The effects of intermittent suckling protocols on reproductive performance in primiparous sows

by

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ABSTRACT

In pig production, sow reproductive efficiency is a key contributor to breeding herd productivity. A major challenge in the implementation of alternative sow housing and piglet management systems is the maintenance of production efficiency. Conventionally, sows are only bred once they come into estrus following piglet weaning. However, the ability to breed sows during lactation creates the potential to improve piglet welfare and maintain annual sow productivity. Lactational management regimes, such as intermittent suckling (IS), allow sows to return to estrus and be bred during lactation, by separating nursing sows and piglets for several approximately 8 hours each day.

For this thesis research, we compared IS protocols to conventional weaning (Control) and their influence on the performance of primiparous (PP) sows. The primary objective was to analyse the effects of inducing lactational estrus and breeding during lactation on PP sow reproductive performance, especially on embryo development and survival. This work involved initially assessing the effects of applying IS protocols in PP sows by evaluating their reproductive responses (e.g. rate of lactation estrus, pregnancy, ovulation, placental development and embryo survival rate) as well metabolic status of sows. Then the effects of IS protocols on conceptus quality were analyzed at a molecular level through embryonic gene expression analysis and the effect of IS on litter sex ratio and sex specific embryonic gene expression.

The results of this research revealed that 61% (35/57) of PP sows exposed to IS exhibited estrus during lactation. Subsequent assessment of IS sows bred during lactation compared to Control sows demonstrated that IS did not negatively impact overall reproductive performance (i.e. pregnancy rates, embryonic survival). It was observed that placental development at day 30 (D30) of gestation, as represented by placental volume, was reduced in IS sows compared to

Control sows. However, IS treatment did not appear to impact embryonic development. Molecular analysis of D30 embryos for IS and Control sows revealed no significant differences in gene expression across treatments and had no impact litter sex ratios. Additionally, metabolic analysis showed that the 39% of sows which did not response to IS were more metabolically challenged during lactation than sows that responded to IS and demonstrated lactation estrus. This result indicates that metabolic status and nutritional management during lactation influence response of sows to IS protocols.

Overall, the results of my thesis research demonstrate that the application of IS protocols to induce estrus and breed PP sows during lactation does not influence embryonic development or negatively impact sow reproductive performance. However, metabolic status and nutritional management during lactation play an important role in the response of sows to IS protocols. Further research is needed to assess the relationship between the metabolic status and response of PP sows to IS, as well as the feasibility of applying IS protocols in large scale commercial production systems.

PREFACE

The research described in this thesis was conducted to assess the effects of inducing lactational estrus and breeding during lactation on primiparous sow reproductive performance, especially on embryo development and reproductive performance and consists of 5 chapters. Chapters 1 and 2 include a general introduction to the research topic, as well as an overview of the related literature and past research in this area. These chapters were prepared by myself with the assistance of my supervisor Dr. Michael Dyck, and has not been published.

Chapter 3 of the thesis describes the animal work, data collection and analysis conducted to assess the effects of inducing lactational estrus in primiparous sows using intermittent suckling and its impact on reproductive parameters. The research concept and experimental design was conceived by Dr. Michael Dyck, Dr, Pieter Langendijk and Dr. George Foxcroft. The animal work, data collection and analysis was conducted primarily by myself, Jennifer Patterson and Rebecca Athorn. The research described in Chapter 3 is not yet published but a manuscript is under preparation

Chapter 4 of the thesis describes analysis to determine the effects intermittent suckling protocols on embryonic quality, using samples described in Chapter 3. The experimental design for these studies was conceived by Dr. Stephen Tsoi and Dr. Michael Dyck. Execution of the molecular analysis was performed by me and Julia Moroni, under the supervision of Dr. Stephen Tsoi. Part of Chapter 4 has been published as Blanes, M. S., S. C. Tsoi and M. K. Dyck, “Accurate and Phenol Free DNA Sexing of Day 30 Porcine Embryos by PCR”. JoVE (Journal of Visualized Experiments): e53301-e53301, 2016. As well as, Tsoi, S., M. Blanes, T. Y. Chen, P. Langendijk, R. Athorn, G. Foxcroft and M. Dyck, “Identification of differentially expressed

genes in sexed pig embryos during post-hatching development in primiparous sows exposed to differing intermittent suckling and breeding strategies", Genomics Data 9: 30-34, 2016.

Dedication

This thesis is dedicated to my mother Cleide, my husband Chris, my sisters Maíra and Lara and late father Edison. Thank you for your unconditional encouragement, enthusiasm, love and support.

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Boar Exposure (BE)

Corpus luteum (CL)

Day (D)

Days (d)

Differentially expressed (DE)

Equine chorionic gonadotrophin (eCG)

Embryo survival (ES)

Farrowing rate (FR)

Follicle stimulating hormone (FSH)

Gonadotropin-releasing hormone (GnRH)

Hour (h)

Human chorionic gonadotrophin (hCG)

Intermittent Suckling (IS)

Luteinizing hormone (LH)

Multiparous (MP)

Ovulation rate (CL count)

Pregnant mare's serum gonadotropin (PMSG)

Pregnancy rate (PR)

Primiparous (PP)

Progesterone (P4)

Stillborn piglets (SB)

Total number (TB)

Weaning-to-estrus interval (WEI)

CHAPTER 1

1. General introduction

In many pig production systems worldwide, sows are housed individually in stalls or crates throughout gestation (~115 d) as well as during lactation (~21 d) and during the weaning-to-rebreeding interval (4 to 7 d). Crates and stall systems allow for individual animal surveillance and management of sow health, estrus detection and feed intake monitoring (Kemp and Soede, 2012). However, despite economic and managerial advantages, these systems lack optimal welfare conditions and have triggered calls for more welfare-friendly alternative pig housing and production systems.

Many consumers prefer pork that has been raised under high animal welfare standards and packers, as well as retailers, are increasingly sourcing product from producers that meet such expectations. Changes in management, such as the implementation of group sow housing, may improve animal welfare. However, a major challenge with the application of alternative sow housing systems is the maintenance of herd reproductive performance. The sow's ability to produce more litters per year has been optimized over the years by decreasing the number of non-productive days, thereby generating more yearly profit. Non-productive days in a breeding female occur when a sow or gilt is neither gestating nor lactating.

As sows are generally in a state of anestrus during lactation, they usually only return to estrus after weaning (Armstrong et al., 1988). Thus, decreasing the weaning age (shortening the lactation length) decreases the inter-farrowing interval and increases the number of litters per sow per year. However, the stress of abruptly weaning at 21 days (d) is often related to decreased nutrient intake, reduced piglet growth, piglet distress behaviors, and changes in gut integrity and function after weaning that reduce piglet viability (Dybkjaer, 1992). For the well-being of the piglet, management strategies that implement longer lactation periods can help address these aspects of animal welfare. However, longer lactation periods are unlikely to be implemented by producers because this would decrease sow productivity by limiting the number of litters a sow is eligible to produce per year.

Alternatively, lactation periods can be extended without affecting sow productivity, by implementing a protocol to induce estrus during lactation. Intermittent suckling (IS) is a

protocol implemented during lactation where the sow and piglets are kept apart for 6-10 hours per day. Sow and piglet separation allows estrus to be induced by reducing the suckling stimulus during lactation (Newton et al., 1987; Gerritsen et al., 2008). Inducing lactational estrus and breeding during lactation create the possibility of increasing lactation lengths and weaning older piglets, thereby increasing piglet welfare without significant losses in sow productivity or piglet performance.

Despite several extensive studies related to IS protocols, the majority of these research findings have been conducted with multiparous (MP) sows (Gerritsen et al., 2005; 2008; 2009; Soede et al., 2012), limited work has been done with primiparous (PP) sows. In addition, the influence of IS on embryo development and survival has not been investigated in depth. It is well documented that stress and sow metabolic status can impact embryonic quality and vitality shortly after fertilization (Vinsky et al., 2006; Peltoniemi et al., 2016) which can affect development and impair critical processes such as maternal recognition of pregnancy. The first 35 days of gestation in the sow is more susceptible to loss of pregnancy than the subsequent fetal period (Peltoniemi et al., 2016). As well, endocrinological models testing maintenance of pregnancy suggest that continued stress lasting for more than two days can lead to abortion and loss of the entire litter. However, the sow may be resistant, in terms of her reproductive function, to acute stress lasting for 8-12 hours or up to a day.

