 1 was affiliated with a different management company and had a larger sow inventory than farms 2 and 3 (approximately 2,500 vs. 1,000 sows). Each farm had a tunnel-ventilated breeding barn. Farm 1 had three sow genotypes: a purebred great grandparent (A, Yorkshire); an F1 grandparent (B, ½ Yorkshire, ½ Landrace); and a three-breed cross (C, ½ Yorkshire, ¼ Landrace, ¼ Duroc). Farm 2 had only one genotype, another three-breed cross (D, ½ Yorkshire, ¼ Landrace, ¼ Chester White). Farm 3 had both three-breed crosses (C and D). On each farm, during two consecutive weeks in each season, approximately 42 lactating sows were weaned on Monday (farm 1) or Thursday (farms 2 and 3) at 07:30 h and moved to individual crates in the breeding barn. A total of 535 different sows (approximately 89/farm/season) were studied. Table 1 contains additional details on these sows, their lactation performance, and the temperature during the summer replicate on each of the three farms.
Detection of estrus and ovulation

On each farm, a mature boar was selected to detect estrus during the spring and summer replicates and, when not in use, boars were housed at least 6 m away from sows. Shortly after morning feeding, sows were checked for estrus in their crates by the back pressure test during nose-to-nose contact with the boar at 08:00 h on day 1 postweaning. From day 2 to 10 postweaning, detection of estrus was performed every 6 h, at 08:00, 14:00, 20:00 and 02:00 h. Once sows exhibited estrus, their ovaries were examined every 6 h at these same times by transabdominal real-time ultrasonography scans with an Aloka 500V equipped with a 5.0 MHz linear convex transducer (Aloka Co. Ltd., Wallingford, CT). Ultrasound transmission gel (Eco-Gel; Therquip, Greensboro, NC) was applied to the transducer and it was placed on the sow’s right flank in the inguinal fold. The urinary bladder was used as a landmark to locate an ovary and the average diameter of the three largest follicles at each scan was recorded. The criteria used to determine when ovulation had occurred were: either the disappearance of large follicles; or a substantial decrease in follicular diameter. An additional scan was performed 6 h later to confirm ovulation. The same trained technician performed all estrous checks and ultrasound scans. The times for onset or end of estrus and ovulation were considered to be 3 h prior to the estrous check and ultrasound scan, respectively, where a change occurred. All sows that had not expressed estrus by day 7 postweaning were scanned to determine their ovarian status. Sows that failed to return to estrus by day 10 postweaning were classified as anestrous.
Insemination

Weaned sows detected in estrus at 08:00 h were moved to empty crates nearby (farms 2 and 3) or in an adjacent barn (farm 1) where they were artificially inseminated according to each farm’s protocol. Sows on farm 1 were inseminated with an 80 mL dose containing $3.0 \times 10^9$ sperm (pooled semen) at 08:00 h on day 1 of estrus (AI 1) and at 08:00 and 15:00 h on day 2 of estrus (AI 2 and AI 3). Sows were inseminated with an 80 mL dose containing $2.25 \times 10^9$ sperm (pooled semen) at 08:00 h on day 1 of estrus (AI 1) and 08:00 h on day 2 of estrus (AI 2) on farm 2 and at 14:00 h on day 1 of estrus (AI 1) and 14:00 h on day 2 of estrus (AI 2) on farm 3. No additional inseminations were administered to sows in estrus beyond day 2 on any of the farms. Each farm was supplied with semen from a company boar stud, which was used within 48 h of collection. A mature boar was penned in the aisle in front of groups of 4 to 6 sows during inseminations on farm 1 whereas boars were placed into crates alongside sows during inseminations on farms 2 and 3. All three farms used Velcro breeding belts (EZ MATE, Hampshire, IL), which were fastened around the sow’s rear flank to hold the insemination catheter and dose in place for several minutes.

Gestation management

On farm 1, mated sows remained in the crates in which they were inseminated until approximately day 110 of gestation, when they were moved to farrowing rooms. Sows on farms 2 and 3 were moved from crates in the breeding barn to crates in the gestation barn at approximately day 30 postmating and then from gestation to farrowing rooms at day 110. Technicians on each of the farms performed daily estrous checks to locate open sows. In
addition, the farm manager (farm 1) or a specialized company technician (farms 2 and 3) confirmed pregnancy via transabdominal real-time ultrasonography (Ultra Scan 45 with a 3.5 MHz sector probe; Alliance Medical U.S.A., Smithville, MO) between day 25 to 30 postmating.

Statistical analyses

Effects of farm (1, 2, 3), season (spring, summer), parity (1, 2, 3, ≥ 4), genotype (A, B, C, D), lactation length (≤ 13, 14 to 15, 16 to 17, 18 to 19, ≥ 20 days), weaning-to-estrus interval (3, 4, 5, 6, 7 to 10 days), and their interactions on weaning-to-estrus interval, duration of estrus (DE), onset of estrus-to-ovulation interval (EOI), percentage of DE at which ovulation occurred (EOI/DE, %), insemination-to-ovulation intervals, total pigs born, and pigs born alive were evaluated using the GLM procedure of SAS (Snedecor and Cochran, 1967; Muller and Fetterman, 2002). Orthogonal contrasts of parity and lactation length classes were run to test for linear effects on weaning-to-estrus interval, DE, and EOI. Orthogonal contrasts of weaning-to-estrus interval classes were run to test for linear effects on DE and EOI. Parity and weaning-to-estrus interval classes were chosen based on evidence of potential physiological differences. Sow reproductive performance tends to increase over the first 3 to 4 parities (Tantasuparuk et al., 2000). Duration of estrus and EOI tend to decrease each day that weaning-to-estrus interval increases from 3 to 6 days postweaning but do not decrease further for sows that return to estrus ≥ 7 days postweaning (Soede and Kemp, 1997). Lactation length classes were chosen arbitrarily in an attempt to divide the dataset into quintiles. The percentage of weaned sows that were: in estrus at each
of the four different estrous check times; in estrus by day 7 postweaning; and in estrus by day 10 postweaning; and the percentage of inseminations in which AI occurred within 24 h pre- to -3 post-ovulation; and that percentage of inseminated sows that were: pregnant at day 25 to 30 postmating; and farrowed; were analyzed with a logistical model for categorical data using the GENMOD procedure of SAS (Koch et al., 1977; Stokes et al., 1995). The percentage of inseminations that occurred within 24 h pre- to -3 h post-ovulation was used to express data on the interval between insemination and ovulation for each AI since insemination within this interval seems to be required to achieve optimal fertilization rates (Kemp and Soede, 1997). In both analyses, independent variables and their interactions were added and removed in a stepwise manner to construct a final model. Unless stated otherwise, second-order and third-order interactions were not significant (P > 0.05). In general, farm, season, and genotype, were included in all models regardless of significance since they were chosen a priori. Effects of season, parity, genotype, and lactation length on DE and EOI reported in the results section are not due to differences in weaning-to-estrus interval since it was included as a covariate in all models except the model used for analysis of weaning-to-estrus interval as a dependent variable. Sow was the experimental unit used to estimate error. The data are expressed as least squares means (± SEM) and percentages, which were separated with F-tests and Chi-square tests, respectively. The contribution of individual independent variables to the variation associated with DE and EOI on each farm, and on all three farms, was also evaluated by forward regression using the REG procedure of SAS (Muller and Fetterman, 2002).
Results

Of the 535 weaned sows assigned to the study, 488 (91.2%) returned to estrus by day 7 postweaning and 506 (94.6%) returned to estrus by day 10 postweaning. However, 5 of these sows were excluded from the analysis because they returned to estrus abnormally early (day 1 to 2 postweaning), and 3 of the 5 had developed multiple, large, follicular cysts. The average weaning-to-estrus interval, DE, EOI, and EOI/DE for the 501 sows that returned to estrus between day 3 and 10 postweaning were 4.6 ± 0.1 days, 55.2 ± 0.5 h, 41.8 ± 0.5 h, and 75.6 ± 0.6%, respectively. Of the 34 sows that were classified as anestrus at day 10 postweaning, 29 (85.3%) had small- to medium-sized follicles (3 to 7 mm diameter) at day 7 postweaning, and based on the farms’ production records, 19 (55.9%) returned to estrus within 18.6 ± 2.0 days postweaning (range, 11 to 46 days). The remaining 15 sows were culled at various postweaning intervals without a recorded estrus.

Considerable variation of DE, EOI (Fig. 1), and EOI/DE (Fig. 2) was evident. Based on forward regression, weaning-to-estrus interval explained 67.5% of the variation of DE and 57.1% of the variation of EOI among sows from all three farms pooled (Tables 2 and 3). Season explained 23.0% of the variation of DE and 34.3% of the variation of EOI among sows from all three farms pooled. Even though weaning-to-estrus interval and season explained approximately 90% of the variation associated with DE and EOI, the \( r^2 \) of the full model for this analysis only reached 0.36 for DE and 0.28 for EOI. The relative contribution of each independent variable to the variation of DE and EOI varied considerably among the three different farms and among the six replicates (Tables 4 and 5).
**Season**

On each farm, onset of estrus in most sows occurred between 02:00 and 08:00 h (Fig. 3). The percentages of sows returning to estrus at 08:00 h in summer and spring were similar on farm 2 (P = 0.32) but were lower in summer than spring on farms 1 and 3 (P < 0.05). The percentages of weaned sows that returned to estrus by day 7 and by day 10 postweaning were lower in the summer than spring on farm 1 (P < 0.05) but not on farms 2 (P > .24) and 3 (P > 0.19; Table 6). There was a farm × season interaction (P < 0.05) for weaning-to-estrus interval since it increased during the summer versus the spring on farms 1 and 2 (P < 0.05) but not farm 3 (P = 0.81). Both DE and EOI increased approximately 8 h during the summer as compared with the spring on farms 1 and 3 (P < 0.001) but did not increase on farm 2 (P = 0.09), resulting in a farm × season interaction (P < 0.01). Consequently, insemination-to-ovulation intervals were greater during the summer that the spring on farms 1 and 3 (P < 0.01) and not different on farm 2 (P = 0.62). In addition, the percentage of inseminations in which AI 1 occurred within 24 h pre- to -3 h post-ovulation was lower in the summer than spring on farms 1 and 3 (P < 0.05) but was not different on farm 2 (P = 0.50). Season did not affect the percentage of inseminations in which AI 2 or AI 3 occurred within 24 h pre- to -3 h post-ovulation on any of the three farms (P > 0.15). The number of AI that occurred post-ovulation during the summer compared to the spring decreased (P < 0.001) on farm 1 and was not different on farms 2 and 3 (P > 0.29). In the summer compared to the spring, conception and farrowing rates were: not different on farms 1 (P > 0.23) and 2 (P > 0.08); and decreased on farm 3 (P < 0.05). Litter size did not differ by season on any of the three
farms \((P > 0.14)\) although both total born and born alive were numerically lower during the summer compared to the spring on each farm.

**Parity**

Data on the effect of parity on DE and EOI are presented by season due to a season \(\times\) parity interaction \((P < 0.05)\) and the lack of a significant farm \(\times\) parity interaction \((P = 0.35;\) Table 7). The percentages of parity 1 sows returning to estrus by day 7 and day 10 postweaning were lower \((P < 0.05)\) compared to parity \(\geq 4\) sows in the spring and summer. Weaning-to-estrus interval decreased linearly \((P < 0.05)\) as parity increased in both the spring and summer. Parity 1 sows had a longer \((P < 0.05)\) weaning-to-estrus interval than parity 3 and \(\geq 4\) sows in the spring and parity 2, 3, and \(\geq 4\) sows in the summer. Both DE and EOI increased linearly \((P < 0.001)\) with increasing parity class in the summer, but not in the spring \((P = 0.40)\). Parity 3 and \(\geq 4\) sows had an approximately 4.5 h longer \((P < 0.05)\) DE and EOI than parity 1 and 2 sows in the summer whereas there were no differences \((P > 0.11)\) among parity classes in the spring. There were no differences \((P > 0.21)\) among parity classes in conception or farrowing rates in the spring or the summer. In the spring, parity 1 sows had a decreased \((P < 0.05)\) number of total pigs born compared to parity \(\geq 4\) sows, but there were no other differences in litter size among the parity classes in the spring or summer.

**Genotype**
A genotype × farm interaction could not be estimated since genotypes were not balanced across farms (Table 8). Multiple genotypes were only present on farms 1 and 3 and there was no significant genotype × season interaction (P > 0.45) for DE or EOI on either farm. Weaning-to-estrus interval did not differ (P > 0.09) among genotypes on farm 1 and was shorter (P < 0.01) for genotype B than genotype D on farm 3 due to the greater (P < 0.001) parity of genotype B than genotype D sows. Genotype did not affect DE on farms 1 or 3 (P > 0.14). The EOI was 4 h shorter (P < 0.05) for genotype B compared to genotype C on farm 1 and EOI tended to be 3 h shorter (P = 0.06) for genotype D compared to genotype B on farm 3. Genotype only affected insemination-to-ovulation intervals on farm 1. Genotype B had shorter (P < 0.05) insemination-to-ovulations intervals and a greater (P < 0.01) number of post-ovulation AI than genotypes A and C on farm 1. The percentage of inseminations in which AI 1 occurred within 24 h pre- to -3 h post-ovulation was also greater (P < 0.05) for genotype B than genotype A or C on farm 1. The opposite was true for AI 3 on farm 1; the percentage of inseminations in which AI 3 occurred within 24 h pre- to -3 h post-ovulation was lower (P < 0.05) for genotype B than genotype A or C. On farm 3, the percentage of inseminations in which AI 1 occurred within 24 h pre- to -3 h post-ovulation was greater (P < 0.05) for genotype D than genotype B and the percentage of inseminations in which AI 2 occurred within 24 h pre- to -3 h post-ovulation was lower for genotype D than genotype B. Conception and farrowing rates did not differ (P > 0.20) among genotypes on farms 1 or 3. The number of total pigs born was lower (P < 0.05) for genotype A compared to genotype C and the number of pigs born alive was lower (P < 0.05) for genotype A compared to genotype B or C on farm 1.
Lactation length

There was no significant farm × lactation length (P = 0.25) or season × lactation length (P = 0.56) interaction for DE or EOI so data are presented across all farms and seasons (Table 9). A greater percentage (P < 0.05) of sows that lactated for ≥ 20 days returned to estrus by 7 days postweaning than sows that lactated for 14 to 15 days. Similarly, a greater percentage (P < 0.05) of sows that lactated for ≥ 20 days returned to estrus by day 10 postweaning than sows that lactated for 14 to 15 or 16 to 17 days. Consequently, weaning-to-estrus interval decreased linearly (P < 0.05) as lactation length increased. As lactation length increased from ≤ 13 to ≥ 20 days, DE decreased linearly (P < 0.01) but EOI did not decrease linearly (P = 0.09). Sows that lactated for 14 to 15 days had a longer (P < 0.01) DE than sows that lactated for 18 to 19 or ≥ 20 days and sows that lactated for ≤ 13 or 16 to 17 days had a longer (P < 0.05) DE than sows that lactated for ≥ 20 days. A consistent effect of lactation length on conception or farrowing rates was not apparent. However, both total pigs born and pigs born alive were lower (P < 0.05) for sows that lactated ≤ 13 days compared to sows that lactated for 18 to 19 or ≥ 20 days.

Weaning-to-estrus interval

Data on the effect of weaning-to-estrus interval on DE and EOI are presented by farm due to a farm × weaning-to-estrus interval interaction (P < 0.05) and the lack of a significant season × weaning-to-estrus interval interaction (P = 0.48; Table 10). There was a linear decrease of DE (P < 0.001) and EOI (P < 0.001) from the 3 day to the 7 to 10 day weaning-
to-estrus interval class on farms 2 and 3. There was also a linear decrease of DE (P < 0.001) and EOI (P < 0.05) from the 3 day to the 7 to 10 day weaning-to-estrus interval class on farm 1. However, as weaning-to-estrus interval increased, the stepwise decrease of DE and EOI was less consistent and covered a narrower weaning-to-estrus interval range on farm 1 (3 to 5 days) than farms 2 and 3 (3 to 6 days). In general, as weaning-to-estrus interval increased on farms 2 and 3, insemination-to-ovulation intervals decreased (P < 0.05) and the number of post-ovulation AI increased (P < 0.05). In addition, the percentages of inseminations that occurred within 24 h pre- to -3 h post-ovulation increased (P < 0.05) for AI 1 and decreased (P < 0.05) for AI 2 as weaning-to-estrus interval increased on farms 2 and 3. On farm 1, there were differences (P < 0.05) among weaning-to-estrus interval classes in insemination-to-ovulation intervals and the percentage of inseminations that occurred within 24 h pre- to -3 h post ovulation, but a consistent pattern, such as on farms 2 and 3, was not evident. There were not enough sows in the 3, 6, and 7 to 10 day weaning-to-estrus interval classes to estimate differences in reproductive performance among the different weaning-to-estrus interval classes within farms. When data from all three farms were pooled, sows in the 3, 4, and 5 day weaning-to-estrus interval classes had a greater (P < 0.05) farrowing rate and a similar (P > 0.40) number of pigs born alive compared to sows in the 7 to 10 day weaning-to-estrus interval class (Fig. 4).

Insemination-to-ovulation interval and subsequent fertility

The percentages of inseminated sows that did not receive an AI within 24 h pre- to -3 h post-ovulation on farms 1, 2, and 3, were 9.3%, 4.1%, and 2.4%, respectively. Sows from
all three farms that received 0, 1, or 2 inseminations within 24 h pre- to -3 h post-ovulation exhibited no differences (P > 0.41) in subsequent farrowing rate or number of pigs born alive (Fig. 5). Regardless of how sows were categorized by insemination-to-ovulation interval (insemination-to-ovulation interval of last pre-ovulation insemination; average insemination-to-ovulation interval of 2 to 3 inseminations), there were no differences (P > 0.12) in total pigs born or pigs born alive among the insemination-to-ovulation interval classes (data not shown).

**Discussion**

In theory, an insemination protocol that consistently introduces a sufficient number of viable spermatozoa into each estrous sow’s uterus within 24 h prior to ovulation should optimize herd reproductive performance. In practice, this task cannot be achieved without multiple inseminations due to the substantial and unpredictable variation of DE and EOI among weaned sows (Flowers, 1998). The mean DE, EOI, and EOI/DE in the 501 sows that returned to estrus between day 3 and 10 postweaning in the present study (55 h, 42 h, and 76%, respectively) were similar to other published estimates of these reproductive parameters (55 h, 41 h, and 68%, respectively; Soede and Kemp, 1997). However, the variation associated with mean DE and EOI is more relevant to insemination strategy. For example, EOI had a range of at least 18 h to 72 h on farm 1, 24 h to 60 h on farm 2, and 18 h to 66 h on farm 3. Since these ranges span more than 24 h (54 h, 36 h, and 48 h, respectively), it is clear that no single timed-insemination could have consistently occurred near ovulation. The primary goal of this study was to identify factors that contribute to this variation of EOI and
DE among weaned sows within a farm. It is reasonable to speculate that such information might be useful for improving insemination protocols and reproductive efficiency.

Weaning-to-estrus interval has already been identified as a key determinant of DE and EOI in sows (Weitze et al., 1994; Kemp and Soede, 1996). In general, both DE and EOI tend to decrease as weaning-to-estrus interval increases from 3 to 6 days (Soede and Kemp, 1997). However, both the literature and the results of this study indicate that the range of weaning-to-estrus interval over which a stepwise decrease of DE and EOI is maintained is farm dependent. Specifically, Kemp and Soede (1996) reported a decrease of 7.7 h for DE and 4.7 h for EOI for each day that weaning-to-estrus interval increased from 3 to 6 days. In contrast, Weitze et al. (1994) observed a decrease of 10.4 h for DE and 7.2 h for EOI for each day that weaning-to-estrus interval increased from 3 to 5 days and then an increase of 6.2 h for DE and 4.9 h for EOI as weaning-to-estrus interval increased from 5 to 6 days. In the present study, the same sort of difference between farms in the range of weaning-to-estrus interval that exhibited a stepwise decrease of DE and EOI resulted in a farm × weaning-to-estrus interval interaction. There was a decrease of approximately 6.0 h for DE and 4.8 h for EOI for each day that weaning-to-estrus interval increased from 3 to 6 days on farms 2 and 3, similar to the findings of Kemp and Soede (1996). In contrast, on farm 1, there was a decrease of 6.1 h for DE and 3.9 h for EOI for each day that weaning-to-estrus interval increased from 3 to 5 days and then an increase of 3.7 h for DE and 4.5 h for EOI as weaning-to-estrus interval increased from 5 to 6 days, similar to the findings of Weitze et al. (1994). A study based on retrospective analysis of DE records also found a farm × weaning-to-estrus interval interaction; there was no significant negative correlation between weaning-
to-estrus interval and DE on 11 of 54 farms (20%) that were surveyed (Steverink et al., 1999). Collectively, these data indicate that farm specific factors can influence the range over which weaning-to-estrus interval has an inverse relationship with DE and EOI. In the present study, the nutrition program, herd size, internal breeding barn layout, sow genetics source, and company that supervised management differed for farm 1 as compared to farms 2 and 3, which were very similar. Results from the present study and the literature also indicate that the range of weaning-to-estrus interval over which DE and EOI may exhibit a significant stepwise decrease is restricted to 3 to 6 days, since DE and EOI did not decrease further as weaning-to-estrus interval increased from 6 to ≥ 7 days.

Seasonal effects on sow reproductive performance have been well documented (Love et al., 1993), but data on the effect of season on DE and EOI are limited (Soede and Kemp, 1997). Results of the present study reveal that season can significantly alter DE and EOI irrespective of weaning-to-estrus interval. Both DE and EOI increased 8 h between the spring and summer replicates on farms 1 and 3, but not on farm 2, resulting in a farm × season interaction. Since the facility design and company that supervised the management of farms 2 and 3 were the same, and the data were collected on each farm during the same year, it is difficult to ascertain why farm 3 but not farm 2 exhibited a seasonal shift in DE and EOI. This effect was not confined to a specific year since data on farms 1 and 3, which both exhibited a seasonal shift, were collected during 2000 and 2002, respectively. In addition, technician and boar effects can be excluded since the same trained technician collected all the data and used the same boar in the spring and the summer on each farm to detect estrus. One difference between the data from farm 2 versus farms 1 and 3 is that summer data were
collected earlier on farm 2, in July instead of August. It is tempting to speculate that the lack of a seasonal shift in DE and EOI on farm 2 was due to this difference, but it is also possible that unidentified farm specific factors were responsible.

There is some evidence that seasonal changes in the environment can cause a shift in estrous behavior. A longer DE during the summer versus the winter months has been reported in cycling gilts (Signoret, 1967) and weaned sows (Nauk and Sekrii, 1983). An increasing photoperiod may be the cue for this seasonal change since sows exposed to 24 h of light versus 12 h of light and 12 h of darkness from day 1 postweaning to 1 day post-estrus had an increased DE (96 vs. 65 h; Perera and Hacker, 1984). Exposure to this constant photoperiod did not affect postweaning serum LH, estrogen, or progesterone concentrations, suggesting that altered sensitivity to similar endocrine signals caused the increased DE (Perera and Hacker, 1984). Seasonal changes in the postweaning sensitivity of the hypothalamo-hypophysial axis to estrogen feedback have been observed in intact primiparous sows (Armstrong et al., 1986) and ovariectomized primiparous sows injected with estradiol benzoate (Cox et al., 1987). In addition, some of the same hypothalamic nuclei involved in estrogen feedback and GnRH surge generation may also mediate behavioral estrus in the sow, as has been demonstrated in the ewe (Blache et al., 1991). Therefore, it is possible that seasonal cues alter hypothalamic behavioral estrus centers that ultimately affect the DE.

In the present study, all three farms had curtain-sided breeding barns and lights were turned on at 06:00 h and off around 16:00 h each day. Based on this schedule and local sunrise-sunset data, weaned sows were exposed to approximately 12 h of light in the spring
and 14 h of light in the summer. It is not known if a gradual 2 h increase in photoperiod could alter DE since the specifics of how swine interpret changes in photoperiod are controversial (Love et al., 1993; Paterson and Foldes, 1994). Daily high temperatures in the breeding barns of the three farms during the study averaged 21.7°C in the spring and 28.5°C in the summer. Temperatures ≥ 27°C can cause heat stress, decrease lactation feed intake, and increase the weaning-to-estrus interval of sows (Armstrong et al., 1986), but these changes would be expected to cause a decrease not an increase in DE (Soede and Kemp, 1997). Overall, it is not clear how a relatively small increase in photoperiod and temperature could result in such a large increase of DE. Furthermore, environmental conditions were apparently similar on farms 2 and 3, which were separated by only 13 km, and yet season affected DE and EOI on farm 3 but not farm 2. Regardless of the cause, the results of this study confirm reports that DE can increase substantially during the summer on some sow farms (Signoret, 1967; Nauk and Sekrii, 1983).

Several other more recent studies did not detect a significant effect of season on the DE (Sterning, 1995; Steverink et al., 1999) or EOI (Knox and Rodriguez Zas, 2001) of weaned sows. Sterning (1995) reported that the DE of 189 weaned primiparous sows in Sweden did not differ between the four quarters of the year based on twice daily detection of estrus. Steverink et al. (1999) analyzed several months of twice daily estrous detection data from 54 farms in the Netherlands and calculated a within farm repeatability of 86% for DE based on 3 consecutive months of data on approximately 30 sows/month/farm. Results of the present experiment do not exclude the possibility that DE within a farm may be quite consistent from month-to-month but instead suggest that a considerable shift in DE can occur
over a longer period (5-month span) on some farms. Knox and Rodriguez Zas (2001) used twice daily detection of estrus and transrectal ultrasound examination to estimate the time of ovulation in 174 weaned sows of mixed parity in the Midwestern US and found that EOI was similar between the four quarters of the year but did not estimate DE. In general, estimates of DE and EOI in these studies were based on morning (08:00 to 10:00 h) and afternoon (14:00 to 16:00 h) estrous checks and ultrasound scans of sows resulting in uneven intervals between examinations. The greater frequency of detection of estrus and ultrasound examination (every 6 h) in the present study should have yielded more accurate estimates of DE and EOI and may explain the inability of other research groups to detect seasonal differences with less frequent observation. However, data from a study on a research station in Germany by Weitze et al. (1994), based on more frequent detection of estrus and transabdominal ultrasound examination 3 times daily, also suggested no significant change of DE or EOI in mixed parity sows weaned in the spring (February to May) versus the summer (July to September). Since one of the three farms in the present experiment also failed to exhibit a seasonal change of DE and EOI, it is quite possible that studies based on only one farm did not detect a seasonal change because the occurrence or timing of this phenomenon is farm dependent. If seasonal photoperiod changes mediate effects on DE and EOI, differences in latitude and building design between the North Carolina sow farms in the present study and those in Germany, the Netherlands, and Sweden may also be related to the conflicting results since they would affect the amount of natural light sows received.

The 8 h increase of DE and EOI between the spring and summer replicates was primarily due to parity ≥ 3 sows and not parity 1 or parity 2 sows. This season × parity
interaction may suggest that there are age and(or) reproductive experience differences in how
the hypothalamus responds to seasonal cues. There is evidence of parity related differences
in ovarian responsiveness to exogenous gonadotropins in lactating (Armstrong et al., 1999)
and weaned sows (Bates et al., 1991), but literature that demonstrated a direct effect of parity
on hypothalamo-hypoophysial function could not be located. In addition, the nocturnal
increase in melatonin secretion changes with age in growing pigs (Klupiec et al., 1993), but it
is not clear if parity-based differences in melatonin secretion exist. Overall, there are
insufficient data to support a physiological explanation of this phenomenon. Nonetheless,
other research groups have noted effects of parity on DE and EOI. In accord with the results
of the present study, Steverink et al. (1997) found that the DE of parity 1 and 2 sows was
shorter than that of parity ≥ 3 sows (55 vs. 62 h), though the season in which their data were
recorded was not reported. Data from Weitze et al. (1994) suggest that parity 1 sows had a
shorter DE and EOI in both the spring and summer compared to parity 2 to 4 sows. However, since only parity 1 sows were treated with equine chorionic gonadotropin in their
study, the effect of parity and hormone treatment cannot be separated. In contrast, several
other recent studies conducted in the Midwestern US (Knox et al., 2002; Knox and
Rodriguez Zas, 2001) and Brazil (Corrêa et al., 2002) found no effect of parity on DE or
EOI. Despite these contradictory findings, results of the present study clearly indicate that
parity can affect DE and EOI during the summer months.

Only farms 1 and 3 had multiple sow genotypes and genotype differences in EOI but
not DE were detected on both farms. On farm 1, genotype B, an F1 grandparent (½
Yorkshire, ½ Landrace), had a 4 h shorter EOI than genotype C, a three-breed cross (½
Yorkshire, ¼ Landrace, ¼ Duroc). On farm 3, genotype D, another three-breed cross (½ Yorkshire, ¼ Landrace, ¼ Chester White), tended to have a 3 h shorter EOI than genotype B. These differences in EOI may be related to the different selection focus of the individual breeds in each composite. Statistical comparisons between farms were avoided due to the numerous farm specific factors that could not be controlled. However, it is interesting that the EOI of genotype B on farms 1 and 3, and the EOI of genotype D on farms 2 and 3 are very similar. One might conclude from this study that DE is influenced mainly by farm and sow specific factors other than genotype, whereas EOI can be affected by genotype. Several experiments on the relationship between insemination-to-ovulation interval and fertilization rate did not detect an effect of genotype on DE or EOI and pooled data from multiple sow genotypes (Soede et al., 1995; Kemp and Soede, 1996; Steverink et al., 1997). However, DE does have a genetic component (e.g., heritability = 0.20; Rydhmer, 2000), and different DE have been reported for different purebreds (Burger, 1952; Bazer et al., 1988; Kopperschmidt, 2000). Only genotype A was a purebred, and it did have a numerically shorter DE than the other two genotypes present on farm 1 (B and C), which each had 100% heterosis.

Sows that lactate for < 21 days often exhibit an extended weaning-to-estrus interval and a subsequent reduction of embryo survival, conception rate, and litter size compared to sows that lactate for longer periods (Varley, 1982). Since weaning-to-estrus interval typically has an inverse relationship with DE (Soede and Kemp, 1997), sows that lactate for a short versus a long period tend to have a shorter DE due to their longer weaning-to-estrus interval. However, it was not known if lactation length affects DE or EOI in addition to this effect of weaning-to-estrus interval. Estimates of DE based on detection of estrus twice daily
(Corrêa et al., 2002) and three times daily (Lucia et al., 1999) on a Brazilian sow farm were not different across lactation lengths of 8 to 20 days and 14 to 23 days, respectively. In contrast, results of the present experiment indicate that as lactation length increased from $\leq 13$ to $\geq 20$ days, there was a linear decrease of DE and a tendency for a linear decrease of EOI. Data from Knox and Rodriguez Zas (2001) on EOI exhibited a similar decrease as lactation length increased from $\leq 16$ to $\geq 32$ days, though there were no significant differences among the individual lactation length classes. In addition, Willis et al. (2003) recently reported that sows that lactated for 14 versus 24 days had a longer DE (52 vs. 46 h). Collectively, these data suggest that an increase of lactation length of as little as 10 days may result in a 4 to 6 h decrease of DE and EOI that is not related to the change in weaning-to-estrus interval.

Since the insemination schedule on each farm was held constant, factors that affected EOI also generally affected insemination-to-ovulation intervals and the percentage of inseminations in which a particular AI occurred with 24 h pre- to -3 h post-ovulation. In most cases, changes in EOI simply resulted in one AI becoming less synchronous with ovulation and another AI in the insemination schedule becoming more synchronous with ovulation. Consequently, the few effects on reproductive performance that were detected within a farm did not appear to be due to sub-optimal insemination timing. For example, as weaning-to-estrus interval increased and EOI decreased from the 3 to the 7 to 10 day class on farms 2 and 3, the percentages of inseminations that occurred within 24 h pre- to -3 h post-ovulation increased for AI 1 and decreased for AI 2. On farm 1, there was no obvious trend in the percentages of inseminations that occurred within 24 h pre- to -3 h post-ovulation as
weaning-to-estrus interval increased due to the inconsistent decrease of DE and EOI. Similar to the findings of Soede and Kemp (1996), the last insemination (AI 2 or AI 3) of sows in the 6 and 7 to 10 day weaning-to-estrus interval class on each farm to occur near and in some cases after ovulation on average (3 h pre- to -4 h post-ovulation). An inverse relationship between weaning-to-estrus interval and subsequent reproductive performance has been reported in the literature. In particular, sows that returned to estrus 7 to 10 days postweaning exhibited lower farrowing rates and litter sizes than sows that returned to estrus in 3 to 6 days (Vesseur et al., 1994; Steverink et al., 1999; Tummaruk et al., 2000). Data from the present study concur to some extent, since sows in the 6 and 7 to 10 day weaning-to-estrus interval classes had lower farrowing rates and similar litter sizes compared to sows in the 3, 4, and 5 day weaning-to-estrus interval classes. However, it does not seem likely that sub-optimal timing of their last insemination explains their reduced farrowing rate given that a previous insemination had occurred within 24 h of ovulation in the majority of these sows.

The percentage of inseminations in which a particular AI occurred within 24 h pre- to -3 h post-ovulation was also affected by genotype and lactation length, which had moderate effects on EOI, and season and parity, which had a large effect on EOI. However, effects of lactation length and parity on insemination timing were not reported since data were pooled across farms with different insemination protocols. The difference in EOI between genotypes B and C on farm 1 and genotypes B and D on farm 3 did influence the percentage of inseminations in which specific AI occurred within 24 h pre- to -3 h post-ovulation, but conception rate, farrowing rate, and litter size were not affected. Season had the largest effect on EOI and the 8 h increase of EOI in the summer as compared to the spring on farms
1 and 3 caused AI 1 to become less synchronous with ovulation. Evidently, the insemination(s) on day 2 of estrus (AI 2 and AI 3, farm 1; AI 2, farm 3) prevented this seasonal shift of EOI from significantly reducing sow reproductive performance. Litter size was not significantly lower in the summer than the spring on either farm 1 or farm 3. However, conception and farrowing rates were lower in the summer than the spring on farm 3. Given the multiple insemination protocols in use, it does not seem likely that this 8 h increase of EOI could be the principal cause of the reduced sow fertility during the summer on farm 3. However, it remains to be determined whether or not seasonal shifts in DE, EOI, and insemination-to-ovulation intervals are a component of summer infertility in swine (Love et al., 1993).

Another seasonal change that may have affected the synchrony between insemination and ovulation was the shift in the time of day that onset of estrus occurred. On each farm, the largest percentage of sows tended to come into estrus between the 02:00 and 08:00 h heat checks. Since initiation of the insemination protocol on each farm was based solely on the 08:00 h heat check, sows in this category had only been in estrus approximately 3 h on farms 1 and 2, and 9 h on farm 3 prior to their first insemination (AI 1) at 08:00 and 14:00 h, respectively. However, during the summer as compared to the spring, the percentages of sows that came into estrus between 02:00 and 08:00 h were reduced on farms 1 and 3, which also exhibited an 8 increase in DE and EOI. Since onset of estrus occurred in more sows after 08:00 h during the summer when EOI was 8 h longer, this change may have reduced the interval between insemination and ovulation in some cases because it delayed initiation of the insemination protocol. In contrast, Flowers (1998) found the time of day of onset of
Estrus was clustered around 08:00 h in the summer and was more evenly distributed throughout the day in the winter on a different North Carolina sow farm. Thus, both farm and season can affect the pattern of the time of day that onset of estrus occurs. The major implication of these data is that even if each farm had added an afternoon estrous check, it would not have significantly improved their accuracy of estimating the onset of estrus because so few sows (3.4 to 10.5%) came into estrus during the workday between 08:00 and 14:00 h. Assuming other farms exhibit a similar onset of estrus pattern, the addition of a late night or early morning estrous check between 14:00 and 02:00 h to the standard morning estrous check would be necessary to more accurately determine the onset of estrus and time inseminations.

Overall, the usefulness of these data to refine insemination protocols is limited since they suggest that DE and EOI can vary considerably among different farms and, more importantly, within an individual farm over time. In addition, adjustment of the multiple insemination protocols currently in use on these farms does not appear to be necessary since they already ensure that at least one AI occurs near ovulation in > 90% of inseminated sows. Farrowing rates of the sows studied on each farm in the spring and in the summer were within the top 10% of US farms (> 82.7%) in a large records database (PigCHAMP®, 2002). Furthermore, sows that received 0, 1, or 2 inseminations within 24 h pre- to -3 h post-ovulation exhibited no significant differences in subsequent farrowing rate or litter size. This finding is in agreement with research that suggested a single insemination within 28 h pre- to -4 h post-ovulation could yield optimal farrowing rates and litter sizes (Nissen et al., 1997). From an applied perspective, these results imply that as long as at least two inseminations
spaced 24 h apart occur during estrus, one insemination will occur near enough to ovulation in the majority of sows to consistently achieve a high level of reproductive performance. Therefore, subtle timing adjustments that would complicate the simple multiple insemination protocols in use on these three farms are not likely to be beneficial since they already effectively manage the variation of DE and EOI among weaned sows. The only insemination protocol change that can be recommended is that the 08:00 h AI on day 1 of estrus on farm 1 could be eliminated or moved to the afternoon so that the 08:00 h AI on day 2 could be eliminated without compromising reproductive performance.