Given the limited research on IS protocols in PP sows and the potential impact of IS on reproductive parameters such as embryonic survival and gene expression, the primary objective of the research presented in this thesis was to analyse the effects of lactational estrus and breeding during lactation on PP sow reproductive performance, especially on embryo development and survival. In Chapter 3 of this thesis, the effects of applying IS protocols in PP sows are assessed by evaluating their reproductive responses (pregnancy, ovulation, and embryo survival rate) as well as their metabolic status. In Chapter 4, the effects of these IS protocols on conceptus quality were analyzed at a molecular level through embryonic gene expression analysis and the effect of IS on litter sex ratio and sex specific embryonic gene expression.

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CHAPTER 2

2. Review of literature

2.1 Alternative housing and reproductive management

Public opinion on animal welfare has the potential to markedly influence the pig production industry. Thus, public reaction to issues such as individual gestation stalls has influenced governments and large corporations to alter codes of practice and regulations, including banning individual sow housing systems in some cases (Barnett et al., 2000, Committee, 2012). Individual sow housing involves two main sow welfare concerns, a lack of movement such as an inability to turn around, or an animal's inability to display normal behavior. For piglets, a major welfare concern is associated with weaning at an early age.

Thus, worldwide, sow housing systems are currently transitioning from individual housing to group housing. However, when analyzing and making decisions regarding animal welfare, moral views and biological facts need to be integrated and addressed, which is not always feasible. Additionally, an important aspect of the transition from individual housing to group housing is the maintenance of sow reproductive performance, as the change to group housing could affect farrowing rate and litter size due to the impact of sow aggression and fighting at mixing of sow social groups. The mixing of animals in dynamic group sow housing causes stress which imposes risks for fertility (Peltoniemi et al., 2016). Also, group housing during lactation increases the chance of lactational estrus occurring due to physiological mechanisms discussed later. If estrus is not detected, and therefore missed, any "missed" sows will not be bred and the producer will have a higher yearly number of non-productive days. In a swine production system, non-productive days occur when a sow or gilt is neither gestating nor lactating and are costly to the producer. Also, extending lactation increases a sow's energy requirements for milk production and, in the absence of adequate feed intake, could further decrease tissue reserves and negatively affect sow reproductive performance (Munsterhjelm et al., 2006). Therefore, new alternative housing systems, such as group housing require special attention to ensure optimal reproductive performance and maintain a reasonable level of profits for the producers (Kemp and Soede, 2012).

In 2003, an European Directive established the minimum weaning age of piglets at 28 d (Colson et al., 2006). In North American ,no minimum weaning age has been set for pig production, however the new Canadian Pig code of practice, 2014 has been recommended a minimum age of 21 days and older (Committee, 2012). Reducing lactation periods to 21 d or less are preferred in order to achieve more litters per sow per year, and consequently higher profits (Worobec et al., 1999). A nursing period of 21 days is considered relatively short, as natural weaning happens gradually and ends at approximately week 14 to 17 of lactation (Jensen and Stangel, 1992). At this point, milk consumption is slowly replaced by the ingestion and digestion of solid food.

Therefore, abruptly weaning at 21d can be stressful for sows and piglets. A study in 2016 by Colson and associates showed that weaning at 21 d has more negative consequences on growth rate and stress endocrine responses than weaning at 28 d (Colson et al., 2006), for example differences in growth rate revealed that weaning at 21 days induced a greater developmental deficit than weaning at 28 days. In addition, the abrupt switch from a milk diet to a solid diet can lead to reduced piglet feed intake, a decrease in growth rate and an increased prevalence of diarrhea (Van Beers-Schreurs et al., 1992). In order to avoid abruptly switching from a milk diet to a solid diet and to reduce abnormal feed intake after weaning, creep feed for piglets should be provided as an adaptation before weaning (Fraser et al., 1994).

2.2 The estrous cycle and lactational anestrus of the sow

The estrous cycle is controlled by the hypothalamo–hypophyseal axis (central nervous system), which releases gonadotropic hormones to regulate endocrine activity in the ovary and uterus (Hughes and Varley, 1980; Senger, 1997), Figure 2.1. Gonadotropin releasing hormone (GnRH) is a small neuropeptide molecule released from neurons in the hypothalamus into the hypothalamo–hypophyseal portal system. GnRH acts on the anterior pituitary stimulating the release of follicle stimulating hormone (FSH) and luteinizing hormone (LH), which in turn acts on the ovaries. Positive and negative feedback mechanisms within this hypothalamo-hypophyseal-ovarian system, mediated by estradiol and progesterone, work to regulate secretion of GnRH and other hormones (Senger, 1997).

Ovarian, uterine, and placental hormones also play a role in regulating the reproductive system. During the follicular phase of the estrous cycle, estradiol secreted by ovarian follicles reaches blood circulation and stimulates increased GnRH pulsatility and promote FSH and LH release. During the luteal phase of the estrous cycle, progesterone (P4) secreted by the corpora lutea strongly inhibits GnRH pulsatility (Senger, 1997). If fertilization does not occur and there is no maternal recognition of pregnancy, prostaglandins are secreted by the uterus, corpora lutea undergo luteolysis, progesterone level decrease and the estrous cycle resumes.

Non-pregnant and non-lactating sows present estrous cycles regularly throughout the year, cyclicity is interrupted only if sows become pregnant. Estrous cycles in sows and gilts, are approximately 21 d, varying from 18-24 d. Crighton and Lamming (1969) found follicular growth and uterine development are suppressed during lactation in sows. Consequently, sows often remain in anestrus during lactation and resume cycling only after weaning (Armstrong et al. (1999). Lactational anestrus in sows is caused by the interplay of various factors. In general, the inhibitory effects of suckling, mediated via the action of opioids at the hypothalamic level, reduce the pulsatility of GnRH and LH release, and suppress lactational estrus (Langendijk et al., 2005a). The suckling stimulus, as well as other nursing stimuli such as auditory, visual and olfactory factors, also stimulates release of oxytocin and somatotropin. (Rushen et al., 1993). Somatotropins produced in the anterior pituitary enhance the sensitivity of the ovaries to gonadotropin stimulation and promote follicular development (Poretsky et al., 1999). Although there is a certain degree of ovarian follicular development during lactation, despite the reduced pulsatility of GnRH, the inhibitory effects of nursing generally do not permit the level of LH to achieve the necessary threshold to stimulate pre-ovulatory follicle development and ovulation. As a result, a nursing litter will usually maintain a sow in anestrus during lactation. It should be noted that along with the hormones discussed above, prolactin is also released in response to nursing but it has not been shown to affect LH secretion and is not considered to play a significant role in lactational anestrus (Varley and Foxcroft, 1990).

Lactational anestrus can also be influence by the metabolic state of the sow. A study by Prunier et al. (2003) showed that metabolic status can affect LH secretion during lactation and the nutritional deficits associated with lactation constitutes an additional inhibitory factor for LH production, and is another major contributor to lactational anestrus (Quesnel and

Prunier, 1995; Zak et al., 1997). A review of related studies on the effects of maternal status on the reproduction of sows (Foxcroft et al., 2007; 2009) suggests that a decrease in body weight, body protein mass and body fat mass negatively influences embryo survival and overall litter development during lactation. Given the importance of sow metabolic status and its effects on sow reproductive performance, they will be discussed further in this Chapter.

Overall, the stimulation of nursing along with the metabolic challenges of lactation result in an inhibition of LH pulsatility and consequently anestrus in the lactating sow. This raises the question whether nursing can be managed in manner that would permit the induction of estrus during lactation. Also, if lactation estrus is induced, can it be done without compromising sow reproductive performance?

2.3 Inducing lactational estrus

As sows usually remain in anestrus during lactation, weaning at D28 instead of D21 of lactation decreases the number of litters a sow is able to produce per year, and is therefore not advantageous to producers. Thus, inducing lactational estrus and breeding while the sow is still nursing creates the possibility of maintaining longer lactation periods and weaning older piglets. This approach reduces losses in sow and piglet productivity and also increases piglet welfare at weaning (Soede et al., 2012).