Conclusions

These data underscore the substantial variation of duration of estrus and onset of estrus-to-ovulation interval among weaned sows and reveal that season, parity, and weaning-to-estrus interval can be important sources of this variation on some farms. Despite the magnitude of this variation, a simple and consistent multiple insemination protocol that delivers two inseminations during estrus, separated by 24 h, is apparently all that is necessary to optimize sow reproductive performance. Considerable time and labor are required to obtain accurate estimates of duration of estrus and onset of estrus-to-ovulation interval and the value of the data are limited since they are often dependent on both the farm and season in which they were collected. Season may also affect the time of day that the onset of estrus occurs and thereby alter the synchrony between initiation of an insemination protocol and ovulation. Improving the accuracy of diagnosing the onset of estrus may not be as simple as
adding a late afternoon estrous check since it appears that few new sows come into estrus after the morning estrous check and before the end of the workday.
Literature Cited


Table 1. Least squares means (± SEM) and range of the parity, pigs weaned, and lactation length of the sows utilized from each farm and the daily high temperature outdoors and in the breeding barn during the summer replicate on each farm

<table>
<thead>
<tr>
<th>Item</th>
<th>Farm 1</th>
<th>Farm 2</th>
<th>Farm 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. sows</td>
<td>176</td>
<td>178</td>
<td>181</td>
</tr>
<tr>
<td>Parity</td>
<td>3.2 ± 0.2 (1 to 9)</td>
<td>3.9 ± 0.2 (1 to 8)</td>
<td>3.0 ± 0.1 (1 to 8)</td>
</tr>
<tr>
<td>Pigs weaned</td>
<td>9.1 ± 0.1 (6 to 15)</td>
<td>10.1 ± 0.1 (7 to 13)</td>
<td>10.7 ± 0.1 (9 to 12)</td>
</tr>
<tr>
<td>Lactation length (days)</td>
<td>15.9 ± 0.2 (11 to 20)</td>
<td>17.3 ± 0.2 (13 to 21)</td>
<td>18.4 ± 0.2 (13 to 23)</td>
</tr>
<tr>
<td>High outdoor temp. (°C)</td>
<td>29.7 ± 0.6 (25.2 to 35.1)</td>
<td>33.1 ± 0.8 (26.3 to 38.2)</td>
<td>32.8 ± 0.5 (28.4 to 36.1)</td>
</tr>
<tr>
<td>High barn temp. (°C)*</td>
<td>29.9 ± 0.4 (26.8 to 33.7)</td>
<td>28.1 ± 0.3 (25.3 to 29.7)</td>
<td>27.7 ± 0.4 (26.2 ± 29.8)</td>
</tr>
</tbody>
</table>

*Farm 1 had internal fans and misters whereas farms 2 and 3 each had evaporative cooling systems.
Table 2. Contribution of individual independent variables to variation of the dependent variable duration of estrus (DE) in all three farms and in each farm based on forward regression

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Percentage</th>
<th>Partial R²</th>
<th>Model R²</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Farms 1, 2, 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weaning-to-estrus interval</td>
<td>67.5%</td>
<td>0.2416</td>
<td>0.2416</td>
<td>155.79</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Season</td>
<td>23.0%</td>
<td>0.0822</td>
<td>0.3238</td>
<td>59.33</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Lactation length</td>
<td>7.4%</td>
<td>0.0264</td>
<td>0.3503</td>
<td>19.82</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Parity</td>
<td>1.3%</td>
<td>0.0047</td>
<td>0.3550</td>
<td>3.55</td>
<td>0.0602</td>
</tr>
<tr>
<td>Farm</td>
<td>0.6%</td>
<td>0.0021</td>
<td>0.3570</td>
<td>1.56</td>
<td>0.2123</td>
</tr>
<tr>
<td>Genotype</td>
<td>0.2%</td>
<td>0.0009</td>
<td>0.3579</td>
<td>0.64</td>
<td>0.4237</td>
</tr>
<tr>
<td><strong>Farm 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weaning-to-estrus interval</td>
<td>51.5%</td>
<td>0.1207</td>
<td>0.1207</td>
<td>20.32</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Season</td>
<td>44.9%</td>
<td>0.1051</td>
<td>0.2258</td>
<td>19.95</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Genotype</td>
<td>2.3%</td>
<td>0.0054</td>
<td>0.2312</td>
<td>1.02</td>
<td>0.3143</td>
</tr>
<tr>
<td>Parity</td>
<td>1.3%</td>
<td>0.0031</td>
<td>0.2343</td>
<td>0.60</td>
<td>0.4413</td>
</tr>
<tr>
<td><strong>Farm 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weaning-to-estrus interval</td>
<td>74.0%</td>
<td>0.1960</td>
<td>0.1960</td>
<td>40.95</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Season</td>
<td>15.2%</td>
<td>0.0401</td>
<td>0.2361</td>
<td>9.00</td>
<td>0.0031</td>
</tr>
<tr>
<td>Lactation length</td>
<td>9.5%</td>
<td>0.0251</td>
<td>0.2612</td>
<td>5.39</td>
<td>0.0215</td>
</tr>
<tr>
<td>Parity</td>
<td>1.3%</td>
<td>0.0035</td>
<td>0.2647</td>
<td>0.79</td>
<td>0.3763</td>
</tr>
<tr>
<td><strong>Farm 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weaning-to-estrus interval</td>
<td>68.0%</td>
<td>0.4088</td>
<td>0.4088</td>
<td>116.86</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Season</td>
<td>26.3%</td>
<td>0.1582</td>
<td>0.5670</td>
<td>61.40</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Lactation length</td>
<td>4.7%</td>
<td>0.0281</td>
<td>0.5951</td>
<td>11.57</td>
<td>0.0008</td>
</tr>
<tr>
<td>Parity</td>
<td>1.0%</td>
<td>0.0059</td>
<td>0.6010</td>
<td>2.46</td>
<td>0.1184</td>
</tr>
</tbody>
</table>
Table 3. Contribution of individual independent variables to variation of the dependent variable onset of estrus-to-ovulation interval (EOI) in all three farms and in each farm based on forward regression

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Percentage</th>
<th>Partial R²</th>
<th>Model R²</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farms 1, 2, 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weaning-to-estrus interval</td>
<td>57.1%</td>
<td>0.1601</td>
<td>0.1601</td>
<td>92.45</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Season</td>
<td>34.3%</td>
<td>0.0963</td>
<td>0.2564</td>
<td>62.71</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Parity</td>
<td>4.0%</td>
<td>0.0113</td>
<td>0.2677</td>
<td>7.42</td>
<td>0.0067</td>
</tr>
<tr>
<td>Lactation length</td>
<td>3.6%</td>
<td>0.0102</td>
<td>0.2779</td>
<td>6.79</td>
<td>0.0094</td>
</tr>
<tr>
<td>Genotype</td>
<td>1.0%</td>
<td>0.0027</td>
<td>0.2805</td>
<td>1.79</td>
<td>0.1821</td>
</tr>
<tr>
<td>Farm 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Season</td>
<td>72.0%</td>
<td>0.1332</td>
<td>0.1332</td>
<td>22.43</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Weaning-to-estrus interval</td>
<td>15.8%</td>
<td>0.0293</td>
<td>0.1625</td>
<td>5.08</td>
<td>0.0257</td>
</tr>
<tr>
<td>Parity</td>
<td>7.1%</td>
<td>0.0131</td>
<td>0.1756</td>
<td>2.29</td>
<td>0.1326</td>
</tr>
<tr>
<td>Genotype</td>
<td>3.5%</td>
<td>0.0064</td>
<td>0.1820</td>
<td>1.12</td>
<td>0.2918</td>
</tr>
<tr>
<td>Lactation length</td>
<td>1.6%</td>
<td>0.0029</td>
<td>0.1849</td>
<td>0.50</td>
<td>0.4792</td>
</tr>
<tr>
<td>Farm 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weaning-to-estrus interval</td>
<td>81.9%</td>
<td>0.2523</td>
<td>0.2523</td>
<td>56.67</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Lactation length</td>
<td>8.9%</td>
<td>0.0274</td>
<td>0.2797</td>
<td>6.49</td>
<td>0.0118</td>
</tr>
<tr>
<td>Season</td>
<td>6.2%</td>
<td>0.0190</td>
<td>0.2987</td>
<td>4.36</td>
<td>0.0383</td>
</tr>
<tr>
<td>Parity</td>
<td>3.1%</td>
<td>0.0096</td>
<td>0.3082</td>
<td>2.28</td>
<td>0.1330</td>
</tr>
<tr>
<td>Farm 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weaning-to-estrus interval</td>
<td>65.4%</td>
<td>0.3745</td>
<td>0.3745</td>
<td>99.99</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Season</td>
<td>27.1%</td>
<td>0.1554</td>
<td>0.5299</td>
<td>54.89</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Lactation length</td>
<td>4.0%</td>
<td>0.0231</td>
<td>0.5530</td>
<td>8.51</td>
<td>0.0040</td>
</tr>
<tr>
<td>Parity</td>
<td>3.4%</td>
<td>0.0195</td>
<td>0.5725</td>
<td>7.46</td>
<td>0.0070</td>
</tr>
</tbody>
</table>
Table 4. Contribution of individual independent variables to variation of the dependent variable duration of estrus (DE) in each replicate in each farm based on forward regression

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Percentage</th>
<th>Partial R²</th>
<th>Model R²</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Spring, Farm 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weaning-to-estrus interval</td>
<td>74.3%</td>
<td>0.1426</td>
<td>0.1426</td>
<td>12.14</td>
<td>0.0008</td>
</tr>
<tr>
<td>Parity</td>
<td>25.7%</td>
<td>0.0492</td>
<td>0.1918</td>
<td>4.39</td>
<td>0.0397</td>
</tr>
<tr>
<td><strong>Summer, Farm 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weaning-to-estrus interval</td>
<td>88.7%</td>
<td>0.1424</td>
<td>0.1424</td>
<td>12.12</td>
<td>0.0008</td>
</tr>
<tr>
<td>Genotype</td>
<td>6.5%</td>
<td>0.0105</td>
<td>0.1529</td>
<td>0.89</td>
<td>0.3487</td>
</tr>
<tr>
<td>Parity</td>
<td>4.8%</td>
<td>0.0078</td>
<td>0.1606</td>
<td>0.66</td>
<td>0.4206</td>
</tr>
<tr>
<td><strong>Spring, Farm 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weaning-to-estrus interval</td>
<td>86.2%</td>
<td>0.2931</td>
<td>0.2931</td>
<td>37.73</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Parity</td>
<td>12.3%</td>
<td>0.0418</td>
<td>0.3349</td>
<td>5.65</td>
<td>0.0195</td>
</tr>
<tr>
<td>Lactation length</td>
<td>1.5%</td>
<td>0.0052</td>
<td>0.3401</td>
<td>0.71</td>
<td>0.4033</td>
</tr>
<tr>
<td><strong>Summer, Farm 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weaning-to-estrus interval</td>
<td>61.6%</td>
<td>0.1593</td>
<td>0.1593</td>
<td>14.21</td>
<td>0.0003</td>
</tr>
<tr>
<td>Lactation length</td>
<td>38.4%</td>
<td>0.0992</td>
<td>0.2585</td>
<td>9.90</td>
<td>0.0024</td>
</tr>
<tr>
<td><strong>Spring, Farm 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weaning-to-estrus interval</td>
<td>93.1%</td>
<td>0.5833</td>
<td>0.5833</td>
<td>116.17</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lactation length</td>
<td>5.5%</td>
<td>0.0346</td>
<td>0.6178</td>
<td>7.42</td>
<td>0.0079</td>
</tr>
<tr>
<td>Parity</td>
<td>1.0%</td>
<td>0.0064</td>
<td>0.6242</td>
<td>1.37</td>
<td>0.2446</td>
</tr>
<tr>
<td>Genotype</td>
<td>0.4%</td>
<td>0.0025</td>
<td>0.6267</td>
<td>0.54</td>
<td>0.4642</td>
</tr>
<tr>
<td><strong>Summer, Farm 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weaning-to-estrus interval</td>
<td>85.4%</td>
<td>0.3176</td>
<td>0.3176</td>
<td>39.09</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lactation length</td>
<td>10.7%</td>
<td>0.0400</td>
<td>0.3576</td>
<td>5.17</td>
<td>0.0255</td>
</tr>
<tr>
<td>Genotype</td>
<td>3.9%</td>
<td>0.0145</td>
<td>0.3721</td>
<td>1.89</td>
<td>0.1730</td>
</tr>
</tbody>
</table>
Table 5. Contribution of individual independent variables to variation of the dependent variable onset of estrus-to-ovulation interval (EOI) in each replicate in each farm based on forward regression

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Percentage</th>
<th>Partial $R^2$</th>
<th>Model $R^2$</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Spring, Farms 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weaning-to-estrus interval</td>
<td>68.5%</td>
<td>0.1389</td>
<td>0.1389</td>
<td>11.62</td>
<td>0.0011</td>
</tr>
<tr>
<td>Parity</td>
<td>20.5%</td>
<td>0.0416</td>
<td>0.1806</td>
<td>3.61</td>
<td>0.0616</td>
</tr>
<tr>
<td>Genotype</td>
<td>6.8%</td>
<td>0.0138</td>
<td>0.1944</td>
<td>0.73</td>
<td>0.3953</td>
</tr>
<tr>
<td>Lactation length</td>
<td>4.2%</td>
<td>0.0085</td>
<td>0.2029</td>
<td>1.20</td>
<td>0.2778</td>
</tr>
<tr>
<td><strong>Summer, Farm 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genotype</td>
<td>100.0%</td>
<td>0.0172</td>
<td>0.0172</td>
<td>1.26</td>
<td>0.2650</td>
</tr>
<tr>
<td><strong>Spring, Farm 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weaning-to-estrus interval</td>
<td>84.0%</td>
<td>0.3006</td>
<td>0.3006</td>
<td>39.11</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Parity</td>
<td>10.6%</td>
<td>0.0380</td>
<td>0.3386</td>
<td>5.17</td>
<td>0.0253</td>
</tr>
<tr>
<td>Lactation length</td>
<td>5.4%</td>
<td>0.0192</td>
<td>0.3579</td>
<td>2.67</td>
<td>0.1059</td>
</tr>
<tr>
<td><strong>Summer, Farm 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weaning-to-estrus interval</td>
<td>91.1%</td>
<td>0.2517</td>
<td>0.2517</td>
<td>25.23</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Lactation length</td>
<td>8.9%</td>
<td>0.0247</td>
<td>0.2764</td>
<td>2.53</td>
<td>0.1161</td>
</tr>
<tr>
<td><strong>Spring, Farm 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weaning-to-estrus interval</td>
<td>88.0%</td>
<td>0.4254</td>
<td>0.4254</td>
<td>60.71</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Lactation length</td>
<td>7.2%</td>
<td>0.0347</td>
<td>0.4601</td>
<td>5.20</td>
<td>0.0252</td>
</tr>
<tr>
<td>Parity</td>
<td>4.8%</td>
<td>0.0232</td>
<td>0.4833</td>
<td>3.59</td>
<td>0.0618</td>
</tr>
<tr>
<td><strong>Summer, Farm 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weaning-to-estrus interval</td>
<td>85.2%</td>
<td>0.3597</td>
<td>0.3597</td>
<td>46.63</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Genotype</td>
<td>9.1%</td>
<td>0.0385</td>
<td>0.3982</td>
<td>5.25</td>
<td>0.0246</td>
</tr>
<tr>
<td>Lactation length</td>
<td>5.6%</td>
<td>0.0238</td>
<td>0.4220</td>
<td>3.33</td>
<td>0.0716</td>
</tr>
</tbody>
</table>
Table 6. Effect of season\(^a\) on postweaning estrus, ovulation, insemination, and subsequent reproductive characteristics of sows in three different farms (least squares means ± SEM and percentages)

<table>
<thead>
<tr>
<th>Item</th>
<th>Farm 1</th>
<th>Farm 2</th>
<th>Farm 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. sows weaned</td>
<td>87</td>
<td>89</td>
<td>91</td>
</tr>
<tr>
<td>Parity</td>
<td>3.0 ± 0.2</td>
<td>3.4 ± 0.2</td>
<td>2.7 ± 0.2(^y)</td>
</tr>
<tr>
<td>Lactation length (days)</td>
<td>15.6 ± 0.3</td>
<td>16.3 ± 0.3</td>
<td>17.3 ± 0.3(^y)</td>
</tr>
<tr>
<td>Estrus by day 7 (%)(^b)</td>
<td>93.1(^z)</td>
<td>78.7(^y)</td>
<td>87.8</td>
</tr>
<tr>
<td>Estrus by day 10 (%)(^b)</td>
<td>94.3(^z)</td>
<td>85.4(^y)</td>
<td>95.5</td>
</tr>
<tr>
<td>Wean to estrus (days)</td>
<td>4.3 ± 0.1(^y)</td>
<td>4.7 ± 0.1(^z)</td>
<td>4.8 ± 0.1</td>
</tr>
<tr>
<td>Duration of estrus (h)</td>
<td>51.9 ± 1.4(^y)</td>
<td>59.2 ± 1.3(^z)</td>
<td>47.2 ± 1.5(^y)</td>
</tr>
<tr>
<td>Estrus to ovulation (h)(^c)</td>
<td>36.0 ± 1.4(^y)</td>
<td>45.9 ± 1.3(^z)</td>
<td>38.5 ± 1.5(^y)</td>
</tr>
<tr>
<td>AI 1 to ovulation (h)(^d)</td>
<td>29.6 ± 1.5(^z)</td>
<td>38.1 ± 1.4(^y)</td>
<td>23.2 ± 1.4(^z)</td>
</tr>
<tr>
<td>AI 2 to ovulation (h)(^d)</td>
<td>5.8 ± 1.5(^z)</td>
<td>14.3 ± 1.4(^y)</td>
<td>3.9 ± 1.4(^z)</td>
</tr>
<tr>
<td>AI 3 to ovulation (h)(^d)</td>
<td>-0.3 ± 1.5(^z)</td>
<td>8.0 ± 1.4(^y)</td>
<td>-0.8 ± 1.4(^z)</td>
</tr>
<tr>
<td>No. post-ovulation AI</td>
<td>0.9 ± 0.1(^y)</td>
<td>0.5 ± 0.1(^z)</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>AI 1 near ovulation (%)(^e)</td>
<td>33.3(^z)</td>
<td>17.7(^y)</td>
<td>44.7(^e)</td>
</tr>
<tr>
<td>AI 2 near ovulation (%)(^f)</td>
<td>62.7(^y)</td>
<td>69.6</td>
<td>23.4</td>
</tr>
<tr>
<td>AI 3 near ovulation (%)(^f)</td>
<td>60.0(^y)</td>
<td>72.1</td>
<td>19.1</td>
</tr>
<tr>
<td>Conception rate (%)(^f)</td>
<td>94.2(^z)</td>
<td>92.5</td>
<td>98.7(^z)</td>
</tr>
<tr>
<td>Farrowing rate (%)</td>
<td>92.8(^z)</td>
<td>86.6</td>
<td>96.1(^z)</td>
</tr>
<tr>
<td>Total born</td>
<td>12.6 ± 0.4</td>
<td>11.8 ± 0.4</td>
<td>12.5 ± 0.4</td>
</tr>
<tr>
<td>Born alive</td>
<td>11.2 ± 0.4</td>
<td>10.9 ± 0.4</td>
<td>11.3 ± 0.4</td>
</tr>
</tbody>
</table>

\(^a\)Spring and summer data collected during March and April versus July and August, respectively.

\(^b\)Percentage of weaned sows that returned to estrus by day indicated.

\(^c\)Onset of estrus-to-ovulation interval.

\(^d\)Insemination-to-ovulation intervals.

\(^e\)Percentage of inseminations that occurred within 24 h pre- to -3 h post-ovulation.

\(^f\)Percentage of inseminated sows that did not return to estrus and were confirmed pregnant at day 25 to 30.

\(^y\), \(^z\)Row values within a farm without a common superscript letter differ (P < 0.05).
Table 7. Effect of parity\(^a\) in two different seasons\(^b\) on postweaning estrus, ovulation, and subsequent reproductive characteristics of sows in three different farms pooled (least squares means ± SEM and percentages)

<table>
<thead>
<tr>
<th>Item</th>
<th>Parity 1</th>
<th>Parity 2</th>
<th>Parity 3</th>
<th>Parity ≥ 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spring</td>
<td>Summer</td>
<td>Spring</td>
<td>Summer</td>
</tr>
<tr>
<td>No. sows weaned</td>
<td>66</td>
<td>46</td>
<td>52</td>
<td>41</td>
</tr>
<tr>
<td>Parity</td>
<td>1.0 ± 0.1(^w)</td>
<td>1.0 ± 0.1(x)</td>
<td>2.0 ± 0.1(^x)</td>
<td>2.0 ± 0.1(x)</td>
</tr>
<tr>
<td>Lactation length (days)</td>
<td>16.2 ± 0.3</td>
<td>17.3 ± 0.4(^w)</td>
<td>16.6 ± 0.3</td>
<td>17.5 ± 0.4(^y)</td>
</tr>
<tr>
<td>Estrus by day 7 (%)(^c)</td>
<td>89.4(^w)</td>
<td>71.1(^w)</td>
<td>98.1(^x)</td>
<td>82.9(^w)</td>
</tr>
<tr>
<td>Estrus by day 10 (%)(^c)</td>
<td>90.9(^w)</td>
<td>82.6(^w)</td>
<td>98.1(^w)</td>
<td>90.2(^w)</td>
</tr>
<tr>
<td>Wean to estrus (days)</td>
<td>4.8 ± 0.2(^w)</td>
<td>5.5 ± 0.2(^w)</td>
<td>4.5 ± 0.2(^w)</td>
<td>4.6 ± 0.2(^w)</td>
</tr>
<tr>
<td>Duration of estrus (h)(^d)</td>
<td>49.8 ± 1.8</td>
<td>53.6 ± 1.8(^w)</td>
<td>52.3 ± 1.6</td>
<td>52.2 ± 1.9(^w)</td>
</tr>
<tr>
<td>Estrus to ovulation (h)(^d)</td>
<td>38.2 ± 1.8</td>
<td>42.5 ± 1.8(^w)</td>
<td>38.8 ± 1.6</td>
<td>41.8 ± 1.9(^w)</td>
</tr>
<tr>
<td>Conception rate, %(^e)</td>
<td>98.1</td>
<td>83.9</td>
<td>95.7</td>
<td>80.6</td>
</tr>
<tr>
<td>Farrowing rate, %</td>
<td>94.4</td>
<td>80.6</td>
<td>93.5</td>
<td>90.6</td>
</tr>
<tr>
<td>Total born</td>
<td>11.2 ± 0.5(^w)</td>
<td>10.4 ± 0.7</td>
<td>11.5 ± 0.5(^w)</td>
<td>10.7 ± 0.5</td>
</tr>
<tr>
<td>Born alive</td>
<td>10.7 ± 0.5</td>
<td>10.0 ± 0.7</td>
<td>10.6 ± 0.4</td>
<td>10.4 ± 0.6</td>
</tr>
</tbody>
</table>

\(^a\)Number of litters farrowed.
\(^b\)Spring and summer data collected during March and April versus July and August, respectively.
\(^c\)Percentage of weaned sows that returned to estrus by day indicated.
\(^d\)Onset of estrus-to-ovulation interval.
\(^e\)Percentage of inseminated sows that did not return to estrus and were confirmed pregnant at day 25 to 30.
\(^w, x, y, z\) Row values without a common superscript letter differ (P < 0.05).
Table 8. Effect of genotype on postweaning estrus, ovulation, insemination, and subsequent reproductive characteristics of sows in three different farms (least squares means ± SEM and percentages)

<table>
<thead>
<tr>
<th>Item</th>
<th>Farm 1</th>
<th>Farm 2</th>
<th>Farm 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. sows weaned</td>
<td>25</td>
<td>49</td>
<td>102</td>
</tr>
<tr>
<td>Parity</td>
<td>3.6 ± 0.4</td>
<td>2.7 ± 0.3</td>
<td>3.3 ± 0.2</td>
</tr>
<tr>
<td>Lactation length (days)</td>
<td>15.7 ± 0.5</td>
<td>16.3 ± 0.4</td>
<td>15.8 ± 0.3</td>
</tr>
<tr>
<td>Estrus by day 7 (%)</td>
<td>92.0</td>
<td>81.6</td>
<td>86.3</td>
</tr>
<tr>
<td>Estrus by day 10 (%)</td>
<td>96.0</td>
<td>87.8</td>
<td>89.2</td>
</tr>
<tr>
<td>Wean to estrus (days)</td>
<td>4.8 ± 0.2</td>
<td>4.3 ± 0.2</td>
<td>4.4 ± 0.1</td>
</tr>
<tr>
<td>Duration of estrus (h)</td>
<td>53.7 ± 2.0</td>
<td>56.6 ± 1.5</td>
<td>56.8 ± 1.2</td>
</tr>
<tr>
<td>Estrus to ovulation (h)</td>
<td>43.4 ± 2.1</td>
<td>39.6 ± 1.5</td>
<td>43.9 ± 1.2</td>
</tr>
<tr>
<td>AI 1 to ovulation (h)</td>
<td>35.5 ± 2.5</td>
<td>29.2 ± 1.9</td>
<td>35.8 ± 1.4</td>
</tr>
<tr>
<td>AI 2 to ovulation (h)</td>
<td>11.6 ± 2.5</td>
<td>5.5 ± 1.9</td>
<td>11.9 ± 1.4</td>
</tr>
<tr>
<td>AI 3 to ovulation (h)</td>
<td>6.4 ± 3.9</td>
<td>-2.6 ± 2.7</td>
<td>5.0 ± 2.0</td>
</tr>
<tr>
<td>No. post-ovulation AI</td>
<td>0.5 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>AI 1 near ovulation (%)</td>
<td>13.6</td>
<td>42.8</td>
<td>20.0</td>
</tr>
<tr>
<td>AI 2 near ovulation (%)</td>
<td>81.8</td>
<td>59.5</td>
<td>65.5</td>
</tr>
<tr>
<td>AI 3 near ovulation (%)</td>
<td>77.3</td>
<td>52.4</td>
<td>70.0</td>
</tr>
<tr>
<td>Conception rate (%)</td>
<td>95.5</td>
<td>93.9</td>
<td>92.6</td>
</tr>
<tr>
<td>Farrowing rate (%)</td>
<td>95.4</td>
<td>90.9</td>
<td>87.7</td>
</tr>
<tr>
<td>Total born</td>
<td>10.8 ± 0.7</td>
<td>12.3 ± 0.6</td>
<td>12.6 ± 0.4</td>
</tr>
<tr>
<td>Born alive</td>
<td>9.1 ± 0.7</td>
<td>11.4 ± 0.6</td>
<td>11.5 ± 0.4</td>
</tr>
</tbody>
</table>

*Genotype A = Yorkshire; B = ½ Yorkshire, ½ Landrace; C = ½ Yorkshire, ¼ Landrace, ¼ Duroc; D = ½ Yorkshire, ¼ Landrace, ¼ Chester White.*

*Percentage of weaned sows that returned to estrus by day indicated.*

*Onset of estrus-to-ovulation interval.*

*Insemination-to-ovulation intervals.*

*Percentage of inseminations that occurred within 24 h pre- to -3 h post-ovulation.*

*Percentage of inseminated sows that did not return to estrus and were confirmed pregnant at day 25 to 30.*

*Row values within a farm without a common superscript letter differ (P < 0.05).*
Table 9. Effect of lactation length\(^a\) on postweaning estrus, ovulation, and subsequent reproductive characteristics of sows in three different farms pooled (least squares means ± SEM and percentages)

<table>
<thead>
<tr>
<th>Item</th>
<th>≤ 13 days</th>
<th>14 to 15 days</th>
<th>16 to 17 days</th>
<th>18 to 19 days</th>
<th>≥ 20 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. sows weaned</td>
<td>36</td>
<td>119</td>
<td>90</td>
<td>160</td>
<td>130</td>
</tr>
<tr>
<td>Parity</td>
<td>3.9 ± 0.3(\textsuperscript{w})</td>
<td>3.5 ± 0.2(\textsuperscript{w})</td>
<td>3.0 ± 0.2(\textsuperscript{v})</td>
<td>3.2 ± 0.2(\textsuperscript{w})</td>
<td>3.6 ± 0.2(\textsuperscript{w})</td>
</tr>
<tr>
<td>Lactation length (days)</td>
<td>11.8 ± 0.1(\textsuperscript{v})</td>
<td>14.5 ± 0.1(\textsuperscript{w})</td>
<td>16.3 ± 0.1(\textsuperscript{x})</td>
<td>18.5 ± 0.1(\textsuperscript{y})</td>
<td>20.4 ± 0.1(\textsuperscript{z})</td>
</tr>
<tr>
<td>Estrus by day 7 (%)(^b)</td>
<td>88.9(\textsuperscript{w})</td>
<td>87.4(\textsuperscript{v})</td>
<td>87.8(\textsuperscript{v})</td>
<td>90.6(\textsuperscript{w})</td>
<td>94.6(\textsuperscript{z})</td>
</tr>
<tr>
<td>Estrus by day 10 (%)(^b)</td>
<td>91.7(\textsuperscript{w})</td>
<td>91.6(\textsuperscript{v})</td>
<td>90.0(\textsuperscript{v})</td>
<td>94.4(\textsuperscript{w})</td>
<td>97.7(\textsuperscript{w})</td>
</tr>
<tr>
<td>Wean to estrus (days)</td>
<td>4.9 ± 0.2(\textsuperscript{w})</td>
<td>4.6 ± 0.1(\textsuperscript{w})</td>
<td>4.6 ± 0.1(\textsuperscript{w})</td>
<td>4.7 ± 0.1(\textsuperscript{w})</td>
<td>4.4 ± 0.1(\textsuperscript{w})</td>
</tr>
<tr>
<td>Duration of estrus (h)</td>
<td>54.4 ± 1.7(\textsuperscript{w})</td>
<td>55.8 ± 1.0(\textsuperscript{x})</td>
<td>53.4 ± 1.1(\textsuperscript{x})</td>
<td>52.0 ± 0.8(\textsuperscript{w})</td>
<td>50.6 ± 1.0(\textsuperscript{y})</td>
</tr>
<tr>
<td>Estrus to ovulation (h)(^c)</td>
<td>42.4 ± 1.8</td>
<td>42.4 ± 1.3</td>
<td>41.1 ± 1.4</td>
<td>41.4 ± 1.3</td>
<td>40.1 ± 1.3</td>
</tr>
<tr>
<td>Conception rate (%)(^d)</td>
<td>91.7(\textsuperscript{w})</td>
<td>96.8(\textsuperscript{x})</td>
<td>92.9(\textsuperscript{w})</td>
<td>93.4(\textsuperscript{w})</td>
<td>88.1(\textsuperscript{w})</td>
</tr>
<tr>
<td>Farrowing rate (%)</td>
<td>87.5</td>
<td>91.6</td>
<td>88.6</td>
<td>91.2</td>
<td>86.4</td>
</tr>
<tr>
<td>Total born</td>
<td>10.3 ± 0.8(\textsuperscript{w})</td>
<td>11.6 ± 0.4(\textsuperscript{w})</td>
<td>11.5 ± 0.4(\textsuperscript{w})</td>
<td>12.1 ± 0.3(\textsuperscript{x})</td>
<td>12.2 ± 0.4(\textsuperscript{y})</td>
</tr>
<tr>
<td>Born alive</td>
<td>9.4 ± 0.7(\textsuperscript{w})</td>
<td>10.4 ± 0.4(\textsuperscript{w})</td>
<td>10.5 ± 0.4(\textsuperscript{w})</td>
<td>11.1 ± 0.3(\textsuperscript{y})</td>
<td>11.3 ± 0.4(\textsuperscript{w})</td>
</tr>
</tbody>
</table>

\(^a\)Number of days from farrowing to weaning.

\(^b\)Percentage of weaned sows that returned to estrus by day indicated.

\(^c\)Onset of estrus-to-ovulation interval.

\(^d\)Percentage of inseminated sows that did not return to estrus and were confirmed pregnant at day 25 to 30.

\(\textsuperscript{v, w, x, y, z}\)Row values without a common superscript letter differ (\(P < 0.05\)).
Table 10. Effect of weaning-to-estrus interval\(^a\) on estrus, ovulation, and insemination characteristics of sows in three different farms (least squares means ± SEM and percentages)

<table>
<thead>
<tr>
<th>Item</th>
<th>Farm 1</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 days</td>
<td>4 days</td>
<td>5 days</td>
<td>6 days</td>
<td>7 to 10 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. sows</td>
<td>28</td>
<td>70</td>
<td>31</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>Parity</td>
<td>3.9 ± 0.4(^x)</td>
<td>3.5 ± 0.2(^x)</td>
<td>2.4 ± 0.3(^w)</td>
<td>3.1 ± 0.5(^wx)</td>
<td>3.1 ± 0.6(^xx)</td>
</tr>
<tr>
<td>Lactation length (days)</td>
<td>17.4 ± 0.6</td>
<td>17.7 ± 0.3</td>
<td>17.1 ± 0.3</td>
<td>16.4 ± 0.6</td>
<td>17.8 ± 1.1</td>
</tr>
<tr>
<td>Duration of estrus (h)</td>
<td>64.9 ± 2.5(^x)</td>
<td>56.8 ± 1.8(^x)</td>
<td>52.7 ± 1.9(^x)</td>
<td>47.6 ± 2.6(^w)</td>
<td>48.3 ± 4.3(^xx)</td>
</tr>
<tr>
<td>Estrus to ovulation (h)(^b)</td>
<td>52.7 ± 2.5(^x)</td>
<td>47.3 ± 1.8(^x)</td>
<td>42.6 ± 1.9(^x)</td>
<td>37.4 ± 2.6(^w)</td>
<td>37.5 ± 4.2(^xx)</td>
</tr>
<tr>
<td>AI 1 to ovulation (h)(^c)</td>
<td>50.6 ± 2.5(^x)</td>
<td>33.5 ± 1.4(^x)</td>
<td>30.8 ± 1.5(^x)</td>
<td>22.9 ± 2.7(^w)</td>
<td>25.4 ± 4.9(^xx)</td>
</tr>
<tr>
<td>AI 2 to ovulation (h)(^c)</td>
<td>6.6 ± 2.5(^x)</td>
<td>9.5 ± 1.4(^x)</td>
<td>6.8 ± 1.5(^x)</td>
<td>-1.1 ± 2.7(^w)</td>
<td>1.4 ± 4.9(^xx)</td>
</tr>
<tr>
<td>No. post-ovulation AI(^d)</td>
<td>0.3 ± 0.1(^wx)</td>
<td>0.2 ± 0.1(^w)</td>
<td>0.2 ± 0.1(^w)</td>
<td>0.6 ± 0.1(^w)</td>
<td>0.6 ± 0.2(^xx)</td>
</tr>
<tr>
<td>AI 1 near ovulation (%)(^d)</td>
<td>21.0(^w)</td>
<td>11.3(^w)</td>
<td>19.0(^w)</td>
<td>52.9(^w)</td>
<td>60.0(^w)</td>
</tr>
<tr>
<td>AI 2 near ovulation (%)(^d)</td>
<td>100.0(^w)</td>
<td>88.7(^wx)</td>
<td>93.1(^y)</td>
<td>70.6(^w)</td>
<td>100.0(^xy)</td>
</tr>
<tr>
<td></td>
<td>Farm 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>71</td>
<td>58</td>
<td>17</td>
<td>5</td>
</tr>
<tr>
<td>Parity</td>
<td>3.9 ± 0.4</td>
<td>4.1 ± 0.2</td>
<td>4.0 ± 0.3</td>
<td>4.1 ± 0.5</td>
<td>3.4 ± 0.9</td>
</tr>
<tr>
<td>Lactation length (days)</td>
<td>17.4 ± 0.6</td>
<td>17.7 ± 0.3</td>
<td>17.1 ± 0.3</td>
<td>16.4 ± 0.6</td>
<td>17.8 ± 1.1</td>
</tr>
<tr>
<td>Duration of estrus (h)</td>
<td>64.9 ± 2.5(^x)</td>
<td>56.8 ± 1.8(^x)</td>
<td>52.7 ± 1.9(^x)</td>
<td>47.6 ± 2.6(^w)</td>
<td>48.3 ± 4.3(^xx)</td>
</tr>
<tr>
<td>Estrus to ovulation (h)(^b)</td>
<td>52.7 ± 2.5(^x)</td>
<td>47.3 ± 1.8(^x)</td>
<td>42.6 ± 1.9(^x)</td>
<td>37.4 ± 2.6(^w)</td>
<td>37.5 ± 4.2(^xx)</td>
</tr>
<tr>
<td>AI 1 to ovulation (h)(^c)</td>
<td>50.6 ± 2.5(^x)</td>
<td>33.5 ± 1.4(^x)</td>
<td>30.8 ± 1.5(^x)</td>
<td>22.9 ± 2.7(^w)</td>
<td>25.4 ± 4.9(^xx)</td>
</tr>
<tr>
<td>AI 2 to ovulation (h)(^c)</td>
<td>6.6 ± 2.5(^x)</td>
<td>9.5 ± 1.4(^x)</td>
<td>6.8 ± 1.5(^x)</td>
<td>-1.1 ± 2.7(^w)</td>
<td>1.4 ± 4.9(^xx)</td>
</tr>
<tr>
<td>No. post-ovulation AI(^d)</td>
<td>0.3 ± 0.1(^wx)</td>
<td>0.2 ± 0.1(^w)</td>
<td>0.2 ± 0.1(^w)</td>
<td>0.6 ± 0.1(^w)</td>
<td>0.6 ± 0.2(^xx)</td>
</tr>
<tr>
<td>AI 1 near ovulation (%)(^d)</td>
<td>21.0(^w)</td>
<td>11.3(^w)</td>
<td>19.0(^w)</td>
<td>52.9(^w)</td>
<td>60.0(^w)</td>
</tr>
<tr>
<td>AI 2 near ovulation (%)(^d)</td>
<td>100.0(^w)</td>
<td>88.7(^wx)</td>
<td>93.1(^y)</td>
<td>70.6(^w)</td>
<td>100.0(^xy)</td>
</tr>
<tr>
<td></td>
<td>Farm 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>68</td>
<td>47</td>
<td>26</td>
<td>18</td>
</tr>
<tr>
<td>Parity</td>
<td>4.5 ± 0.5(^x)</td>
<td>3.6 ± 0.2(^x)</td>
<td>2.6 ± 0.3(^w)</td>
<td>2.4 ± 0.4(^w)</td>
<td>1.9 ± 0.5(^w)</td>
</tr>
<tr>
<td>Lactation length (days)</td>
<td>18.9 ± 0.6(^x)</td>
<td>18.9 ± 0.3(^x)</td>
<td>18.3 ± 0.4(^wx)</td>
<td>18.1 ± 0.5(^wx)</td>
<td>17.2 ± 0.6(^w)</td>
</tr>
<tr>
<td>Duration of estrus (h)</td>
<td>62.4 ± 2.6(^x)</td>
<td>56.8 ± 1.4(^x)</td>
<td>53.4 ± 1.8(^x)</td>
<td>43.9 ± 2.2(^w)</td>
<td>41.6 ± 2.6(^w)</td>
</tr>
<tr>
<td>Estrus to ovulation (h)(^b)</td>
<td>49.8 ± 2.6(^x)</td>
<td>47.6 ± 1.5(^x)</td>
<td>44.3 ± 1.8(^x)</td>
<td>36.2 ± 2.2(^w)</td>
<td>34.6 ± 2.6(^w)</td>
</tr>
<tr>
<td>AI 1 to ovulation (h)(^c)</td>
<td>31.7 ± 3.0(^y)</td>
<td>32.6 ± 1.4(^y)</td>
<td>28.2 ± 1.6(^y)</td>
<td>19.8 ± 2.3(^w)</td>
<td>15.5 ± 2.8(^w)</td>
</tr>
<tr>
<td>AI 2 to ovulation (h)(^c)</td>
<td>7.7 ± 3.0(^y)</td>
<td>8.6 ± 1.4(^y)</td>
<td>4.2 ± 1.6(^y)</td>
<td>-4.2 ± 2.3(^w)</td>
<td>-8.5 ± 2.8(^w)</td>
</tr>
<tr>
<td>No. post-ovulation AI(^d)</td>
<td>0.2 ± 0.1(^w)</td>
<td>0.2 ± 0.1(^w)</td>
<td>0.3 ± 0.1(^w)</td>
<td>0.8 ± 0.1(^w)</td>
<td>0.9 ± 0.1(^w)</td>
</tr>
<tr>
<td>AI 1 near ovulation (%)(^d)</td>
<td>7.1(^w)</td>
<td>10.6(^w)</td>
<td>34.0(^w)</td>
<td>76.9(^w)</td>
<td>87.5(^w)</td>
</tr>
<tr>
<td>AI 2 near ovulation (%)(^d)</td>
<td>92.8(^y)</td>
<td>93.9(^y)</td>
<td>76.6(^y)</td>
<td>57.7(^xy)</td>
<td>31.2(^w)</td>
</tr>
</tbody>
</table>

\(^a\)Number of days from weaning to onset of estrus.