As a result, researchers have been investigating protocols to induce estrus during lactation and its consequences for sow fertility. This has led to several intermittent suckling (IS) protocols being developed to induce lactational estrus (Soede et al., 2012). An IS protocol is generally applied on the last 7d of a 28d lactation to induce estrus before weaning. During IS, the sow and piglets are kept apart for 8-12h per day during the last week of lactation and, and by reducing the suckling stimulus, estrus is induced (Newton et al., 1987; Gerritsen et al., 2008a). At a physiological level, when piglets are temporarily separated from the sows for several hours a day, the inhibitory suckling effect decreases, LH pulsatility increases and induction of estrus occurs (Armstrong et al., 1988). Varley et al. (1990) demonstrated that LH secretion increases when the number of piglets suckling decreases from 10 to 5 piglets. The increase in gonadotropin release and ovarian follicular development associated with IS can ultimately lead to estrus and ovulation in sows. Even though lactational follicle development,

estrus behavior and ovulation occurs during IS, sows' responses to IS are not consistent. The success of inducing lactational estrus varies greatly from no observed estrus induction (Grinwich and McKay, 1985) to 100% induction (Newton et al., 1987). This variation is generally associated with the impact of IS on the reproductive physiology of the sows, which is affected by various other physiological and management considerations that will be discussed here.

2.3.1 Response to induction of lactational estrus

Given that the sow is generally in anestrus during lactation, one would expect that the profile of reproductive hormones of a sow experiencing lactational anestrus to differ from that of a sow post-weaning. Gerritsen et al. (2008b) compared hormone profiles and follicular dynamics in sows conventionally weaned and sows showing lactational estrus when exposed to IS. The LH surge and the subsequent progesterone (P4) concentrations were lower in IS sows compared to sows abruptly weaned after 21d of lactation, despite normal follicular development, plasma estradiol concentrations and ovulation (Gerritsen et al., 2008b). Lower P4 concentrations in IS sows may be related to decreased LH concentrations, as peak LH concentrations are associated with luteinization and the formation of corpora lutea (Einarsson and Rojkittikhun, 1992). Therefore, triggering pre-ovulatory follicle development and establishing pregnancy during lactation may be responsible for lower progesterone concentrations and reduced embryo development in IS sows (Gerritsen et al., 2009).

Langendijk et al. (2007) examined the profiles of LH, estradiol, and cortisol for sows subjected to an IS regime with 12h-a-day separation from piglets, commencing at approximately 2 weeks of lactation until D27 when the piglets were weaned. Separation was performed either by moving the sows to a different unit (total separation), or by providing physical separation where piglets were inhibited from suckling using only a physical barrier (sows and piglets in close proximity). With total separation, 100% of the sows ovulated, while physical separation resulted in only 50% of sows ovulating. Further, the LH secretion patterns differed between the two treatments. Throughout sampling the period sows exposed to total separation had lower LH pulse amplitude compared with physically separated sows. Sows from the total separation treatment also had higher cortisol levels after separation, although the difference was not observed between ovulating and non-ovulating sows. Thus, Langendijk et

al. (2007) concluded that total separation of sows and piglets results in a more continuous increase in LH secretion, which increases the probability of ovulation. Moreover, Langendijk et al. (2007) also confirmed that LH secretion during suckling was lower than during non-suckling periods. Although their study had a limited number of animals it was concluded that non-tactile stimuli, such as auditory, olfactory and visual contact between sow and piglets, contributed to reduced LH secretion (Langendijk et al., 2007).

Although IS protocols are generally associated with longer lactation periods (i.e. 28d), investigation of IS with shorter lactation has also been explored. In one study, when IS was started on D14 of lactation, 87% of the sows developed pre-ovulatory follicles, compared to 100% of sows when IS was started at D21 of lactation. Further, the incidence of ovulation within 8d after onset of IS was lower (75%) when IS began at D14 of lactation compared with when IS was started at D21 (94%); (Gerritsen et al., 2008a). In other research, Kuller et al. (2004) reported that only 22% of sows ovulated when separated for 12h per day starting from D14 of lactation, while Langendijk et al. (2005) observed a 90% ovulation rate with a similar approach.

In a recent comprehensive study, Soede et al. (2012) evaluated the effect of three IS regimes on lactational estrus and subsequent fertility. Control sows were weaned at D26 ± 2 of lactation. For one IS treatment group, IS started at D19 ± 1 of lactation with the sows being weaned either 7d (IS19-7D) or 14d later (IS19-14D) while in another IS group was started at D26 ± 1 of lactation and sows were weaned 7 d later (IS26-7D). For this study the IS period consisted of 10 hours of daily separation. For the IS19-7D, IS19-14D and IS26-7D treatments, 50%, 64% and 61% of sows exhibited lactational estrus and ovulation, respectively. Of the remaining sows, 100%, 93%, and 69% exhibited estrus in the initial week after weaning. When sow age was evaluated, only 23% of the PP sows exhibited lactational estrus which was significantly less than older parities (68%). In general, sows bred during lactation had comparable pregnancy rates and litter sizes to sows bred after weaning. Hence, (Soede et al., 2012) concluded that regardless of the stage of lactation, implementation of IS can induce lactational estrus in 50-64% of sows submitted to a 26 ± 2 d lactation.

These results suggest that induction of estrus during lactation can by influenced by various factors and a comprehensive review of the literature reveals a wide range of

management considerations including, lactation length, the duration and type of sow-piglet separation, and the number of days of IS and suckling frequency (Armstrong et al., 1988). Another factor often overlooked in these types of studies is boar contact and its role in estrus induction (Kemp and Soede, 2012).

2.3.2 Boar stimulation and induction of lactational estrus

Boar contact is an important consideration for inducing and detecting estrus regardless of whether the sow has been weaned or is being exposed to IS. Building off previous research, work in Australia described encouraging results when an IS regime is associated with boar exposure (Downing et al., 2007). During estrus, sows respond to boar stimuli by showing a standing response (lordosis), the exact neuro-endocrine pathways associated with lordosis response is not yet well understood (Langendijk et al., 2005b). However, boar stimuli are important for stimulating follicle development needed to induce first estrus in gilts and advance estrus in sows (Langendijk et al., 2005b). The differences in sows' standing response to olfactory and tactile boars' stimuli can be due to individual variation. Total physical boar contact with sows (boar and sows in the same pen) is always more potent than fenced boar stimuli, however total physical contact is not always practical.

A study conducted by Van Wettere et al. (2013) evaluated the efficacy of applying P.G.600® (a combination of equine chorionic gonadotrophin (eCG) and human chorionic gonadotrophin (hCG); Merck Animal Health, USA) associated with boar exposure to induce estrus during lactation. The experiment used Large White/Landrace sows (parity 1.9 ± 0.14 litters) to evaluate the effects of boar exposure (BE) versus no boar exposure (non-BE) and PG600 versus NoPG600 on the occurrence of lactation estrus and ovulation. Boar exposure consisted of full physical boar contact for 15 min daily. Administration of PG600 occurred on D1 of lactation when boar exposure was initiated. Boar exposure increased the proportion of sows ovulating during lactation (0.61 versus 0.09) compared to non-BE sows. There was no effect of PG600 on any reproductive parameters, and no interactions with the BE treatment. It was demonstrated that moving lactating sows to an estrus detection area for 15 min of daily physical boar contact increases the incidence of lactation ovulation within 22d following parturition (Van Wettere et al., 2013).

Previously, Newton et al. (1987) showed the combination of litter separation and boar exposure increased basal and pulsatile secretion of LH in both multiparous (MP) and primiparous (PP) sows. A later study explored the effect of full physical BE and IS on the incidence of lactation estrus within a large commercial breeding herd of MP and PP sows (Terry et al., 2014). It was concluded boar exposure is effective at stimulating lactational estrus in MP sows, whereas PP sows require, in addition to BE, a reduction in suckled litter size to from 12 to 7 piglets. For better success in inducing lactational estrus, Terry et al. (2013) suggested combining boar exposure with an IS protocol.

2.3.3 Effect of sow parity and genotype on lactational estrus induction

Further to the breeding management considerations already discussed, the effect of sow parity as well as genetic background on response to lactational estrus induction has also been considered (Kemp and Soede, 2012a). In general, there have been many studies evaluating the reproductive performance of PP sows, as young female pigs are especially sensitive to body reserve depletion. This sensitivity is due to the fact that PP sows often do not have enough body reserves at first farrowing and PP sows feed intake capacity is not sufficient to maintain a positive metabolic status during lactation. In addition, young sows have not reached physical maturity and are still growing. The lack of body reserves and continued growth make young sows more sensitive to the effects of a negative energy balance on reproduction (Prunier et al., 2003). This phenomenon, often referred to as "second-litter syndrome" is discussed further below. With this maturity factor in mind, sow parity is also a major consideration when applying IS protocols to induce lactational estrus, although the number of recent studies on this topic are limited.