\(^b\)Onset of estrus-to-ovulation interval.

\(^c\)Insemination-to-ovulation intervals.

\(^d\)Percentage of inseminations that occurred within 24 h pre- to -3 h post-ovulation.

\(^w, x, y, z\)Row values without a common superscript letter differ (P < 0.05).
Figure 1. Frequency distribution of the postweaning duration of estrus (DE) and onset of estrus-to-ovulation interval (EOI) among 501 sows in three different farms pooled.
Figure 2. Frequency distribution of the postweaning percentage of duration of estrus at which ovulation occurred (EOI/DE, %) among 501 sows in three different farms pooled.
Figure 3. Effect of season on the percentage of sows detected in estrus by time of day of onset of estrus in three different farms. Spring and summer data collected during March and April versus July and August, respectively. * Percentages differ by season, P < 0.05.
Figure 4. Effect of weaning-to-estrus interval on subsequent farrowing rate (percentages) and pigs born alive (least squares means ± SEM) of sows from three different farms pooled. Number of sows in the 3, 4, 5, 6, and 7 to 10 day weaning-to-estrus interval categories = 51, 198, 133, 49, and 12, respectively. a, b, and x, y, z Values within a row without a common letter differ (P < 0.05).
Figure 5. Effect of number of inseminations within 24 h pre- to –3 h post ovulation on subsequent farrowing rate (percentages) and pigs born alive (least squares means ± SEM) of sows from three different farms pooled. Number of sows in the 0, 1, and 2 AI categories = 24, 298, and 141, respectively. No significant differences (P > 0.05).
CHAPTER 3

URINARY EXCRETION OF COLLAGEN DEGRADATION MARKERS BY SOWS DURING POSTPARTUM UTERINE INVOLUTION
Abstract

Incomplete uterine involution is the putative cause of the increased embryo mortality and reproductive failure often exhibited by sows that lactate for less than 21 days. Since such short lactation lengths are currently common in American swine production, an effective technique to monitor the involution process and test this hypothesis might be valuable. The objective of this study was to characterize postpartum excretion of two markers of collagen degradation. In experiment I, urine samples were collected from five sows every other day from the day before parturition (day -1), through a 21-day lactation, to day 8 postweaning. The collagen crosslinks hydroxylysyl pyridinoline (HP), which is present in many tissues, and lysyl pyridinoline (LP), which is primarily concentrated in bone, were assayed by both ELISA and HPLC. Urinary levels of both free (ELISA) and total (HPLC) HP and LP increased (P < 0.001) approximately 2-fold during lactation. The mean molar ratio of total HP:LP increased (P < 0.001) from 6.6 ± 1.6 at day 1 to a maximum of 10.2 ± 1.5 at day 7 postpartum and averaged 9.1 ± 0.3 for the entire sampling period. These data are consistent with a postpartum increase of soft tissue collagen catabolism since bone has a low HP:LP ratio of 4 and soft tissues like the uterus have a high HP:LP ratio because they contain only trace amounts of LP. Only the ELISA technique was used in experiment II since HPLC (total) and ELISA (free) crosslinks estimates were highly correlated (r = 0.85 to 0.91, P < 0.001) in experiment I. Urine samples were collected from 21 sows every third day from day 1 to 19 of lactation. Sows from this larger group exhibited one of four distinct crosslinks excretion patterns: peak on day 1 (n = 3), peak on day 7 (n = 4), peak on day 10, 13 or 16 (n = 7), or no peak (n = 7). None of the sow or litter variables that were recorded
explained this variation among sows. Overall, data from experiments I and II indicate that urinary HP does increase postpartum in a pattern temporally consistent with uterine involution. However, significant variation among sows in the magnitude and timing of peak HP excretion was evident.

**Introduction**

Tissues of the postpartum uterus must undergo a degenerative and regenerative process known as involution to return to the non-gravid state and prepare a suitable environment to establish the next pregnancy. Based on histological and morphological data, uterine involution appears to be complete within approximately 14 to 21 days postpartum in the sow (Palmer et al., 1965a, 1965b; Graves 1967; Svajgr et al., 1974; Hays et al., 1978). Recent records surveys of US sow herds suggest an average weaning age (lactation length) of approximately 18 days and 75% of litters are weaned at less than 21 days (USDA, 2001; PigCHAMP® Inc., 2002). Sows that lactate for less than 21 days often exhibit a subsequent reduction of embryo survival, conception rate, and litter size compared to sows that lactate for longer periods (Svajgr et al., 1974; Hays et al., 1978; Marsteller et al., 1997). This reduced sow reproductive performance following short lactation lengths is typically attributed to incomplete uterine involution, though a causal relationship has not been established. A simple, repeatable, and non-invasive means to monitor uterine involution in the sow would therefore be a useful research tool with potential management applications.

Large amounts of collagen are added to the extracellular matrix of the gravid reproductive tract, particularly during late gestation, to support the increasing fetal load (rat:
Harkness and Harkness, 1954; woman: Morrione and Seifter, 1962; cow: Kaidi et al., 1995). Rapid and extensive postpartum degradation of this collagen parallels the decrease in uterine weight during involution (rat: Harkness and Moralee, 1956; Gunja-Smith et al., 1989; woman: Woessner and Brewer, 1963). In sows, the uterus typically exhibits a 90% decrease in wet weight from approximately 2.7 to 0.3 kg during the first three weeks postpartum (Palmer et al., 1965a), though data on collagen turnover are not available. Urinary excretion of hydroxylysyl pyridinoline, a molecular marker of collagen degradation, was reported to increase during the period of postpartum uterine involution in dairy cows (Kaidi et al., 1991; Liesegang et al., 2000) and women (Stone and Franzblau, 1995; Naylor et al., 2000). Despite the diffuse and non-invasive attachment of the chorioallantois to the uterus during gestation and limited tissue sloughing during involution in the sow, we hypothesized that there should be a substantial increase in postpartum collagen degradation, as observed in other mammals with more invasive placentation. The objective of this study was to characterize the urinary excretion of hydroxylysyl pyridinoline and lysyl pyridinoline by postpartum sows.

**Materials and Methods**

*Sows and urine sampling*

A total of 26 pregnant sows at the North Carolina State University Swine Education Unit were monitored in two separate experiments. Sows were housed in individual crates and were moved from the gestation to the farrowing barn at 110 days post-mating. Sows had ad libitum access to water and were fed a corn-soybean meal based diet (15.8% crude protein, 0.85% lysine, and 3,307 kcal ME/kg) twice daily. On average, sows consumed 6.3
to 8.2 kg of this diet daily during lactation. Data on sow and litter lactation performance were recorded.

In experiment I, five purebred Yorkshire sows (parity 1 and 2) that farrowed on the same day were selected for a preliminary study. Mid-stream urine samples were collected every other day from the day before farrowing (day –1 prepartum), through lactation (days 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 postpartum), and on days 2, 4, 6, and 8 postweaning (days 23, 25, 27, 29 postpartum). Sows were monitored continuously between 06:00 and 08:00 h but approximately 10% of the urine samples could not be obtained until later in the day, especially during early lactation and postweaning, when urination was infrequent. Samples were protected from UV light, frozen, and stored at -20°C.

In experiment II, 21 Yorkshire × Landrace sows (parity 1 to 10, mean ± SEM, 3.8 ± 0.6) were housed and fed as described, but their litters were split-weaned with 2 to 4 pigs removed at day 14 and the remainder at final weaning. Since this larger group of sows farrowed over a period of several days, lactation length at weaning ranged from 17 to 24 days (21.3 ± 0.3 days). Mid-stream urine samples were collected every third day during lactation (days 1, 4, 7, 10, 13, 16, 19 postpartum) and again, most samples were collected between 06:00 and 08:00 h but approximately 25% of the samples were not obtained until later in the day, between 08:00 and 15:00 h.

*Free HP and LP assays*

Urine samples from experiments I and II were assayed in duplicate for the collagen crosslinks hydroxylysyl pyridinoline (HP) and lysyl pyridinoline (LP) with two commercially
available competitive, enzyme-linked immunosorbent assays (ELISA; Quidel Corp., Santa Clara, CA, USA). The monoclonal antibody used in the LP ELISA (Metra™ Dpd) is specific for LP whereas the monoclonal antibody in the HP ELISA (Metra™ Pyd) binds HP and LP with equal affinity. The antibodies used in both assays bind free crosslinks but not glycosylated or peptide-bound forms (< 2.5% reactivity). Data from human research suggests that 40 to 50% of the total crosslinks in urine are in the free state (Robins et al., 1994; Gomez et al., 1996). Both assays were performed based on the manufacturer’s instructions with the exception that higher dilution ratios were required for the unknowns. Optical density was measured at 405 nm with a microplate spectrophotometer linked to a PC and analysis software (SpectraMax® 250 and SoftMax® Pro; Molecular Devices Corp., Sunnyvale, CA, USA). Serial dilutions of a sow urine pool yielded a standard curve parallel to the human standard curve for both assays over a 100-fold dilution range. Intra- and inter-assay coefficients of variation for six assay runs were 6.4% and 7.3%, respectively, for the HP and LP ELISA and 4.9% and 5.7%, respectively, for the LP ELISA based on the sow urine pool, which contained 1,414.8 nmol/L free HP and LP and 124.0 nmol/L free LP.

Total HP and LP assay

Urine sub-samples from experiment I were sent to an independent laboratory (TNO Prevention and Health, Leiden, The Netherlands) for analysis of total (free and peptide-bound) HP and LP by high-performance liquid chromatography (HPLC; Black et al., 1988; ‘t Hart et al., 1998). In short, urine samples were hydrolyzed in 6 M HCl and the resulting acid hydrolysates were diluted in acetic acid (final volume 50% acetic acid) and injected on an
HPLC system equipped with on-line sample purification on CC31 cellulose using a solid phase extractor (Prospekt™; Spark Holland, Emmen, The Netherlands). Retained crosslinks were eluted from the CC31 material and on-line separated on a cation exchange column (Partisil® SCX; Whatman International Ltd., Kent, UK). Elution of crosslinks was detected with a fluorometer at 295(ex.) / 400(em.) nm (Model FP-920; Jasco, Tokyo, Japan) and an HP:LP HPLC calibrator was used as a standard (Quidel Corp., Santa Clara, CA, USA). Intra- and inter-assay coefficients of variation for three assay batches were 5.6% and 0.9%, respectively, for HP and 6.9% and 2.2%, respectively, for LP based on 6 to 7 control samples per batch. The sow urine pool, which contained 2,912.8 nmol/L total HP and 344.3 nmol/L total LP, yielded inter-assay coefficients of variation of 3.5% for HP and 5.0% for LP. Even though the HPLC technique quantifies HP and LP separately, in some cases HP and LP data were summed to compare to data from the ELISA that measured both HP and LP.

Creatinine assay

Crosslinks data are expressed as a ratio to creatinine (CR) to standardize samples for variation in urine excretion volume (HP or LP nmol:CR mmol). Urinary CR concentrations for experiments I and II were estimated in duplicate with a quantitative, colorimetric assay based on a modification of the Jaffé method which utilizes the reaction between picric acid and CR that yields a colored product (Metra™ Creatinine, Quidel Corp., Santa Clara, CA, USA). Intra- and inter-assay coefficients of variation for five assay runs were 2.5% and 2.2%, respectively, based on the sow urine pool, which contained 9.7 mmol/L CR. An additional CR assay was performed on the samples from experiment I by the independent lab
using a dry slide autoanalyser (VITROS 250; Ortho-Clinical Diagnostics, Inc., Raritan, NJ, USA) and their estimates were highly correlated to our CR estimates \((r = 0.93, P < 0.0001)\). However, the average of the two CR assays was used to adjust both the ELISA and HPLC crosslinks data from experiment I to eliminate any effect of CR differences between the two assays (labs).

*Statistical analyses*

Pearson’s correlation coefficients were computed to compare the ELISA (free) and HPLC (total) HP and LP estimates from experiment I. Normality plots and statistics generated from experiment I and II data indicated parametric tests were appropriate. The effect of time (day relative to parturition) on free and total urinary HP and LP:CR ratios, and the percentage of free HP and LP, was examined by repeated measures analysis of variance through use of the mixed procedure of SAS (Littell et al., 1998). The least squares day means generated were compared with Student’s t-tests. A similar repeated measures analysis was performed on the urinary HP and LP ratios from experiment II except individual sows were categorized into four classes based on when and whether or not they exhibited a peak in HP and LP during lactation. A peak occurred if the maximum HP and LP:CR ratio during lactation exceeded the sow’s mean HP and LP:CR ratio by 1.5 standard deviations \((peak = mean + 1.5 (Std. Dev.))\). The effects of time (day), peak class, and their interaction were included in this model. The effect of peak class on sow and litter lactation variables was examined by analysis of variance (Snedecor and Cochran, 1967) through use of the general
linear models procedure of SAS (Muller and Fetterman, 2002). The least squares means generated were compared with f-tests.

Results

Experiment I

Total and free HP and LP nmol:CR mmol, total and free LP nmol:CR mmol, and molar ratio of total HP:LP data for each of the five sows monitored in experiment I were plotted initially to examine the variation of crosslinks excretion among sows (Fig. 1a, 1b, and 1c, respectively). There was an effect of time (P < 0.01; day relative to parturition) on urinary excretion of both total and free HP and LP (Fig. 2a and 2b). The mean ratio of total crosslinks increased (P < 0.001) 2.3-fold from 228.4 ± 49.5 to 535.0 ± 46.7 HP and LP nmol:CR mmol between day 1 and 11 postpartum and had decreased (P < 0.05) by day 2 postweaning. The mean ratio of free crosslinks also increased (P < 0.001) 2.3-fold from 76.2 ± 19.9 to 174.6 ± 18.1 HP and LP nmol:CR mmol between day 1 and 7 postpartum and decreased (P < 0.05) temporarily at day 15 postpartum. In comparison, the mean ratio of total LP increased (P < 0.001) 1.9-fold from 32.0 ± 9.0 to 60.5 ± 8.7 LP nmol:CR mmol between day 1 and 21 postpartum and had decreased (P < 0.01) by day 2 postweaning. The mean ratio of free LP increased (P < 0.001) 2.3-fold from 7.5 ± 2.5 to 17.1 ± 2.4 LP nmol:CR mmol between day 1 and 15 postpartum and had decreased (P < 0.05) by day 21 postpartum. The mean molar ratio of total HP:LP averaged 9.1 ± 0.3 for the sampling period. There was an effect of time (P < 0.01) on the mean total HP:LP ratio since it increased (P < 0.001) from
6.6 ± 1.6 to 10.2 ± 1.5 between day 1 and 7 postpartum and then tended to decrease during the remainder of lactation (Fig. 2c).

The HPLC estimates of the concentration of total (free and peptide-bound) HP and LP in the experiment I urine samples were highly correlated to the ELISA estimates of the concentration of free HP and LP in the samples (r = 0.91, P < 0.001 and r = 0.85, P < 0.001; Fig. 3a and 3b, respectively). The slope of the regression equations for figures 3a and 3b indicate that approximately 32% of HP and LP crosslinks and 21% of LP alone were in free as compared to peptide-bound forms. The mean concentrations of free (ELISA) versus total (HPLC) HP and LP crosslinks (1,721.6 vs. 5,069.6 nmol/L) and LP alone (139.7 vs. 548.3 nmol/L) also suggest similar percentages of crosslinks were in the free versus peptide-bound form (34% and 25%, respectively). There was an effect of time (P < 0.05) on both the mean percentage of free HP and LP (mean ± SEM, 34.1 ± 1.3%; range, 26.5 to 37.7%) and the mean percentage of free LP (26.8 ± 1.2%; 19.3 to 33.7%). This effect was due to the divergent pattern of total versus free crosslinks excretion during the postweaning period, which resulted in a 6% higher percentage of free crosslinks postweaning than during lactation.

Experiment II

The 21 sows in experiment II exhibited one of four HP and LP excretion patterns during lactation: peak on day 1 postpartum (early, n = 3), peak on day 7 postpartum (mid, n = 4), peak on day 10, 13, or 16 postpartum (late, n = 7), or no obvious peak (no peak, n = 7; Fig. 4a, 4b, 4c, and 4d respectively). This classification of sows resulted in an effect of time
(P < 0.01), peak class (P < 0.05), and a time × peak class interaction (P < 0.001) on urinary HP and LP excretion. Sows that exhibited an early peak had a greater mean free HP and LP ratio than sows that had a mid, late, or no peak on day 1 (293.2 ± 51.0 vs. 65.4 ± 27.5, 77.3 ± 23.8, or 52.8 ± 20.8 HP and LP nmol:CR mmol, respectively, P < 0.001) and on day 4 postpartum (260.5 ± 31.8 vs. 135.9 ± 30.9, 95.7 ± 20.8, or 85.9 ± 23.9 HP and LP nmol:CR mmol, respectively, P < 0.01; Fig. 5a). On day 7 postpartum, sows that exhibited a mid peak had a greater mean free HP and LP ratio than sows that had an early, late, or no peak (261.1 ± 27.5 vs. 149.6 ± 31.8, 142.3 ± 20.8, or 89.4 ± 20.8 HP and LP nmol:CR mmol, respectively, P < 0.01). On day 10 postpartum, sows that exhibited a late peak had a greater mean free HP and LP ratio than sows that had no peak (195.1 ± 22.1 vs. 114.2 ± 22.1 HP and LP nmol:CR mmol, respectively, P < 0.05) but not sows that had an early or mid peak (P > 0.05).

There was no effect (P > 0.05) of peak class on urinary LP excretion but effects of time (P < 0.01) and a time × peak class interaction (P < 0.001) were detected. Sows that exhibited an early peak in HP and LP excretion also had a greater mean free LP ratio than sows that had a mid, late, or no peak on day 1 (35.6 ± 7.9 vs. 13.8 ± 4.5, 11.0 ± 3.6, or 9.9 ± 3.4 LP nmol:CR mmol, respectively, P < 0.05), day 4 (35.1 ± 5.2 vs. 11.0 ± 5.0, 12.6 ± 3.4, or 7.2 ± 3.8 LP nmol:CR mmol, respectively, P < 0.01), and day 10 postpartum (34.0 ± 5.2 vs. 12.8 ± 4.5, 16.3 ± 3.6, or 10.5 ± 3.6 LP nmol:CR mmol, respectively, P < 0.01; Fig. 5b). The only other LP excretion differences between the peak classes were that sows that exhibited a mid peak in HP and LP had a greater mean free LP ratio than sows that had no peak at day 7 postpartum (21.7 ± 4.5 vs. 10.0 ± 3.4 LP nmol:CR mmol, P < 0.05) and sows
that had an early peak at day 19 postpartum (16.9 ± 4.5 vs. 1.0 ± 6.0 LP nmol:CR mmol, P < 0.05).