Studies of MP sows reveal that more than 80% of sows exhibited lactational estrus as a result of two primary methods, sow and piglet separation and boar exposure (McDonald et al., 2013). While, a recent study comparing induction of lactational estrus in MP vs PP sows demonstrated that MP sows are more likely to exhibit a lactation estrus than PP sows (Terry et al., 2014). During lactation, PP sows experience additional metabolic challenges which may compromise subsequent reproduction (Foxcroft et al., 2007). In PP sows, during the last week of lactation, catabolism increases and appears to reduce embryonic survival and development by D30 of gestation in the following litter (Foxcroft, 1996). In order to support the practice of

longer lactation periods in PP sows, Hidalgo (2014) showed that PP sows which had lactated for 35d had larger litters compared with sows that lactated for 21d, suggesting that PP sows would benefit from longer lactation periods to improve litter size at next farrowing.

The discussion around genotype and response to lactational estrus induction has also occurred, particularly in relation to the changes in genetics and pressures in genetic selection over time. Gerritsen et al. (2009) suggested that genetic selection since the 1970's has changed the physiology of the modern sow, from a group that was usually in anestrous during lactation to one that is more likely to cycle during lactation. Similarly, sows have been genetically selected for a shorter weaning-to-estrus interval (WEI) which may explain why modern sows respond differently to IS than they have in previous studies (Gerritsen et al., 2008b). Research studies have been shown that WEI can also be influenced by factors such as sow breed and parity during a non-lactational estrus (Vesseur et al., 1994). Given the changes in reproductive performance of the modern sow due to the selection pressure to reduce the WEI phenotype, as well the limited research in the induction of lactational anestrus in PP, there is a need for work in this area.

2.4 Intermittent suckling and sow reproductive performance

If IS can be used to successfully induce lactational estrus, not only is it important for the sow to exhibit mating behaviour and to ovulate, it is also important that the follicles that develop and oocytes produced are of good quality in order to produce viable embryos and large healthy litters. Therefore, one must also consider the effects of IS and lactational estrus induction on some of the downstream indicators of sow reproductive performance including: WEI, pregnancy rate (PR), farrowing rate (FR), ovulation rate (CL count), embryo survival (ES), total number (TB) and number of stillborn piglets (SB).

Over the past decades, various investigations of the effect of IS on sow reproductive performance have been carried out. Despite some conflicting results among these various studies (Kuller et al., 2004; Gerritsen et al., 2008a; 2009; Van Wettere et al., 2013), it is generally accepted that IS can be used to induce lactational estrus in sows without dramatically affecting pregnancy rate and early embryonic survival.

In addition to assessing estrus induction, previous research has compared the effects of initiating IS at D14 versus D21 on embryo survival as well as pregnancy rate. When starting IS at D14, pregnancy rate was not affected. However, P4 and embryo survival at D7 post-ovulation were reduced. Lower P4 and reduced embryo survival before D21 were attributed to an incomplete uterine involution and altered hormonal pathways, as reviewed by Gerritsen et al. (2009). It has also been shown that lactation during the first 2d to 9d of gestation does not have a negative effect on fertility (Soede et al., 2012). These researchers determined that reproductive characteristics such as PR, FR and LS, do not differ between sows bred during lactational estrus and sows bred after weaning. In addition, a previous study found that there were no difference in follicle development, ovulation rate or estradiol level, or for onset of estrus or time of ovulation relative to the start of IS and weaning (Gerritsen et al., 2005). Later, Gerritsen et al. (2008a) also concluded that when IS continues into pregnancy, embryo development and survival can be affected. Lactation impairs P4 secretion which is needed to the maintenance of sow pregnancy (Soede et al., 2012). Therefore, the use of an IS protocol and the time at which sows are bred during lactation are important to consider in order to achieve optimal reproductive responses.

The development of anovulatory or cystic follicles is another concern with IS protocols. Researchers found that sows submitted to IS which develop anovulatory ovarian follicles are not responsive to the increase in LH secretion (Langendijk et al., 2009). In the study by Langendijk and associates, trans-rectal ultrasonography was performed to detect ovulation and sows that did not ovulate were designated as anovulatory. On D1 of IS follicle size ranged from 1 to 5 mm among treated sows, but on D6 of IS was 5.4 mm on average with a range between 3.5 and 7.6 mm. Anovulatory sows presented a varying degree of follicle development, without ovulation. Langendijk et al. (2009) suggested that the metabolic condition of a sow may be one of the factors that contributed to ovulation failure.

Cystic ovaries may also account for anovulatory follicles in IS sows. Gerritsen et al. (2008b) described that 5% of control sows, 25% of the sows exposed to 12h IS, and 19% of the sows to 6h IS protocol presented with cystic ovaries. It was noted that a LH surge was not present or it was very low in sows with cystic ovaries. As such, intermittent suckling for 6h may not be enough to stimulate a LH surge and resulting in a higher risk of disrupting the LH

surge and ovulation. Although it is generally agreed that IS can be used to induce lactational estrus in sows without dramatically affecting reproductive performance, the metabolic status of the sow is a major factor that can influence reproductive performance and must also be considered when applying IS protocols.

2.4.1 Sow metabolic status and reproductive performance

Mechanisms linking maternal nutritional state to embryonic survival and prenatal programming of litters have been extensively investigated in various species as reviewed by Foxcroft et al. (2009). Maternal nutrition and metabolic state are considered to play an indirect role in limiting placental size and, therefore, embryonic and fetal development. Latent effects of sow metabolic state on oocyte quality and on early embryonic development have also been reported, in addition to the effects of previous sow catabolism on the embryo (Foxcroft et al., 2009).

Sow's metabolic status is, therefore, important to consider when implementing an estrus induction protocol, as an extended lactation will increase a sow's energy requirements. Sow reproductive potential, even despite IS protocol implementation, is negatively impacted by reduction of body weight, body protein, and body fat during lactation (Foxcroft et al., 2007; Foxcroft et al., 2009). Further, extending lactation periods increases the sow's energy requirement for milk production and, in the absence of adequate feed intake, further decreases tissue reserves which may negatively affect the sow's reproductive performance (Schenkel et al., 2010). As described by Vinsky et al. (2006), the metabolic status of the sow and in turn the maternal environment during breeding can affect embryonic gene expression and influence sex ratio within the litter. Furthermore, as reviewed by Foxcroft et al. (2007), lactational catabolism is related to the development of immature follicles and oocytes, as well as to reduced fertility when PP sows are bred at the first post-weaning estrus.

2.4.2 Intermittent suckling and breeding at a second estrus "Skip-a-heat"

As mentioned, reproductive performance in PP sows can be problematic and second-litter syndrome is a phenomenon that is usually present in most commercial breeding herds. A key aspect of second-litter syndrome is a decrease in embryo viability which are mainly predisposed by the demands of the first lactation (Werlang et al., 2011). In order to address

second litter syndrome, approaches to increase the interval between weaning and breeding have been investigated (Clowes et al., 1994; Patterson et al., 2007). Clowes et al. (1994) found that by delaying the breeding of weaned PP sows until their second post-weaning estrus (skip-a-heat breeding) the subsequent litter size was increased. Patterson et al. (2007) showed that breeding weaned PP sows at the second post-weaning estrus increased the number of embryos at D30 of gestation by 2.3 live embryos. Hence, breeding on the second post-weaning estrus could negate the effects of the second litter syndrome. The development of immature follicles and oocytes, and reduced fertility of first parity sows when bred at the first post weaning estrus are considered to be related to the effects of lactation (Foxcroft et al., 2007).

2.5 Embryonic development and survival

As already stated, a significant factor in reproductive performance is the ability to produce viable embryos that will survive, implant and produce a quality litter. Embryo survival can be linked to the competence of the oocytes shed at ovulation, and oocyte competence is profoundly impacted by the follicular environment in which the oocyte matures (Krisher, 2004; Ferguson et al., 2007). Nutritional status can greatly influence the follicular environment (Hunter, 2000; Ferguson et al., 2003; 2007) thus, a highly catabolic sow may potentially ovulate poorer quality oocytes during lactation leading to reduced embryo survival and subsequent litter size. In particular, both under-nutrition and over-nutrition of the sow during the prenatal period can have significant negative effects on fetal growth (Dziuk, 1992). Due to the high metabolic demand during lactation it is likely the negative energy balance of the nursing that has a detrimental effect on the development and survival of embryos conceived during lactation.