None of the sow lactation variables that were recorded differed among peak classes, though several litter characteristics did (Table 1). Sows that exhibited a late peak suckled fewer pigs after day 2 cross fostering than sows that had an early peak in HP and LP excretion (P < 0.05). Fewer pigs and thus lower litter weights were weaned at day 14 from sows that exhibited a late or no peak compared to sows that had a mid peak in HP and LP excretion (P < 0.01). These differences between peak classes were apparently due to the number of pigs suckled after day 2 cross fostering and are related to differences in management, not sow performance. Sows that had no peak in HP and LP excretion did however wean a lower litter weight at final weaning compared to sows that exhibited a late peak (P < 0.01), and the number of pigs weaned was not different (P > 0.05) between these groups.

**Discussion**

The pyridinium crosslinks HP and LP are small trivalent molecules that form on mature collagen and link two of the three non-helical amino-terminal or carboxy-terminal ends of one collagen molecule to a site on the helical portion of an adjacent collagen molecule (review: Eyre, 1996). The HP crosslink is somewhat ubiquitous in that it is present on the collagen of many tissues, whereas most of the LP in the body is concentrated in bone and dentin (Eyre, 1996). When collagen is degraded, these crosslinks are released connected to peptide fragments, which may be further cleaved in the liver or kidneys. The crosslinks
themselves, however, are not metabolized and are excreted (Eyre, 1996). Consequently, the measurement of urinary crosslinks excretion has been developed and extensively studied as a clinical tool to monitor levels of bone collagen catabolism in humans (review: Fraser, 1998).

The present experiments provide novel data on urinary excretion of HP and LP by postpartum sows that are consistent with increased catabolism of a soft, non-bone collagen source. Within 7 to 11 days postpartum, urinary excretion of total and free HP and LP increased 2.3-fold in the lactating sows in experiment I. Levels of total and free LP alone also increased approximately 2-fold but did so over a longer interval of 15 to 21 days. In addition, there was much less LP than HP present in postpartum sow urine. In fact, the molar ratio of total HP:LP during lactation averaged 9 and consistently exceeded the HP:LP ratios of 3 to 5 reported for porcine bone collagen (Nicodemo et al., 1998). The uterus contains virtually no LP (Kaidi et al., 1991) and like most other soft tissues has a high HP:LP ratio (Fraser, 1998). Thus, the abundance of HP relative to LP in lactating sow urine suggests that catabolism of some soft, non-bone tissue is primarily responsible for the postpartum increase in crosslinks excretion. The uterus is a likely candidate given the extensive and rapid collagen degradation that occurs during postpartum involution (Woessner and Brewer, 1963; Gunja-Smith et al., 1989), which represents the fastest rate of non-pathological collagen catabolism known (Harkness and Harkness, 1954; Harkness and Moralee, 1956).

A somewhat larger increase in total HP excretion and HP:LP ratios as high as 10 to 20 have been noted during the postpartum period in dairy cows (Kaidi et al., 1991; Liesegang et al., 2000) and women (Stone and Franzblau, 1995). Urinary HP increased 3 to 3.5-fold within 6 to 14 days postpartum in dairy cows and returned to prepartum levels by 20 days in
one study (Kaidi et al., 1991) but not until 120 days postpartum in another (Liesegang et al.,
2000). In women, urinary HP increased 5-fold within 14 to 21 days postpartum and
decreased to prepartum levels by 42 days postpartum (Stone and Franzblau, 1995). In the
present study, urinary HP excretion did not return to peripartum (day –1 and 1) levels by
weaning (21 days postpartum) or by day 8 postweaning (29 days postpartum) in sows. Two
other studies reported that urinary HP increased only 1.5 to 2-fold by day 14 to 28
postpartum in women (Yamaga et al., 1996; Naylor et al., 2000). Similar to our results,
Naylor et al. (2000) found that the mean molar ratio of total urinary HP:LP increased from
4.7 to 7.8 by day 14 postpartum. Comparison of results among studies is difficult due to
differences in the timing and frequency of sample collection relative to parturition, and
species differences in the type of uterus and placentation. In general, our results are similar
to those reported for dairy cows and women. It is noteworthy that the peak in HP excretion
around days 7 to 11 in experiment I occurred just after the first week postpartum, when the
majority of uterine weight loss in sows is known to occur (Palmer et al., 1965a; Graves et al.,
1967). In addition, the gradual increase and peak in LP excretion that occurred at days 15 to
21 postpartum is consistent with the gradual increase in nutrient demands and resulting tissue
catabolism that occurs as lactation progresses in sows (Whittemore and Morgan, 1990),
which may have increased degradation of bone collagen. Thus, the postpartum change in
excretion of each type of crosslink seems to match known patterns of tissue catabolism in the
sow.

Previously, the only available data on urinary HP and LP in swine were from a single
time point or were averages from several time points in growing pigs (Pointillart et al., 1997,
Weiler et al. (2001) reported total urinary HP and LP in 70 kg barrows averaged 139 and 12 nmol:CR mmol, respectively, whereas the 230 kg sows in experiment I excreted a total HP and LP average of 368 and 45 nmol:CR mmol, respectively. Differences in physiological state but not body weight between growing pigs and lactating sows should account for their different levels of crosslinks excretion since the data are adjusted to CR, which is directly related to muscle mass and body weight (Deguchi, 1997). Free urinary LP excreted by the sows in experiment I averaged 11 nmol:CR mmol and was similar to the free urinary LP ratios of 11 and 21 nmol:CR mmol reported for 20 and 10 kg growing pigs, respectively (Pointillart et al., 1997, 2000). Collectively, these data suggest that excretion of HP and catabolism of non-bone tissue is considerably elevated in postpartum sows as compared to growing pigs.

The ELISA used in the present experiments represent a reliable means to measure free HP and LP in sow urine based on the parallelism of serial dilutions of sow urine to their human standard curves, their low intra- and inter-assay variation (< 8%), and their positive correlation with HPLC estimates of total HP and LP (r = 0.85 to 0.91). Therefore, data from experiment I support human studies that found comparable performance between these immunoassays and the HPLC technique, which is considered the ‘gold standard’ for pyridinium crosslinks determination (Robins et al., 1994; Kamel et al., 1995; Gomez et al., 1996). Since the antibodies in these ELISA can only bind free crosslinks forms and only the HPLC technique was preceded by an acid hydrolysis step that frees up peptide-bound crosslinks, comparison of these two techniques provided an estimate of the amount of free versus total crosslinks. Free HP and LP expressed as a percentage of total HP and LP was
approximately 25 to 35% in postpartum sow urine, whereas estimates of free HP and LP in human urine are typically 40 to 50% (Robins et al., 1994; Kamel et al., 1995; Gomez et al., 1996). The reduced percentage of free HP and LP in this study as compared to human studies may be related to the predominance of soft tissue catabolism instead of bone collagen catabolism in postpartum sows. Another explanation would be a species or physiological state-based difference in the hepatic or renal metabolism of peptide-bound crosslinks (Eyre, 1996). However, it should also be noted that since the ELISA were not calibrated against the HPLC assay in this study, the crosslinks estimates they generated could differ in part because the two techniques are based on different principles. Despite the 6% postweaning increase of the percentage of free HP and LP in experiment I, the percentage of free HP and LP remained relatively constant during lactation and we chose to use the less technical ELISA technique to estimate urinary crosslinks in a second experiment.

Based on the results of experiment I, sampling was concentrated on the lactation period and a larger number of sows were monitored in experiment II to estimate variation of postpartum crosslinks excretion among sows. Plots of ELISA crosslinks estimates from day 1 to 19 of lactation revealed four distinct excretion patterns. Surprisingly, one third of the sows (7 out of 21) failed to exhibit a peak (section 2.3) in free HP and LP excretion during lactation. There was a significant increase in crosslinks excretion between day 1 and 10 postpartum in these sows, but it was not large enough to constitute an HP and LP peak, such as those that occurred during early (day 1), mid (day 7), or late (day 10, 13, or 16) lactation in the other two-thirds of the sows. It is difficult to ascertain the cause of this variation among sows in postpartum crosslinks excretion since the only differences between the peak
classes were related to variation in litter management and not the sows themselves. One exception was the early peak in HP and LP on day 1 postpartum in three sows, which was primarily due to their abnormally low CR levels on day 1 and 4 postpartum compared to the remainder of lactation (2.4 vs. 6.8 mmol/L). One of the three sows that exhibited an early HP and LP peak did require some manual assistance during farrowing but since the other two farrowed normally, this abnormal CR excretion pattern was not necessarily related to dystocia. Levels of CR were relatively stable throughout lactation in all the other peak classes. In theory, sows that gestated the largest litters should have had the most collagen to degrade and crosslinks to excrete postpartum since fetal mass dictates how much collagen is deposited in the uterus during gestation (Harkness and Harkness, 1954). However, no significant correlation between total pigs born (range; 6 to 16) or litter weight at day 2 (range; 7.9 to 18.7 kg) and subsequent crosslinks excretion was found.

A number of factors can introduce variation into estimation of urinary HP and LP (review: Vesper et al., 2002). There is some diurnal variation in urinary crosslinks excretion, so a portion of the variation within and among sows is no doubt related to the fact that we were not able to obtain samples for all of the sows during the same time period each day. The inherent inaccuracy of adjusting crosslinks estimates to CR excretion likely introduced some variation into the dataset as well. The remainder of the unexplained variation among sows may represent biological variation in the rate of uterine collagen degradation and(or) in the metabolism and excretion of collagen degradation products. The amount of variation in the rate of uterine involution among sows is not well defined, though it is interesting that a histological study by Geissinger et al. (1980) indicates it can be substantial. Overall, data
from this experiment, like those from experiment I, indicate that the catabolism of non-bone collagen increases during the postpartum period. The source of the variation among sows in the postpartum increase of free HP excretion warrants further investigation given the potential research and production applications of this technique.

**Conclusions**

Based on the amount and type of pyridinium crosslinks excreted by lactating sows, these data suggest that the degradation of collagen from some soft, non-bone tissue dominates the postpartum period when uterine involution occurs. Relating the excretion of these collagen degradation markers to some physical measure of involution will be necessary to establish their validity and may reveal why such significant variation among sows in the magnitude and timing of peak HP excretion exists. Should this technique prove sound, it would provide researchers with a novel tool to study the effects of uterine involution on subsequent embryo survival and might provide pork producers with a sow management tool to base weaning and pharmaceutical intervention decisions on.
Literature Cited


Table 1. Effect of time of peak urinary hydroxylysyl pyridinoline (HP) and lysyl pyridinoline (LP) to creatinine (CR) ratio during lactation (peak class) on sow and litter performance (least squares means ± SEM)

<table>
<thead>
<tr>
<th>Peak Class</th>
<th>Early</th>
<th>Mid</th>
<th>Late</th>
<th>No Peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak day</td>
<td>1</td>
<td>7</td>
<td>10, 13, 16</td>
<td>----</td>
</tr>
<tr>
<td>No. sows</td>
<td>3</td>
<td>4</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Parity</td>
<td>3.3 ± 1.5</td>
<td>2.0 ± 1.3</td>
<td>4.6 ± 1.0</td>
<td>4.1 ± 1.0</td>
</tr>
<tr>
<td>Sow weight prepartum (kg)</td>
<td>240.4 ± 14.9</td>
<td>217.1 ± 12.9</td>
<td>229.9 ± 9.7</td>
<td>231.0 ± 9.7</td>
</tr>
<tr>
<td>No. total pigs born</td>
<td>10.7 ± 2.0</td>
<td>11.3 ± 1.7</td>
<td>11.9 ± 1.3</td>
<td>11.3 ± 1.3</td>
</tr>
<tr>
<td>No. pigs born alive</td>
<td>10.7 ± 1.8</td>
<td>10.3 ± 1.5</td>
<td>8.7 ± 1.2</td>
<td>10.7 ± 1.2</td>
</tr>
<tr>
<td>Litter weight day 2 (kg)</td>
<td>15.0 ± 1.7</td>
<td>17.0 ± 1.5</td>
<td>14.1 ± 1.1</td>
<td>14.7 ± 1.1</td>
</tr>
<tr>
<td>No. pigs suckled day 2a</td>
<td>12.0 ± 0.8y</td>
<td>10.8 ± 0.7xy</td>
<td>9.4 ± 0.5x</td>
<td>10.4 ± 0.5xy</td>
</tr>
<tr>
<td>*No. pigs weaned day 14</td>
<td>3.7 ± 0.7y</td>
<td>4.8 ± 0.6y</td>
<td>2.3 ± 0.4x</td>
<td>2.1 ± 0.4x</td>
</tr>
<tr>
<td>*Litter weight weaned day 14 (kg)</td>
<td>17.5 ± 3.6y</td>
<td>24.1 ± 3.1y</td>
<td>10.1 ± 2.4x</td>
<td>10.0 ± 2.4x</td>
</tr>
<tr>
<td>No. pigs weaned final</td>
<td>5.3 ± 0.3</td>
<td>5.3 ± 0.3</td>
<td>5.6 ± 0.2</td>
<td>5.1 ± 0.2</td>
</tr>
<tr>
<td>*Litter weight weaned final (kg)</td>
<td>38.2 ± 1.9y</td>
<td>38.0 ± 1.6xy</td>
<td>40.9 ± 1.2y</td>
<td>34.5 ± 1.2s</td>
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<tr>
<td>Lactation length (day)</td>
<td>21.3 ± 0.9</td>
<td>22.5 ± 0.7</td>
<td>20.9 ± 0.5</td>
<td>21.1 ± 0.5</td>
</tr>
<tr>
<td>Sow weight at weaning (kg)</td>
<td>206.5 ± 14.6</td>
<td>207.3 ± 12.6</td>
<td>205.4 ± 9.5</td>
<td>211.1 ± 9.5</td>
</tr>
<tr>
<td>Sow weight change (kg)</td>
<td>-33.9 ± 9.7</td>
<td>-9.9 ± 8.4</td>
<td>-24.5 ± 6.3</td>
<td>-19.8 ± 6.3</td>
</tr>
<tr>
<td>*Mean HP &amp; LP:CR (nmol:mmol)b</td>
<td>149.2 ± 18.1y</td>
<td>135.9 ± 15.7y</td>
<td>132.4 ± 11.9y</td>
<td>84.6 ± 11.9x</td>
</tr>
<tr>
<td>*Std. Dev. HP &amp; LP:CR (nmol:mmol)c</td>
<td>88.6 ± 12.9x</td>
<td>69.1 ± 11.2yz</td>
<td>53.4 ± 8.4y</td>
<td>24.2 ± 8.4x</td>
</tr>
<tr>
<td>Mean LP:CR (nmol:mmol)b</td>
<td>20.5 ± 3.4y</td>
<td>14.8 ± 2.9xy</td>
<td>14.4 ± 2.2yv</td>
<td>9.6 ± 2.2x</td>
</tr>
<tr>
<td>*Std. Dev. LP:CR (nmol:mmol)c</td>
<td>16.4 ± 2.1y</td>
<td>5.6 ± 1.8x</td>
<td>5.1 ± 1.4x</td>
<td>3.8 ± 1.4x</td>
</tr>
</tbody>
</table>

*aSignificant effect of peak class in model (P < 0.05).
*bNumber of pigs suckled after day 2 cross fostering.
*cMean of sow standard deviations across days 1, 4, 7, 10, 13, 16, and 19 postpartum.
*x, y, z Means within a row without a common superscript letter differ (P < 0.05).
Figure 1. HPLC (total) and ELISA (free) urinary hydroxylysyl pyridinoline (HP) and lysyl pyridinoline (LP) ratios excreted by five different sows relative to parturition. (a) HP and LP:CR; (b) LP:CR; (c) Molar ratio of HP:LP. Vertical dashed lines mark parturition (day 0) and weaning (day 21).
Figure 2. Least squares means (- SEM) of HPLC (total) and ELISA (free) urinary hydroxylsyl pyridinoline (HP) and lysyl pyridinoline (LP) ratios excreted by five sows relative to parturition. (a) HP and LP:CR; (b) LP:CR; (c) Molar ratio of HP:LP. w, x, y, z Day means within a plotted line without a common letter differ (P < 0.05). Vertical dashed lines mark parturition (day 0) and weaning (day 21).
Figure 3. Correlation between HPLC (total) and ELISA (free) estimates of hydroxylsyl pyridinoline (HP) and lysyl pyridinoline (LP) concentration in sow urine samples from experiment I. (a) ELISA HP and LP versus HPLC HP + LP; (b) ELISA LP versus HPLC LP. Correlation (r) significant at P < 0.001 for both (a) and (b).
Figure 4. ELISA (free) urinary hydroxylysyl pyridinoline (HP) and lysyl pyridinoline (LP) ratios excreted by lactating sows that exhibited a peak in HP and LP on: (a) day 1 (early, n = 3); (b) day 7 (mid, n = 4); (c) day 10, 13, or 16 (late, n = 7); or (d) no significant peak (no peak, n = 7). Vertical dashed lines mark parturition (day 0).
Figure 5. Least squares means (- SEM) of ELISA (free) urinary hydroxylysyl pyridinoline (HP) and lysyl pyridinoline (LP) ratios excreted by lactating sows that exhibited a peak in HP and LP on: day 1 (early, n = 3); day 7 (mid, n = 4); day 10, 13, or 16 (late, n = 7); or no significant peak (no peak, n = 7). (a) Free HP and LP ratios by peak class; (b) free LP ratios by peak class. Means marked with a *, **, and *** differ P < 0.05, P < 0.01, and P < 0.001, respectively, from one or more of the other day means at that time point. Vertical dashed lines mark parturition (day 0).
CHAPTER 4

POSTPARTUM CHANGES IN THE MORPHOLOGY OF THE CERVIX, UTERUS, AND OVARIES OF SOWS
Abstract

Despite the potential influence of postpartum uterine involution on subsequent reproductive success in the sow, techniques to measure the involution process have not been developed. The primary objective of this preliminary study was to evaluate techniques to measure postpartum change in the morphology of the cervix and uterus. The cervical penetration depth of a spiral-tipped and a blunt-tipped AI catheter was measured daily in 12 mixed parity sows from day 1 postpartum until weaning (20.6 ± 0.4 day lactation). Daily transabdominal ultrasonography was used to measure the distance between the abdominal wall and intestines (“gut loops”), which sandwich the uterus. Day postpartum affected (P < 0.001) both catheter penetration depth and abdomen-to-gut loop distance. Penetration depth only decreased (P < 0.05) from day 1 to 3 postpartum for the spiral-tipped catheter (420.4 ± 17.6 to 317.5 ± 17.6 mm). Abdomen-to-gut loop distance decreased (P < 0.05) from 125.4 ± 2.9 mm at day 1 to 38.7 ± 2.9 mm by day 18 postpartum. The average follicle diameter (FD) of the three largest follicles present was also estimated daily postpartum and every 8 h postweaning by the same ultrasonography technique. Day postpartum affected (P < 0.001) average FD because it decreased (P < 0.05) from 7.2 ± 0.4 mm at day 1 to 3.9 ± 0.4 mm by day 12 postpartum. Detection of estrus every 8 h postweaning revealed a weaning-to-estrus interval × day postpartum interaction (P < 0.001) for average FD. Sows that returned to estrus ≤ 3 days postweaning had greater (P < 0.05) average FD on several different days postpartum than sows that returned to estrus ≥ 5 days postweaning. Sows that returned to estrus ≥ 5 days postweaning: had lower (P < 0.05) parity; suckled more (P < 0.05) pigs; and weaned more (P < 0.05) pigs than sows that returned to estrus ≤ 3 days postweaning. There
was a negative correlation between average FD and weaning-to-estrus interval during the last 3 days preweaning ($r = -0.69$, $P < 0.05$) and during the first 3 days postweaning ($r = -0.80$, $P < 0.01$). These data indicate that abdomen-to-gut loop distance, but not AI catheter penetration depth, could be a suitable means to monitor postpartum involution of the reproductive tract. The average FD data suggest that follicle size in the days just prior to weaning and just after weaning is related to the length of weaning-to-estrus and weaning-to-ovulation intervals.