Embryo development is complex and embryos undergo major morphological changes before implantation, including cleavage, morula compaction, embryonic genome activation, blastocyst formation, and hatching (Østrup et al., 2009). Even though morphological events during embryo and fetal development have been well documented, the molecular processes underlying porcine embryo development are not yet fully understood. Despite the numerous IS and sow reproductive efficiency studies currently in the literature, the effect of IS and pregnancy during lactation on embryo quality and development has yet not been explored at a molecular level. Gerritsen et al. (2008a) found that embryo development in a 6 h IS treatment

was significantly compromised. The impaired embryo development observed may be related to effects of lactation of the reproductive physiology of the sow. For example, hormones related to suckling, such as oxytocin, prolactin, and endogenous opioids are known to affect pregnancy performance in sows. During the suckling period, the pathways of oxytocin and prolactin are unclear, but, they are thought to negatively affect embryo parameters in other species (Fortun-Lamothe and Prunier, 1999).

It is only after weaning that recruitment and selection of follicles occur and sows resume cycling. The impact of lactation on the reproductive axis is also influenced by physiological and metabolic requirements for milk production. Furthermore, a sow's negative energy balance influences follicular development. Feed intake as well as protein restriction during lactation have also been shown to negatively impact oocyte quality and embryo development (Quesnel, 2009). Although the application of IS between 2 to 9 d of gestation has not been shown to have a significant negative effect on fertility (Soede et al., 2012), IS protocols may still have an effect on embryo development and viability.

At a molecular level, nutritional and metabolic states of the sow and gilt have been shown to have epigenetic effects on embryo development. Litter prenatal programming is influenced by environmental factors which affect oocyte quality and the developing embryo (Foxcroft et al., 2006; 2007). In addition, several studies have demonstrated that maternal nutrition during gestation reduces piglet weight which is primarily related to muscle development (Handel and Stickland, 1987; Dwyer and Stickland, 1994). A review by Robinson et al. (1999) outlined the clear effects of maternal nutritional during gestation on embryo and fetal development, and demonstrates the influence of the maternal environment on the conceptus.

In addition to metabolic status, the various other factors that influence the success of IS protocols such as breed, parity, boar exposure and stress on the sows must also be considered. Although lactational follicle development and ovulation occurs during intermittent suckling, the number of sows which respond to IS varies, likely due to these other factors (Kuller et al., 2004; Soede et al., 2012). As mentioned, it is thought PP sows could benefit from skipping a heat and breeding at the estrus following application of an IS protocol. Given the expected metabolic status of PP sows, it could be would anticipated that breeding at the second estrus could benefit embryo development.

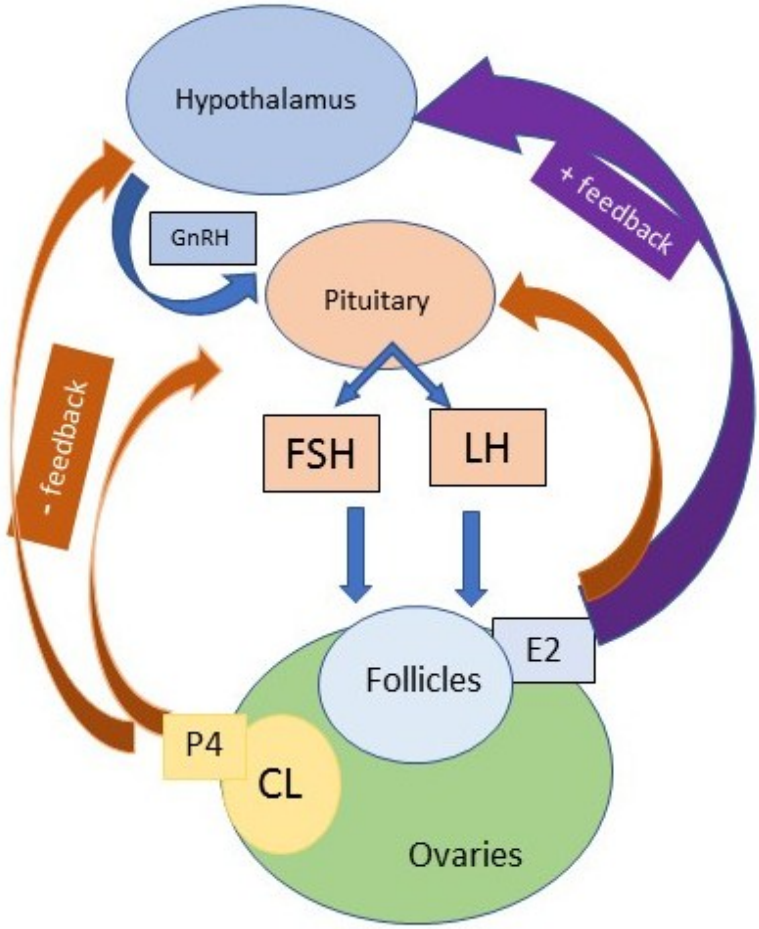
Studies in mice suggest maternal diet may have sex-specific effects and result in gender imbalance (Rosenfeld and Roberts, 2004). Similarly, in sows, metabolic programming is known to affect embryonic development, alter gene expression and may also induce gender-specific loss of embryos by D30 of gestation (Foxcroft et al., 2007). An imbalance in sex ratio has been reported in pigs related to paternal breed (Gorecki, 2003), uterine capacity (Chen and Dziuk, 1993), and sows' metabolic status at weaning (Vinsky et al., 2006). In the Vinsky et al. (2006) study, feed restriction of PP sows during the last week of lactation resulted in decreased embryonic growth and female embryo survival to D30 of gestation. A follow up study showed that a compromised maternal metabolic state prior to breeding resulted in maternally inherited epigenetic defects that appeared to cause female embryonic loss and reduced growth before D30 of gestation (Vinsky et al., 2007).

Given the information available, our interest is to evaluate whether metabolic state of the sow during IS and breeding during lactating have an effect on gene expression in the developing embryo and in doing so alters litter size and possibly litter sex ratio. Advances in molecular biology tools for studying gene expression have resulted in the availability of a variety of options for gene expression profiling such as DNA micro-arrays, and next generation sequencing (NGS) technologies (Pariset et al., 2009). A porcine embryo-specific microarray platform, EmbryoGENE Porcine Array Version1 (EMPV1), has been recently developed in our research group (Tsoi et al., 2012). The EMPV1 micro-array is a custom Agilent 4X44K platform with the only porcine-early embryo specific gene expression micro-array that allows for parallel analysis of many samples. This platform allows for the assessment of embryonic quality at a molecular level and provides insights into the impact of the maternal environment that cannot be assessed otherwise. Furthermore, sexing of individual embryos is a fundamental aspect of most investigations related to genotype, epigenetics and X chromosome-inactivation of sexual dimorphism during early embryo development (Gutiérrez-Adán et al., 2006). Since differences observed in embryos and litters can be influenced by sexual dimorphism, one needs to be aware of embryo sex and sex ratios before drawing conclusions particularly when assessing gene expression patterns.

2.6 Objectives and hypothesis

As discussed, PP sows face extra metabolic challenges during lactation which can compromise subsequent reproductive capacity. Although there have been recent studies on the application of IS in MP sows, there has been limited research in the induction of lactational estrus in PP sows and the potential impact of IS on reproductive parameters such as embryonic survival and gene expression. Therefore, the primary objective of the research presented in this thesis was to analyse the effects of lactational estrus and breeding during lactation on PP sow reproductive performance, especially on embryo development and reproductive performance. This research involved evaluating the effects of inducing estrus and breeding during lactation (first estrus - ISFE), breeding at the subsequent estrus following first estrus during lactation (skip-a-heat- second estrus - ISSE) and at a first delayed estrus (IS28) on the establishment of pregnancy, conceptus quality and embryonic gene expression. The general hypothesis of this study was that IS can be used to induce estrus during lactation and breeding during the last week of gestation which will influence reproductive performance in primiparous sows. In Chapter 3 of this thesis, the effects of these treatments were determined by analyzing the reproductive traits of the sow (pregnancy, ovulation, and embryo survival rate), conceptus quality. The effects of IS protocols on conceptus quality was analyzed at a molecular level through embryonic gene expression analysis and the effect of IS on litter sex ratio and gender specific embryonic gene expression was also assessed, as described in Chapter 4. For this study, it was hypothesised that breeding PP sows at the subsequent estrus following first estrus during lactation (skip-a-heat) could improve reproductive outcomes including embryo development and survival when combined with an IS estrus induction protocol.