**Introduction**

Postpartum involution of the cervix and uterus is essentially a tissue turnover process that allows these reproductive tract components to regress to their non-gravid state. Some degree of involution is likely a prerequisite for subsequent reproduction and studies suggest the process is complete within 14 to 21 days postpartum in sows (review: Kiracofe, 1980). Sows can reproduce successfully after lactations of only a few days, but embryo survival and conception rate are substantially reduced compared to levels achieved after longer lactations (reviews: Varley, 1982; Varley and Foxcroft, 1990). In general, lactation lengths less than 21 days result in a lower subsequent farrowing rates and litter sizes than longer lactations (Mabry et al., 1996; Gaustad-Aas et al., 2003). Given that lactation lengths among US swine breeding farms currently average approximately 18 days (USDA, 2001; PigCHAMP® Inc., 2002), incomplete postpartum involution may be limiting reproductive performance.

Despite the potential influence of postpartum uterine involution on subsequent reproductive success in the sow, techniques to measure the involution process have not been
developed. Traditionally, physical measurements of uterine weight and length and/or histological measurements of different uterine tissues from excised reproductive tracts at different postpartum intervals have been used to assess the state of the involution process (Palmer et al., 1965a, 1965b; Graves 1967; Svajgr et al., 1974; Hays et al., 1978; Geissinger et al., 1980). The major limitations of these methods are that they are not repeatable within an individual and they preclude any observation of subsequent reproductive function. Thus, incomplete postpartum involution has only been associated with and not causally linked to increased embryo mortality. More recently, indirect methods to monitor postpartum uterine involution by real-time ultrasonography (Martinat-Botté et al., 2000; Lee et al., 2000) and by excretion of biochemical markers (Belstra et al., 2003) have been investigated. These new techniques require validation through correlation with more direct measures of postpartum changes and with postmortem measurements of the reproductive tract. Therefore, the primary objective of this preliminary study was to evaluate techniques to measure postpartum changes in the morphology of the cervix and uterus. An additional aim was to monitor ovarian follicle size postpartum and postweaning to determine if and when follicle size is related to the weaning-to-estrus interval.

**Materials and Methods**

*Animals*

A group of 12 pregnant Landrace (n = 7) and Yorkshire (n = 5) sows (parity 1 to 9) at the North Carolina State University Swine Education Unit were utilized. Sows were moved at approximately 110 days of gestation to the farrowing barn where they were hand-washed.
and loaded into farrowing crates. Sows had ad libitum access to water and were fed approximately 3.6 kg of a corn-soybean meal based diet (15.8% crude protein, 0.85% lysine, and 3,307 kcal ME/kg) twice daily. Sows farrowed from November 19th to the 22nd of 2003. Sow and litter lactation performance data were recorded. Beginning on the day after parturition (day 1 postpartum), three different reproductive tract measurements were made daily between 07:30 and 08:30 h on each sow until the day of weaning (mean ± SEM, 20.6 ± 0.4 days; range, 17.5 to 22.0 days).

Postpartum cervical measurement

First, obstetrical lubricant (O B Lube; Agri Laboratories, Ltd., St. Joseph, MO, USA) was applied to a blunt-tipped AI catheter (Goldenpig®; IMV International Corp, Maple Grove, MN, USA; tip outer diameter × length = 21 × 31 mm) and it was introduced into the vagina and gently advanced as far into the cervix as possible. In case the vagina had been stretched, the shaft of the catheter was released briefly to allow it to spring back. Then, the shaft of the catheter was grasped where it protruded from the vulva and it was gently removed. A measurement of the depth of catheter penetration was taken in millimeters with a 45 cm ruler. This process was then repeated with a spiral-tipped AI catheter (Original Spirette™; Minitube of American, Verona, WI, USA; tip outer diameter × length = 15 × 83 mm), which was advanced with a counter-clockwise rotation. Both disposable AI catheters were bathed in a pan of soap and hot water between examinations and replaced daily.
Postpartum uterine measurement

Next, uterine size was estimated via transabdominal real-time ultrasonography using an Aloka 500V equipped with a 5.0 MHz linear convex transducer (Aloka Co. Ltd., Wallingford, CT, USA). The transducer was coated with ultrasound transmission gel (Eco-Gel; TheraQuip, Greensboro, NC, USA) and placed on the sow’s right rear flank in the inguinal fold. An image of the intestines (“gut loops”) was captured and the maximum distance between the inner edge of the abdominal wall and intestines that sandwich the uterus was measured as described by Martinat-Botté et al. (2000; Fig. 1). An effort was made to only apply enough pressure on the transducer to obtain a clear image and avoid compression of the abdominal cavity.

Postpartum ovarian measurement

Finally, the same transabdominal ultrasonography technique was used to estimate ovarian follicular diameter. Using the bladder as a landmark, an ovary was located, examined, and an image that maximized the diameter of three largest follicles was captured. The diameters of these three largest follicles were recorded (Fig. 2). Digital copies of all the uterine and ovarian images that were captured were saved to a floppy drive in a connected color printer (Sony® FVP-1/1E; Sony Corp., Tokyo, Japan) and later archived in a PC.

Postweaning detection of estrus and ovulation

Piglets were weaned on December 11th of 2003 at 08:00 h and the sows were moved to the breeding barn, where they were housed in individual crates. A mature boar was
selected to detect estrus. On day 1 postweaning at 08:00 h, the same ultrasonography technique was used to estimate diameter of the three largest follicles, and sows were checked for estrus in their crates by the back pressure test during nose-to-nose boar contact. From day 2 postweaning on, ovarian examination and detection of estrus were performed every 8 h, at 06:00, 02:00, and 10:00 h. Ovarian examination and detection of estrus continued until ovulation occurred and estrus ended, respectively. The criteria used to determine when ovulation had occurred were: either the disappearance of large follicles; or a substantial decrease in follicular diameter. An additional scan was performed 8 h later to confirm ovulation. The times for onset or end of estrus and ovulation were considered to be 4 h prior to the estrous check and ultrasound scan, respectively, where a change occurred. On day 10 postweaning, sows that had not expressed estrus were classified as anestrus and the study was terminated.

Statistical analyses

The effects of time (day relative to parturition) on AI catheter penetration depth, abdomen-to-gut loop distance, and average follicle diameter (FD) were examined by repeated measures analysis of variance through use of the mixed procedure of SAS (Littell et al., 1998). The least squares day means generated were compared with Student’s t-tests. A similar repeated measures analysis was performed on postpartum and postweaning average FD except individual sows were categorized into three classes based on their weaning-to-estrus interval (≤ 3 days, 4 days, ≥ 5 days). The effects of time (day), weaning-to-estrus interval class, and their interaction were included in this model. Pearson’s correlation
coefficients were computed to compare relationships among dependent variables. The effects of weaning-to-estrus interval and parity class (1, 2, ≥ 3) on the dependent variables total pigs born, pigs born alive, birth weight, pigs suckled, lactation length, pigs weaned, weight weaned, duration of estrus (DE), estrus onset-to-ovulation interval (EOI), weaning-to-ovulation interval (WOI), average FD on different days postpartum and postweaning, and the change in average FD were also examined by analysis of variance (Snedecor and Cochran, 1967) through use of the general linear models procedure of SAS (Muller and Fetterman, 2002). Sow was the experimental unit used to estimate error. The least squares means generated were compared with f-tests. Average FD data from one sow had to be omitted for the analyses because it developed multiple, large (15 to 25 mm), follicular cysts during lactation and did not return to estrus or ovulate postweaning.

**Results**

*Catheter penetration depth*

There was an effect of time (day postpartum; P < 0.001) on the cervical penetration depth of both the spiral-tipped and blunt-tipped AI catheters (Fig. 3a). However, penetration depth only decreased (P < 0.05) from day 1 to 3 postpartum for the spiral-tipped catheter (420.4 ± 17.6 to 317.5 ± 17.6 mm). Neither weaning-to-estrus interval (P > 0.25) nor parity (P > 0.35) class affected penetration depth of the spiral-tipped and blunt-tipped catheters. There was a positive correlation between the decrease of penetration depth of the spiral-tipped catheter from day 1 to 3 postpartum and average FD at day 1 and days 1 to 3 postpartum (r = 0.65 and r = 0.60, P < 0.05).
Abdomen-to-gut loop distance

There was an effect of time (P < 0.001) on abdomen-to-gut loop distance, which decreased (P < 0.05) from 125.4 ± 2.9 mm at day 1 to 38.7 ± 2.9 mm by day 18 postpartum (Fig. 3b). Neither weaning-to-estrus interval (P = 0.18) nor parity (P = 0.35) class affected abdomen-to-gut loop distance. There tended to be a negative correlation between the decrease of abdomen-to-gut loop distance from day 1 postpartum to weaning and average FD during the last 6 days, last 3 days, and last day preweaning (days 5 to 0, days 2 to 0, and day 0; r = -0.52 to -0.56, P < 0.10).

Average follicle diameter

Average FD was also affected by time (P < 0.001) because it decreased (P < 0.05) from 7.2 ± 0.4 mm at day 1 to 3.9 ± 0.4 mm by day 12 postpartum (Fig. 3c). Neither weaning-to-estrus interval (P = 0.31) nor parity (P = 0.46) class affected the average FD. However, there was a weaning-to-estrus interval × time interaction (P < 0.001). Sows that had a weaning-to-estrus interval of ≤ 3 days tended (P < 0.10) to have greater average FD at days 11, 12, 15, 19, and 20 postpartum, and had greater (P < 0.05) average FD at day 7 and 21 postpartum compared to sows that had a weaning-to-estrus interval of ≥ 5 days (Fig. 4a). When average FD data were reorganized relative to the day of weaning, there was still a weaning-to-estrus interval × time interaction (P < 0.05), but sows that had a weaning-to-estrus interval of ≤ 3 days only had a greater (P < 0.05) average FD than sows that had a weaning-to-estrus interval of ≥ 5 days at day -8 preweaning (Fig. 4b). There was an effect of
time (P < 0.001) and weaning-to-estrus interval class (P < 0.05) on average FD during the postweaning period, but no weaning-to-estrus interval × time interaction was detected (P = 0.39; Fig. 4c). Sows that had a weaning-to-estrus interval of ≤ 3 days had greater (P < 0.05) average FD than sows that had a weaning-to-estrus interval of ≥ 5 days at 2.3, 2.6, 2.9, 3.3, 3.6, 3.9, 4.3, 4.6, 4.9, and 5.3 days postweaning. Sows that had a weaning-to-estrus interval of ≤ 3 days had greater (P < 0.05) average FD than sows that had a weaning-to-estrus interval of 4 days at 2.3, 2.6, 2.9, and 3.6 days postweaning. Sows that had a weaning-to-estrus interval of 4 days only had greater (P < 0.05) average FD than sows that had a weaning-to-estrus interval of ≥ 5 days at 5.6 days postweaning. Average FD data for individual sows in the ≤ 3 day, 4 day, and ≥ 5 day weaning-to-estrus interval classes during the preweaning (Fig. 5a, b, c) and postweaning (Fig. 6a, b, c) periods were plotted to illustrate the variation of average FD among sows.

Sows that returned to estrus ≤ 3 days postweaning had greater (P < 0.05) parity and more (P < 0.05) pigs born alive than sows that returned to estrus ≥ 5 days postweaning (Table 1). Sows that had a weaning-to-estrus interval of ≥ 5 days: suckled more (P < 0.05) pigs at day 2 postpartum than sows that had a weaning-to-estrus interval of 4 days; and weaned more (P < 0.05) pigs and more (P < 0.05) litter weight than sows that had a weaning-to-estrus interval of ≤ 3 days. Sows that returned to estrus ≤ 3 days postweaning had a longer (P < 0.05) DE and EOI than sows that returned to estrus 4 and ≥ 5 days postweaning. Sows that returned to estrus ≤ 3 days postweaning had a greater (P < 0.05) average FD at day 2 and day 3 postweaning than sows that returned to estrus 4 and ≥ 5 days postweaning. Even though average FD did not differ (P > 0.05) among weaning-to-estrus interval classes at any of the
other time periods examined in this analysis, there was a negative correlation between sow weaning-to-estrus interval and average FD during the last 3 days preweaning (days 2 to 0, and day 0; \( r = -0.69 \) and \(-0.67, P < 0.05\)) and during the first 3 days postweaning (day 1, 2, and 3; \( r = -0.74 \) to \(-0.80, P < 0.01\)). Likewise, there was a negative correlation between sow WOI and average FD during the last 3 days and last day preweaning (days 2 to 0, and day 0; \( r = -0.72 \) and \(-0.71, P < 0.05\)) and during the first 3 days postweaning (day 1, 2, and 3; \( r = -0.72 \) to \(-0.76, P < 0.01\)).

When sows were categorized by parity, there were too few sows in the parity 1 and 2 classes to detect any difference among parity classes (Table 2) or estimate a parity \( \times \) weaning-to-estrus interval interaction. However, there was clearly a negative relationship between parity and weaning-to-estrus interval (\( r = -0.64, P < 0.05 \)). The number of pigs weaned and weaning-to-estrus interval had a strong positive correlation (\( r = 0.81, P < 0.01 \)).

**Discussion**

The largest obstacle to testing the effects of postpartum involution on subsequent embryo survival and reproductive performance is that traditional postmortem measures of involution are not repeatable within an individual and they preclude subsequent evaluation of reproduction (review: Kiracofe, 1980). Novel techniques that circumvent this problem by indirectly measuring postpartum involution have recently been developed for the sow (Martinat-Botté et al., 2000; Lee et al., 2000; Belstra et al., 2003), but still require postmortem validation. Results of the present study suggest that estimation of the abdomen-
to-gut loop distance via transabdominal ultrasonography, but not AI catheter penetration depth, warrants further investigation and correlation to postmortem measurements.

Measurement of the postpartum decrease of cervical diameter or increase of cervical tone via palpation per rectum has been used as an indicator of the involution process in cows (Kiracofe, 1980). The cervix, a portion of the uterus, and the ovaries can also be palpated per rectum in multiparous sows (Bollwahn, 1972; Cameron, 1977; Meredith, 1977). We had intended to utilize a small, hand-worn, electronic caliper to make a transrectal measurement of cervical diameter (Hindson et al., 1984; Watson, 1985; Tian and Noakes, 1991). Prior to the experiment, we attempted rectal palpation in several sows after manual evacuation of the fecal material present and found that the cervix could be easily located and grasped. However, we also found that there was very little space to maneuver and that making a measurement in the same location at each examination would be quite difficult in the sow. We also attempted to use transrectal ultrasonography to obtain measurements of the cervix (Knox and Althouse, 1999). A small linear transducer, stabilized by an angled plastic handle, was lubricated and introduced into the rectum. Portions of the cervix could be identified, but since the transducer face was much smaller than the cervix, and was located directly on top of and parallel to the cervix during examination, it was not possible to view large sections or obtain a transverse sectional diameter. Again, consistently making a measurement in the same location at each examination would have been very difficult.

A graduated series of different diameter conical rods, called cervical sounds, has been used to measure dilation of the cervix in gilts and sows (Zarrow et al., 1956; Smith and Nalbandov, 1958; Kertiles and Anderson; 1979). Based on these studies, we hypothesized
that it might be possible to indirectly estimate postpartum changes in cervical diameter and (or) how distensible the cervix was by simply measuring the depth that different sized AI catheters could penetrate the lumen of the cervix. The present results indicate that the depth of AI catheter penetration only decreased from day 1 to 3 postpartum and only for the spiral-tipped, not the blunt-tipped catheter. This decrease and difference between catheter types occurred because on day 1 and 2 postpartum, the spiral-tipped catheter could be passed completely through the cervix in most sows, while the blunt-tipped catheter usually could not. There was a large amount of variation in the postpartum penetration depth of each catheter both within and among sows. Though effort was made to use a similar amount of force to introduce each catheter type at each examination, it was not possible to control this likely source of variation. Sometimes the catheter tip may have gotten past a cervical fold that it had gotten caught on in other examinations and it is likely that this added variation to the measurements. In addition, a large portion of the catheter penetration depth was vaginal not cervical, and postpartum changes in vaginal length could have affected the measurement. Overall, this simple measurement is too subjective and probably lacks the precision to be useful. However, it is interesting that the decrease in cervical penetration depth of the spiral-tipped catheter from day 1 to 3 postpartum was positively associated with average FD during this period. Since estrogen tends to increase the tone of the cervix and make it more rigid (Smith and Nalbandov, 1958; Kunavongkrit et al., 1983), it seems logical that larger, more estrogenic follicles might decrease the diameter of the lumen of the cervix and decrease catheter penetration depth.
Real-time ultrasonography has been used to monitor postpartum changes in uterine echotexture (Irie, 1987), uterine wall thickness (Lee et al., 2000), and uterine size (Martinat-Botté et al., 2000) in the sow. In the present study, we observed an 85 mm decrease of abdomen-to-gut loop distance from day 1 to 18 postpartum that was similar both in magnitude and timing to the decrease in this measure reported by Martinat-Botté et al. (2000). Since the uterus occupies the area between the abdominal wall and intestines, this decrease of abdomen-to-gut loop distance should be related to the postpartum decrease in uterine size that occurs during this same interval (Palmer et al., 1965a; Graves et al., 1967). Indeed, transabdominal ultrasonography estimates of uterine size in peripubertal gilts by measurement of abdomen-to-gut loop distance, the area occupied by the uterus (Martinat-Botté et al., 2003), and transverse sectional area of uterine horns (Kauffold et al., 2004) have all been found to be highly correlated to postmortem uterine weights. However, it will also be necessary to confirm that abdomen-to-gut loop distance is related to the size of the larger uterus of postpartum sows. Variation of abdomen-to-gut loop distance among sows was relatively minor considering the imprecise nature of this measurement. For example, the profile of the gut loops across the captured ultrasound image was rarely horizontal and only one measurement of the maximum distance between the abdomen and gut loops was used. In addition, the presence of the bladder in some images could not be avoided and the size of the bladder could have affected the distance that the gut was displaced from the abdomen. Nonetheless, these data suggest that postmortem validation of abdomen-to-gut loop distance, as a measure of postpartum uterine size would be worthwhile.
Since there is little published information available on follicle development during lactation in the sow (Britt et al., 1985), and new hypotheses on how follicle development prior to weaning affects the weaning-to-estrus interval have been suggested (Lucy et al., 2001), postpartum and postweaning follicle data were collected. Average FD decreased from 7.2 to 3.9 mm from day 1 to 12 postpartum in the present experiment. The suckling-induced suppression of GnRH release is supposed to reduce follicle size within a few days postpartum (Varley and Foxcroft, 1990). The present results indicate that average FD remained above 5 mm for at least the first 7 to 8 days postpartum on average. In general, the average FD that we measured during lactation was greater than published reports of average follicle size during this period (Palmer et al., 1965a; Kunavongkrit et al., 1982). This discrepancy may be due to the fact that we measured the diameter of the three largest follicles present via ultrasonography whereas earlier studies measured the diameter of all of the follicles present (≥ 2 mm) at postmortem examination.

Soede et al. (1998) used transrectal ultrasonography to examine ovarian follicles and reported that average follicle size at day 3 postweaning was negatively correlated with weaning-to-estrus interval (r = -0.24) and WOI (r = -0.36) in a group of 115 sows. In this much smaller group of sows (n = 11), we observed a greater negative correlation between average FD and weaning-to-estrus interval during the first 3 days postweaning (r = -0.74 to −0.80) and also during the last three days preweaning (r = -0.67 to −0.69). In fact, sows that returned to estrus ≤ 3 days tended to have a greater average FD at several time points during lactation than sows that required ≥ 5 days to return to estrus postweaning. It is clear that sow parity and litter size suckled had an affect on the average FD reached just prior to weaning.
and the subsequent weaning-to-estrus interval. Since there was no time × weaning-to-estrus interval interaction for average FD postweaning, the results of this study concur with those of Lucy et al. (2001) and Bracken et al. (2003). Their transrectal ultrasonography observations indicate that follicles develop at similar rates postweaning for sows that have different weaning-to-estrus intervals, follicles simply initiate development at different times postweaning. However, results of this study revealed more variation in average FD in the days just prior to weaning among sows than reported by Lucy et al. (2001) and this variation explained a significant amount of the variation of their weaning-to-estrus interval and WOI. It is noteworthy that Lucy et al. (2001) and Bracken et al. (2003) measured all of the follicles ≥ 3 mm present at each scan whereas our observations were based on only the largest three follicles we could locate. These data support the conclusion of Soede et al. (1998), accurate prediction of postweaning estrus and ovulation based on follicle size is not likely. However, these data suggest that it may be possible to identify sows that will have a short (≤ 3 day) or a long (≥ 5) weaning-to-estrus interval a few days prior to weaning or at weaning.

Conclusions

These data indicate that abdomen-to-gut loop distance, but not AI catheter penetration depth, could be a suitable means to monitor postpartum involution of the reproductive tract. Comparison of abdomen-to-gut loop distances to postmortem measurements of uterine size and weight will be necessary to validate this technique. Average follicle diameter in the days just prior to weaning and just after weaning is related to the length of weaning-to-estrus and weaning-to-ovulation intervals. Identifying sows that are likely to have a very short or very
long weaning-to-estrus interval prior to weaning would give pork producers the opportunity to apply appropriate management.
**Literature Cited**


Table 1. Sow and litter lactation performance, sow postweaning estrus and ovulation, and postpartum and postweaning follicular diameter by weaning-to-estrus interval class (least squares means ± SEM)

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<th>Item</th>
<th>Weaning-to-estrus interval</th>
<th>Model P-value</th>
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<td>4 days</td>
</tr>
<tr>
<td>No. sows</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td>6.3 ± 1.3z</td>
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<td>Total pigs born</td>
<td>15.3 ± 1.8</td>
<td>12.6 ± 1.4</td>
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<td>Pigs born alive</td>
<td>14.0 ± 1.5x</td>
<td>11.6 ± 1.1xz</td>
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<td>Birth weight (kg)</td>
<td>18.5 ± 1.9</td>
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<td>Pigs suckled, day 2</td>
<td>10.0 ± 0.6xz</td>
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<td>Lactation length (days)</td>
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<td>Pigs weaned</td>
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<td>8.8 ± 0.6xz</td>
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<td>Weight weaned (kg)</td>
<td>40.2 ± 5.5y</td>
<td>56.6 ± 4.2xz</td>
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<td>Wean-to-estrus interval (days)</td>
<td>2.3 ± 0.4y</td>
<td>4.2 ± 0.3yz</td>
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<td>Duration of estrus (h)</td>
<td>71.3 ± 4.3z</td>
<td>57.6 ± 3.3yz</td>
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<td>Estrus-to-ovulation interval (h)</td>
<td>60.7 ± 4.8x</td>
<td>41.6 ± 3.8yz</td>
</tr>
<tr>
<td>Wean-to-ovulation interval (h)</td>
<td>115.1 ± 6.7x</td>
<td>142.9 ± 5.2xz</td>
</tr>
<tr>
<td>FD day 1 postpartum (mm)a</td>
<td>7.8 ± 1.0</td>
<td>5.9 ± 0.8</td>
</tr>
<tr>
<td>FD day 1 to 3 postpartum (mm)a</td>
<td>7.6 ± 0.9</td>
<td>6.3 ± 0.7</td>
</tr>
<tr>
<td>FD day 1 to 6 postpartum (mm)a</td>
<td>7.0 ± 0.8</td>
<td>6.3 ± 0.7</td>
</tr>
<tr>
<td>FD day 5 to 0 preweaning (mm)a</td>
<td>4.9 ± 0.7</td>
<td>3.7 ± 0.5</td>
</tr>
<tr>
<td>FD day 2 to 0 preweaning (mm)a</td>
<td>4.9 ± 0.6</td>
<td>3.5 ± 0.5</td>
</tr>
<tr>
<td>FD day 0 weaning (mm)a</td>
<td>4.8 ± 0.7</td>
<td>3.7 ± 0.6</td>
</tr>
<tr>
<td>FD lactation average (mm)a</td>
<td>5.8 ± 0.7</td>
<td>4.7 ± 0.5</td>
</tr>
<tr>
<td>FD lactation change (mm)ab</td>
<td>-2.7 ± 0.8</td>
<td>-2.7 ± 0.6</td>
</tr>
<tr>
<td>FD day 1 postweaning (mm)b</td>
<td>5.3 ± 0.7</td>
<td>3.8 ± 0.5</td>
</tr>
<tr>
<td>FD day 2 postweaning (mm)b</td>
<td>6.5 ± 0.7z</td>
<td>4.2 ± 0.5y</td>
</tr>
<tr>
<td>FD day 3 postweaning (mm)b</td>
<td>6.9 ± 0.6z</td>
<td>4.7 ± 0.5y</td>
</tr>
<tr>
<td>FD postweaning change (mm)ac</td>
<td>2.0 ± 0.3</td>
<td>1.2 ± 0.2</td>
</tr>
</tbody>
</table>

aAverage follicle diameter (FD) of the three largest follicles present.
bBased on average FD day 1 to 3 postpartum minus average FD day 2 to 0 preweaning.
cBased on average FD day 3 postweaning minus average FD day 2 to 0 preweaning.
x, y, zRow values without a common superscript letter differ (P < 0.05).
Table 2. Sow and litter lactation performance, sow postweaning estrus and ovulation, and postpartum and postweaning follicular diameter by parity class (least squares means ± SEM)

<table>
<thead>
<tr>
<th>Item</th>
<th>Parity Model</th>
<th>1</th>
<th>2</th>
<th>≥ 3</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. sows</td>
<td></td>
<td>2</td>
<td>2</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td>1.0 ± 1.3*y</td>
<td>2.0 ± 1.0*y</td>
<td>5.6 ± 0.7*z</td>
<td>&lt; 0.02</td>
</tr>
<tr>
<td>Total pigs born</td>
<td></td>
<td>13.0 ± 2.6</td>
<td>11.3 ± 2.1</td>
<td>13.9 ± 1.4</td>
<td>= 0.62</td>
</tr>
<tr>
<td>Pigs born alive</td>
<td></td>
<td>11.5 ± 2.1</td>
<td>10.0 ± 1.7</td>
<td>12.6 ± 1.1</td>
<td>= 0.48</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td></td>
<td>14.6 ± 2.6</td>
<td>15.4 ± 2.1</td>
<td>17.6 ± 1.4</td>
<td>= 0.52</td>
</tr>
<tr>
<td>Pigs suckled, day 2</td>
<td></td>
<td>11.0 ± 1.0</td>
<td>10.3 ± 0.8</td>
<td>9.7 ± 0.5</td>
<td>= 0.52</td>
</tr>
<tr>
<td>Lactation length (days)</td>
<td></td>
<td>20.5 ± 1.0</td>
<td>20.0 ± 0.8</td>
<td>20.9 ± 0.5</td>
<td>= 0.68</td>
</tr>
<tr>
<td>Pigs weaned</td>
<td></td>
<td>10.5 ± 1.3</td>
<td>9.0 ± 1.0</td>
<td>7.9 ± 0.7</td>
<td>= 0.22</td>
</tr>
<tr>
<td>Weight weaned (kg)</td>
<td></td>
<td>60.7 ± 10.0</td>
<td>55.5 ± 8.1</td>
<td>50.4 ± 5.3</td>
<td>= 0.64</td>
</tr>
<tr>
<td>Wean-to-estrus interval (days)</td>
<td></td>
<td>5.3 ± 0.8</td>
<td>4.9 ± 0.8</td>
<td>3.3 ± 0.4</td>
<td>= 0.12</td>
</tr>
<tr>
<td>Duration of estrus (h)</td>
<td></td>
<td>48.0 ± 6.6</td>
<td>56.0 ± 6.6</td>
<td>63.7 ± 3.6</td>
<td>= 0.16</td>
</tr>
<tr>
<td>Estrus-to-ovulation interval (h)</td>
<td></td>
<td>36.0 ± 8.3</td>
<td>44.0 ± 8.3</td>
<td>50.0 ± 4.4</td>
<td>= 0.36</td>
</tr>
<tr>
<td>Wean-to-estrus interval (h)</td>
<td></td>
<td>162.0 ± 12.9</td>
<td>161.6 ± 12.9</td>
<td>130.6 ± 6.9</td>
<td>&lt; 0.08</td>
</tr>
<tr>
<td>FD day 1 postpartum (mm)a</td>
<td></td>
<td>6.3 ± 1.5</td>
<td>7.8 ± 1.5</td>
<td>6.9 ± 0.8</td>
<td>= 0.76</td>
</tr>
<tr>
<td>FD day 1 to 3 postpartum (mm)a</td>
<td></td>
<td>6.8 ± 1.2</td>
<td>7.8 ± 1.2</td>
<td>6.9 ± 0.6</td>
<td>= 0.80</td>
</tr>
<tr>
<td>FD day 1 to 6 postpartum (mm)a</td>
<td></td>
<td>6.3 ± 1.0</td>
<td>6.7 ± 1.0</td>
<td>6.6 ± 0.5</td>
<td>= 0.96</td>
</tr>
<tr>
<td>FD day 5 to 0 preweaning (mm)a</td>
<td></td>
<td>2.6 ± 0.8</td>
<td>3.8 ± 0.8</td>
<td>4.3 ± 0.4</td>
<td>= 0.27</td>
</tr>
<tr>
<td>FD day 2 to 0 preweaning (mm)a</td>
<td></td>
<td>2.7 ± 0.9</td>
<td>3.6 ± 0.9</td>
<td>4.2 ± 0.5</td>
<td>= 0.37</td>
</tr>
<tr>
<td>FD day 0 weaning (mm)a</td>
<td></td>
<td>2.6 ± 0.9</td>
<td>3.3 ± 0.9</td>
<td>4.2 ± 0.5</td>
<td>= 0.32</td>
</tr>
<tr>
<td>FD lactation average (mm)a</td>
<td></td>
<td>4.0 ± 0.9</td>
<td>4.8 ± 0.9</td>
<td>5.2 ± 0.5</td>
<td>= 0.49</td>
</tr>
<tr>
<td>FD lactation change (mm)ab</td>
<td></td>
<td>-4.1 ± 1.1</td>
<td>-4.2 ± 1.1</td>
<td>-2.8 ± 0.6</td>
<td>= 0.40</td>
</tr>
<tr>
<td>FD day 1 postweaning (mm)b</td>
<td></td>
<td>3.0 ± 0.9</td>
<td>4.2 ± 0.9</td>
<td>4.5 ± 0.5</td>
<td>= 0.38</td>
</tr>
<tr>
<td>FD day 2 postweaning (mm)b</td>
<td></td>
<td>3.5 ± 1.1</td>
<td>4.9 ± 1.1</td>
<td>5.2 ± 0.6</td>
<td>= 0.41</td>
</tr>
<tr>
<td>FD day 3 postweaning (mm)b</td>
<td></td>
<td>3.7 ± 1.0</td>
<td>5.4 ± 1.0</td>
<td>5.7 ± 0.5</td>
<td>= 0.25</td>
</tr>
<tr>
<td>FD postweaning change (mm)ac</td>
<td></td>
<td>1.1 ± 0.5</td>
<td>1.8 ± 0.5</td>
<td>1.6 ± 0.2</td>
<td>= 0.48</td>
</tr>
</tbody>
</table>

*yAverage follicle diameter (FD) of the three largest follicles present.

*bBased on average FD day 1 to 3 postpartum minus average FD day 2 to 0 preweaning.

*cBased on average FD day 3 postweaning minus average FD day 2 to 0 preweaning.

*x, y, zRow values without a common superscript letter differ (P < 0.05).
Figure 1. Transabdominal ultrasound images from one sow from day 1 to 20 postpartum. The maximum distance from the abdomen (top of each image) to the gut loops (mid to lower portion of each image) was measured in millimeters.
Figure 2. Transabdominal ultrasound images from one sow from day 1 to 20 postpartum (top panel) and from day 1 to 5.3 postweaning (bottom panel). The diameter of each of the three largest follicles present was measured in millimeters. The average diameter of these three follicles is listed on each image.
Figure 3. Least squares means (± SEM) of AI catheter penetration depth (a), abdomen-to-gut loop distance (b), and average follicle diameter (c) by day postpartum.
Figure 4. Least squares means (± SEM) of average follicle diameter by day postpartum (a), day preweaning (b), and day postweaning (c) for sows that had a ≤ 3 day (n = 3), 4 day (n = 5), or ≥ 5 day (n=3) weaning-to-estrus interval. Means marked with a * differ P < 0.05 from one or more of the other day means at that time point.
Figure 5. Average follicle diameter by day preweaning for sows that had a ≤ 3 day (a), 4 day (b), or ≥ 5 day (c) weaning-to-estrus interval. Exact weaning-to-estrus interval for each sow listed in legend.
Figure 6. Average follicle diameter by day postweaning for sows that had a ≤ 3 day (a), 4 day (b), or ≥ 5 day (c) weaning-to-estrus interval. Exact weaning-to-estrus interval for each sow listed in legend.
CHAPTER 5

GENERAL CONCLUSIONS
Profitable pork production in many systems involves maximizing the number of piglets produced per sow per unit time and minimizing the variation of the number of piglets produced over time. Unfortunately, reproductive performance among different sows and among different sow farms is typically quite variable and the underlying causes of this variation are largely unknown. Complex interactions between environment, health status, and management factors probably determine the extent to which individual sows achieve their reproductive potential. The observational studies described in this dissertation focused on estimation of the amount of variation in reproductive processes among sows and identification of factors associated with such variation. Swine breeding operations could benefit from practical methods to reduce or cope with this variation and increase the consistency and efficiency of sow reproduction.

In the first experiment (Chapter 2), weaning-to-estrus interval, season, parity, and lactation length explained approximately 65%, 25%, 5%, and 5%, respectively, of the variation of duration of estrus (DE) and onset of estrus-to-ovulation interval (EOI) among sows in three different farms. Weaning-to-estrus interval clearly had the largest affect on DE and EOI. However, adjustment of insemination service timing based on weaning-to-estrus interval and anticipated DE and EOI is not necessarily a wise strategy. The inverse relationship between DE, EOI, and weaning-to-estrus interval is somewhat inconsistent among different farms. In addition, average DE and EOI can undergo large seasonal shifts on some farms. Complex timing adjustments of multiple insemination schedules based on weaning-to-estrus interval would need to be farm specific and may need to be changed periodically within a farm to accommodate shifts in herd DE and EOI. This would require
intermittent characterization of each farm’s DE and EOI characteristics, which is not feasible due to the labor involved. Furthermore, the results of this experiment indicate that a simple multiple insemination schedule that delivers two services during estrus, separated by 24 hours, can yield high levels of reproductive performance despite the variation of EOI among sows. There is evidence that the interval prior to ovulation in which insemination occurs can affect the percentage of oocytes fertilized in sows. However, the present study found little evidence of an effect of insemination-to-ovulation interval on subsequent farrowing rate or litter size. Overall, the observations from these three sow farms indicate that the variation of EOI among sows can be overcome and high levels of reproductive performance can be achieved by the consistent administration of two inseminations, one on each of the first two days of estrus.

Detection of estrus is typically performed once daily during the early morning hours on most sow farms. Since the initiation of an insemination schedule is based solely on this heat check, the time of day that onset of estrus occurs in a sow can have a substantial effect on the number of hours remaining until ovulation occurs. In this experiment, onset of estrus occurred in less than 10% of the sows after the morning heat check and before the end of the workday. Among the three farms, onset of estrus occurred in approximately 20%, 20%, and 50% of the sows between 2 pm and 8 pm, 8 pm and 2 am, and 2 am and 8 am, respectively. The time of day that onset of estrus occurred in the majority of sows also differed among farms and seasons. These data suggest that addition of an evening or late night heat check would be required to increase the accuracy of diagnosing the onset of estrus. However, a
second heat check does not appear to be necessary based on the reproductive performance of these three farms.

Even though lactation length had little effect on DE or EOI when weaning-to-estrus interval was accounted for in the first experiment, lactation length can have considerable effects on postweaning reproductive performance. In general, sows that lactate for less than 21 days often exhibit an increased weaning-to-estrus interval, increased embryonic mortality, and a decreased farrowing rate and litter size compared to sows that lactate for longer periods. Variation among sows in the amount of uterine involution completed by weaning may be an important determinant of their subsequent embryo mortality and reproductive performance. However, this hypothesis cannot be tested because repeatable techniques to monitor postpartum uterine involution in sows have not been developed and validated. In the second set of experiments (Chapter 3), a postpartum increase in the urinary excretion of a marker of collagen degradation (hydroxylysyl pyridinoline, HP) by sows occurred and was consistent with the known pattern of postpartum uterine weight loss. However, since HP is not uterine specific, and there was considerable unexplained variation in the timing and magnitude of the postpartum increase of HP excretion among sows, it is not known if this marker accurately reflects the degenerative aspects of uterine involution. In the last experiment (Chapter 4), transabdominal ultrasonography estimates of the distance between the abdominal wall and gut loops, which sandwich the uterus, also decreased postpartum in pattern consistent with the known rate of uterine weight loss. Comparison of the results of these two techniques to postmortem uterine measurements is necessary to establish their validity. However, even if these techniques prove to be an accurate means to monitor the
postpartum decrease of uterine size, they may not be directly related to the recovery of uterine function. Certainly, better methods to gauge the postpartum recovery of uterine function and the ability of the maternal system to maintain synchrony with embryo development are needed. Given that postpartum uterine involution may not be complete at the lactation lengths less than 21 days currently common in swine breeding, research in this area should be a priority.

In summary, the results of these experiments indicate that there is considerable variation of postweaning, and possibly postpartum, reproductive processes among sows. The first experiment is a prime example of how management practices, like consistent detection of estrus and a multiple insemination protocol, can account for this variation and maintain a high level of sow reproductive performance. The remaining experiments suggest that repeatable measures of postpartum uterine involution can be developed, but it remains to be determined if the amount of involution completed by weaning is a key determinant of subsequent sow reproductive performance.