Figure 2.1 Hypothalamus-hypophyseal-ovarian endocrine system



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CHAPTER 3

3. Induction of lactational estrus in primiparous sows using intermittent suckling and its impact on reproductive parameters

3.1 Introduction

Under natural conditions, piglets are gradually weaned over a couple of months with complete weaning achieved by approximately 12 to 17 weeks of age (Bøe, 1991). Since sows are typically in anestrus during lactation (Armstrong et al., 1999), in an attempt to optimize sow productivity, commercial pig producers have reduced the duration of the suckling period from several weeks to 21-28 days (d) at which point piglets are abruptly weaned. Thus, by decreasing the length of lactation, the annual productivity of a breeding sow can be improved by increasing the potential number of litters per year. However, the industry practice of abruptly weaning piglets is often associated with decreased nutrient intake, reduced piglet growth, as well as displays of piglet distress behavior, and changes in gut integrity and function after weaning that increase the susceptibility for diarrhea (Dybkjaer, 1992). Considering the detrimental effects of conventional weaning on the post-weaning performance of piglets, the stressful weaning period is a concern from both an overall productivity and animal welfare point of view. The pig industry has seen an increase in public concerns for animal welfare which have led to changes in production systems. In particular, in Europe the minimum piglet weaning age has been set at 28 d of age (Colson et al., 2006). Also, the new Canadian Pig code of practice, recommends a minimum weaning age of 21 days or older (Committee, 2012).

As changes in animal production practices are made to address welfare concerns, producers must also maintain production efficiency of their herds in order to remain economically viable. In this case, the well-being of the piglets can be addressed by providing an extended lactation period, but because the sow expresses anestrus during lactation, an extension of lactation length reduces sow productivity. In response, the induction of estrus during lactation has been explored using protocols such as intermittent suckling (IS) to induce estrus during lactation. Inducing lactational estrus and breeding during lactation allows for the weaning of older piglets, whilst still maintaining sow yearly productivity and producing as many litters per year as possible (Soede et al., 2012). Intermittent suckling consists of

separating sows and piglets for a number of hours a day during lactation (Newton et al., 1987; Gerritsen et al., 2008b).

There has been significant interest in inducing lactational estrus and although a number of extensive studies related to IS protocols and sow reproductive performance have been conducted, the majority of this this work has been carried out with multiparous sows (MP) (Gerritsen et al., 2005; 2008a; 2009; Soede et al., 2012). Only a few studies have explored IS with primiparous (PP) sows (Terry et al., 2014; Turpin et al., 2016) which can be problematic to manage as the young sows are more sensitive to the effects of a negative energy balance on reproduction (Prunier et al., 2003). This is an important consideration with IS because prolonging the lactation period also increases the sow's energy requirement for milk production, further decreasing tissue reserves, which may negatively affect the sow's reproductive performance (Schenkel et al., 2010). Earlier sow studies established that embryonic and fetal development, as well as placental size, are all affected by maternal nutrition and sow metabolic condition before and during pregnancy (Foxcroft, 1996). Likewise, sow catabolism affects the subsequent reproductive cycle by reducing oocyte quality and decreasing early embryonic development (Foxcroft et al., 2009).

Therefore, the objective of the study presented here was to analyse the effects of inducing lactational estrus and establishing early pregnancy during lactation on PP sow reproductive performance, especially embryo development and survival. As a part of this research, sow metabolic stage before breeding and its influence on reproductive traits was also considered. For this study, it was hypothesised that breeding PP sows at the subsequent estrus following first estrus during lactation would improve reproductive outcomes, when combined with an 8h separation IS protocol.

3.2 Materials and methods

3.2.1 Animals

This study involved 105 PP sows and was conducted from November 2012 to December in 2013 at the University of Alberta Swine Research and Technology Centre (SRTC), in accordance with Canadian Council on Animal Care guidelines and with the

approval of the Faculty Animal Policy and Welfare Committee – Livestock at the University of Alberta (AUP00000521).

During November and December of 2012, five groups of Landrace x Large White terminal line gilts (Hypor, Regina, SK, Canada) were delivered to the SRTC at approximately 150 d of age. Starting at approximately 164 d of age, puberty was induced in the boar exposure area (BEAR) of the SRTC using boar stimuli for all gilts and hormonal treatment (P.G. 600®, pregnant mare serum gonadotropin (PMSG) and chorionic gonadotropin, Merck Animal Health) to induce pubertal estrus where necessary. Gilts were assigned to treatments based on pubertal age, weight, and PG600 administration in order to have homogenous groups of animals. Average age at puberty was 183 ± 1.44 d (SEM) and the average weight at puberty was 109 ± 1.98 kg (SEM) across all five groups. Pubertal estrus was skipped and the number of subsequent estrous cycles completed before breeding varied by experimental group as follows: Group 1 = 1.62, Group 2= 1.46, Group 3 = 1.99, Group 4 = 2.1 and Group 5 = 3.76.

Bred gilts were housed in individual stalls and from D18 of gestation were exposed to fence-line contact with a mature boar in order to check for return to estrus following breeding. Pregnancy was confirmed by real-time ultrasound scanning at approximately D25 of pregnancy and gilts confirmed pregnant were moved to a group housing system at D30 of gestation. A total of 4 sows did not become pregnant and therefore they were not considered for the trial. At approximately D110 of gestation, gilts were moved to individual farrowing crates. Within 48 h of farrowing, litters were standardized to a minimum of 12 piglets via cross-fostering in order to accommodate for any losses and ensure that at least 8 piglets were nursed throughout lactation by each sow until weaning. At processing (i.e. ear notching, cutting teeth and tails, and iron injection), piglets were identified and individual weights recorded. Water was provided *ad libitum* for sows and litters throughout the experiment.

3.2.2 Treatment of primiparous sows

Within each of the five animal groups in our study, PP sows were subjected to an IS protocol which consisted of 8 h/d separation from their litters. IS was applied during the last 7d of a 28d or 35d lactation period. As stated above, treatments were assigned based on PG600 use and weight, as well as back fat similarities from farrowing to D20 of lactation to ensure

consistency among sows allocated to various treatments. An overview of the study's treatments is provided in Figure 3.1. At D20 of lactation, sows were assigned to one of three treatments: 1) Control treatment (C28 n = 19) where piglets were weaned at D28 of lactation but were not exposed to IS, 2) IS treatment from D21 of lactation until weaning at D28 (IS21), 3) IS treatment from D28 of lactation until weaning at D35 (IS28).

During the IS period, sows allocated to the IS21 and IS28 treatments were moved away from the piglets to another room within the barn. During the 8h of separation, sows were housed together in group pens. Twice daily, sows received fence-line boar exposure for 20 min in the BEAR using mature high-libido boars with an established reproductive record. Back-pressure testing was performed during boar exposure to detect first standing estrus. Sows were considered to be in estrus if they exhibited a standing reflex and rigid stance when firm pressure was applied to their backs. Within treatments, sows were further classified based on the response to estrus induction during IS. Sows that responded to IS21 treatment and were detected in estrus during lactation were bred at either the first induced estrus (FE) during lactation (IS21FE n = 18) or were "skipped" and bred at the second estrus (SE) which occurred after final weaning at D28 (IS21SE n = 17). "Non-responders" (NR) refers to sows that did not show estrus during lactation and were only considered for further study if bred within 8d after final weaning (IS21NR, n = 23). All sows, within each of the five groups, were weaned on the same day of each week, approximately 28d after farrowing. Once weaned and bred, all sows were transferred to a breeding room and housed in individual stalls until checked for pregnancy.

Sows showing estrus were bred by artificial insemination (AI) starting 12h after the onset of standing heat every 24 hours until no longer in standing heat. Artificial insemination was performed with pooled semen, collected and processed on-site from three known-fertile boars. Semen doses used for AI contained 3.0 X 10^9 morphologically normal sperm per 50 mL dose and were stored for a maximum of 3d following semen processing. Any issues associated with the insemination procedure, such as excessive backflow post insemination or an extended period required for insemination, were recorded for all breedings. Two weeks following insemination, sows were also exposed to fence line boar exposure twice daily for 10 min to detect returns to estrus.

One day after weaning, Control and IS21 non-responders received twice daily 10 min fence-line boar exposure (0800 and 1500) for stimulation and estrus detection. Back-pressure testing was performed during boar exposure to detect first standing estrus after weaning and the WEI was recorded. If they displayed estrus within 8d of weaning they were bred according to the same protocol used for the other treatments. If not bred within 8d after weaning (n=4), sows were removed from the experiment. For all treatments, the day of the last insemination was considered to be day zero (DO) of gestation (embryo development). It should be noted that for the final group of sows who were exposed to IS from D28 to D35 of lactation (IS28 n = 14), there was limited response to induction of estrus and 10 were non-responders. The number of sows that responded to IS28 treatment (n=4) was not adequate to be statistically representative, so these animals were not included in further analyses. A total of 11 sows were removed during the experiment for various reasons including: lameness, mastitis, small litter and sows that were not pregnant at the time of assigning the treatments.

3.2.3 *Feeding*

Sow diets were based on NRC requirements. Starting at farrowing, all sows were fed 3.5 kg of a standard lactation diet (14.1 MJ DE kg^{-1} 20% Crude Protein and 1.04% Lysine). After farrowing, sows were fed using a standardized step-up feeding regimen: feed was increased by 0.5 kg /day if there was less than 0.5 kg feed not consumed and 12 kg was set as the maximum feed allowance. Feed was provided twice a day, with half in the morning and the remainder in the afternoon or when IS sows returned to the farrowing room after the IS period. Sows were not fed during the 8h of separation from their litters associated with IS.

After weaning, until sows were bred and/or completed a second estrus, they were provided *ad libitum* access to a dry sow diet (13.0 MJ DE kg^{-1} 15% Crude protein and 0.65% Lysine). On a daily basis, the amount of feed not consumed was weighed before the morning feed and feed intake (FI) was considered to be equal to feed disappearance, therefore feed wastage or spillage was not considered. Feed intake was calculated as total feed offered less feed weighed back. Piglets were offered creep feed which was introduced on D19 of lactation for the Control and IS21 groups and on D26 for IS28 groups. The amount of creep feed offered and weighed back was recorded.

3.3 Measurements

Sows body weight, back-fat depth (BFD) and loin depth (LD) (Newcom et al., 2002) were recorded on D1 of lactation (farrowing), D20 of lactation, at weaning, at second estrus for IS21SE sows and at D30 of the subsequent gestation. Piglets (n=1162) were individually weighed on D20 of lactation and at weaning. Sow BFD and LD were measured by real-time ultrasonography using a 3.5 – MHz probe (Mylab30, Esaote). Ultrasound images of BFD and LD were captured by placing the probe parallel to and at approximately 7 cm from the midline of the sow's back, from the last rib back towards the third or fourth last rib. From farrowing until weaning, if a piglet was removed (n=177) from the litter for any reason (e.g. death, injury, transfer, etc.) the date and weight were recorded.

3.3.1 Collection of reproductive tissues and embryos

All pregnant sows (n = 62) from C28, IS21, IS21NR, IS21SE and IS28 and IS28NR were euthanized on D29 ± 0.5 (SEM) of gestation. Reproductive tracts and embryos were recovered as described by Almeida *et al.* 2014. Number of corpora lutea (CL) per ovary was counted to determine ovulation rate. Immediately after slaughter, uteri were gently dissected to expose the allanto-chorionic placenta and enclosed embryos. All embryos within their extraembryonic placental membranes were gently separated from the underlying uterine wall. Allanto-chorionic volume and wet weight were used as measures of placental development. Viable embryos were removed from the enclosing amniotic membranes, crown-rump length and weight recorded and embryos were wrapped in aluminum foil and snap frozen in liquid nitrogen.

3.3.2 Metabolic parameters

Metabolic parameters for lactation efficiency were calculated as described by Bergsma et al. (2009). Energy balance was characterized using the same methodology as described by Patterson et al. (2011). In summary, energy input is considered to be the energy from total feed intake and body tissue mobilization minus energy required for maintenance of the sow. Energy output was calculated as energy needed for piglet growth and maintenance. Lactation efficiency is characterized as an energy competence of sows while lactating (Figure 3.2). The higher the lactation efficiency, the more energy available through feed intake and mobilization

from body stores above maintenance of the sow (input) is used for piglet growth and sow energy maintenance (output) (Bergsma et al., 2009). Estimates of energy inputs from feed and tissue mobilization, and energy outputs for sow maintenance plus litter maintenance and growth are shown in Table 3.3.

3.3.3 Statistical analyses

Sow was considered the experimental unit for all variables within the models analyzed. Analysis of reproductive traits was performed by comparing data from C28, IS21FE, IS21SE and IS21NR sow treatments only. In order to better understand the effect of metabolic state on reproductive responses, a retrospective analysis was completed comparing sows from the Control group and the sows that either responded or did not to the IS treatment. For the metabolic analysis, sows were re-grouped in categories as Control (C28), IS21FE and IS21SE were categorized as IS responders (ISR) and IS21NR as IS non-responders (ISN).

Statistical analyses were performed using SAS software and all variables were tested, using untransformed data, for normality and homogeneity of variance by histograms, gplots, and formal statistical tests as part of the MIXED procedure of SAS 9.4 (Statistical Analysis System Institute, Inc., Cary, NC, USA). Ovulation rate data was the only parameter that was not normally distributed and required log transformation. For presentation of the ovulation data, the results of least squares means were back-transformed and an error term for the original units was assessed using the untransformed data.

Effects of treatment on ovulation rate, the number and weight of embryos, embryonic survival, and metabolic energy inputs and outputs were analyzed by a mixed model in SAS. Day of euthanasia (based on day of gestation) was used as a co-variate in the analysis of embryo weight, crown-rump length and allanto-chorionic fluid volume. Embryo number was tested, but rejected as a co-variate in the analysis as it was found not to be significant. The effect of treatment (Control, IS-first estrus, IS-second estrus, IS-non-responders) was included in the model as a fixed effect and blocked by animal group (1 to 5) was considered a random effect. The generalized logit function (PROC CATMOD) of SAS was used to analyze separately the categorical data (proportion of sows bred and pregnancy rate).

3.4 Results

3.4.1 Sow fertility, embryonic survival, and development

As presented in Table 3.1, overall 35 out of 57 sows (61%) showed estrus during lactation in response to IS. In general, sows in the C28 treatment and sows that did not respond to estrus stimulation during lactation (non-responders (ISN)) displayed estrus 5.5 ± 0.3 d (SEM) after final weaning (D0), compared to -2.2 and -2.7 ± 0.3 d ($P < 0.05$) for sows that did respond to IS treatments (IS21FE and IS21SE).

Ovulation rate was lower in IS21SE sows (19.3 ± 0.8 SEM) than in C28 andIS21FE sows (23.3 ± 0.8, 22.7 ± 0.9). Despite this fact, considering sows generally do not ovulate during lactation, sows bred during IS period had an average of 20.0 ± 0.9 ovulations (IS21FE and IS21SE) and an average of 17.5 ± 1.1 live embryos (IS21FE) per sow at D30 after artificial insemination. Regarding pregnancy rates, IS21FE and IS21SE treatment groups were significantly different (83.3% vs 100%, respectively). The ISN groups had lower pregnancy rate than IS21FE (76.5% vs 100%, respectively).

For the embryonic analyses, embryo weight, and placental volume were adjusted for gestation length, that is, the day of gestation the measurements were collected, which ranged from 28 d to 31 d, with an average of 29.1 ± 0.5 d. Embryo survival (number of embryos/number of ovulations) for treatment IS21SE was 88.1% ± 4.6, compared to survival rates observed for the C28 which was 72% ± 4.5 (Table 3.1). Embryonic weight for the IS21FE treatment was lower than that of the C28 and NR treatment sows. Additionally, placental development as represented by the mean volume of allantochorion fluid present was significantly lower in the both IS groups assessed (IS21FE and IS21SE) compared to the C28 treatment.

As stated previously, in the IS28 group, only 4 of 14 sows exhibited estrus during lactation and all were pregnant at slaughter. Additionally, 6 of the remaining 10 sows displayed estrus within 7d of final weaning and were classified as IS28 non-responders (IS28NR); these 6 were bred and all (100%) were pregnant at slaughter.

3.4.2 Metabolic status

As shown in Table 3.2, among treatments the sow weights at farrowing, D20 of lactation and at weaning did not differ and, similarly, BFD measurements at these same time points were not different ($P > 0.05$). Although sow weights did not differ, litter growth rates from D20 to D28 for C28 sows was greater than for the ISR sows (3172.38 g and 2817.71 g, respectively; $P < 0.05$). Moreover, during the IS period (D20 – D28), the ISR sows lost more weight than the C28 sows (-6.53 kg and -4.38 kg respectively; $P < 0.05$).

When inputs from the sow were assessed for the period from farrowing to D20 of lactation, ISR sows mobilized less tissue (3.34 ± 1.0 MJ ME/d) when compared to ISN group (6.7± 1.2 MJ ME/d), while C28 and ISN tissue mobilization were not different. Non-responders (ISN) had higher input energy from tissue mobilization from farrow to D 20 than the IS sows. In addition, sow energy metabolized from diet, energy maintenance and sow energy inputs were not different among treatments. From D20 to weaning, sow energy output of Controls was 54.62 ± 1.4 MJ ME/d while ISR was 49.35 ± 1.1 MJ ME/d. Sow energy input of Controls was 101.8 ± 5 MJ ME/d and ISR 73.48 ± 3.5 MJ ME/d.

From D20 to weaning, the input energy from body reserves was higher in ISR sows than C28 sows (14.5 ± 3.3 and 7.4 ± 3.7 MJ ME/d, respectively; Table 3.3). Energy from feed intake (ME from diet) in C28 sows was higher than ISR and ISN (110.5 ± 4.2, 82.2 ± 3.1 and 88.9 ± 4 MJ ME/d, P-value < 0.05). The ISR sows had higher input energy from tissue mobilization than C28 sows. Moreover, C28 sow energy output for litter growth and total energy output to the litter was higher when compared to ISR sows (Table 3.3).

3.5 Discussion and Conclusions

3.5.1 Sow fertility – ability of IS to induce estrus during lactation

Inducing lactational estrus and breeding during estrus offers the potential to increase lactation lengths, weaning age, and piglet weight without affecting sow productivity (Soede et al., 2012). The results of the current study demonstrated that implementing an IS protocol with PP sows, lactational estrus can be induced, but in this study only 61% of sows exposed to IS experience estrus while lactating. Also, sows in the C28 treatment and those that did not

respond to IS (ISR) displayed estrus 5.3 ± 0.3 d after weaning compared to -2.3 ± 0.3 d ($P < 0.05$) for sows that did respond to the IS protocol. Previous IS studies have shown that, in some circumstances, only 23% of PP sows had estrus induced during lactation. For example, Soede et al. (2012) evaluted three IS treatments: Control sows were weaned at 26D ± 2 of lactation and IS treatments started either at D19 or D26 and continued until weaning at D26 and D33, respectively. Sows and litters were separated for 10 h per day and housed separately, preventing visual and auditory contact between sows and litters.

Regardless of which IS treatment was applied, the percentage of sows with lactation estrus was greatly affected by parity with 23% (8/26) of first parity (PP) showing estrus, while 85% (22/26) of second parity and 61-65% of older parity sows showed lactational anestrus. This response to estrus induction is much less than the 61% of PP sows that exhibited standing estrus during lactation in the present study The reason for the higher rate of lactation estrus in PP in our study relative to other studies has not been elucidated, but could be related to genetic background or one of the many factors that can influence response to induction of lactational estrus listed above. It has been suggested that selection pressure with lines of breeding females has resulted in modern sows with a shorter WEI (Gerritsen et al. 2008b) that are more likely to exhibit lactational estrus than previous lines (Gerritsen et al. 2009). However, given the differences in genetic selection strategies across commercial pig breeding companies, the genetic background of sows may influence their response to estrus induction during lactation.

3.5.2 Effects of IS on ovulation rate, pregnancy rate, embryonic survival

One must keep in mind that even if IS can be used to successfully induce lactational estrus, it is also important that the follicles that develop and oocytes produced are of good quality to produce viable embryos and adequately sized litters of robust piglets. In the present study, ovulation rate and embryo survival between sows bred at first estrus during lactation (IS21FE) and sows bred at the subsequent estrus following lactational estrus (skip-a-heat-IS21SE) differed significantly. The difference could be due to metabolic status during lactation. However, the number of viable embryos at d30 were similar among treatments (Table 3.1). This is in agreement with the findings of Gerritsen et al. (2008) who that found sows bred during lactation had similar pregnancy rates and litter sizes to sows bred after weaning as long as the sows are well managed and they are challenged metabolically.

3.5.3 The effects of skipping a heat (IS21SE) and IS

In the present study, it was hypothesized that breeding PP sows at the subsequent estrus following their first estrus during lactation (IS21SE) would improve reproductive outcomes, including embryo survival, when combined with an IS estrus induction protocol. Interestingly, sows bred during lactation (IS21FE) had significantly decreased placental development and lower embryonic weight at D30 when compared to C28 sows. These results indicate that breeding at the IS-induced estrus during lactation may be linked to issues of subsequent litter quality, which are partly corrected by breeding at the next estrus after final weaning as in the ISSE group.

The results of this study showed no difference in embryo survival between C28 and IS21FE, but interestingly embryo survival in IS21SE sows was higher when compared to C28. This effect on embryo survival appears to be the first demonstration of this response to IS as a review of the literature reveals no other studies comparing IS protocols, skipping lactational estrus and breeding at subsequent estrus. Higher embryo survival in IS21SE sows has been addressed by Patterson et al. (2007) in that breeding first parity weaned sows at 2nd post-weaning estrus can negate the effects of the "second parity dip". Although skipping the lactational estrus and breeding at subsequent estrus resulted in greater rate embryonic survival, it did not translate into a difference in the number of live D30 embryos or pregnancy rates for the Control, IS21FE and IS21SE sows.

Despite the lack of difference in the number of live embryos across treatments, the IS21SE sows exhibited a lower ovulation rate that IS21FE and C28 sows, but a higher embryonic survival than the C28 sows. Also, the embryonic weight for the IS21FE sows was lower than the C28 sows and the placental volume, as indicated by the amount of allanto-chorioc fluid present, was less in the IS21SE and IS21 sows. This observation raises the question whether there may be an impact of the IS protocols on embryonic development, which is addressed in Chapter 4 of this thesis.

In the present study, skip-a-heat breeding (IS21SE) compared to breeding at the lactational estrus did not result in a significant overall improvement reproductive outcomes in PP sows. Based on these results, the hypothesis that breeding PP sows at the subsequent estrus

following first estrus during lactation would improve reproductive outcomes is rejected. However, the effect of lactational estrus on subsequent litter development requires further examination.

3.5.4 Metabolic changes during and after IS and their reproduction outcomes

Farrowing to D20

The relationship between maternal metabolic state and subsequent embryonic and fetal development in domestic animals has received growing attention, as maternal metabolic state is directly related to reproductive outcomes. Sarr et al. (2010) reported that maternal nutritional status during gestation can affect fetal growth and tissue development, which in turn can influence piglets' phenotypes, including the amount of fat deposition and body weight.

In our study, assessment of energy inputs from the sow and energy outputs to the litter proved to be quite revealing. C28 sows consumed more feed than ISR and ISN sows. Although not significant, C28 sows also numerically partitioned more energy to litter growth than the ISR sows. Interestingly, ISN sows mobilized more energy from tissue when compared to IS sows, suggesting that ISN sows were more metabolically challenged than the ISR sows but not as challenged as the C28 sows. From farrowing to D20 the total energy out to the litter was similar between treatments.

From D20 to weaning

Lactation efficiency in term of energy input and output to the litter was described by Bergsma et al. (2009) in terms of growth. In the present study, piglets' average daily gain for C28 sows was higher at 3172.38 ± 87.85 g than ISR at 2926.43 ±7 6.08 g. Litter growth was similar when comparing C28 and ISN and IS and ISR (Table 3.2). However, total energy output to the litter has higher in C28 sows (56.6 ± 1.4) than IS sows 49.4 ± 1.1 MJ ME/d. Furthermore, retrospective analysis found ISR sows were less catabolic than the ISN sows at D20 (when the treatments were assigned). Thus, the response to IS could be explained by the metabolic stage of the sow. On the other hand, ISN sows were able to return to estrus promptly after weaning – similar to the C28 sows, due to the fact that ISN had a 28d lactation instead of a 21d lactation which allowed these sows to recover metabolically. Even though ISN were able to return to estrus promptly after weaning, the ISN pregnancy rate was lower than IS21SE (76.5% vs 100%, respectively. Additionally, the weight loss data in Table 3.2 shows that ISN weight loss from D20 to farrowing fall between that of the C28 and the ISR sows.

The ISN sows in the current study recruited more energy from their own body resources and were therefore were more metabolically challenged and were not able to respond to the IS and did not displayed estrus during lactation. As reviewed by Foxcroft (1996), a sow's metabolic state can indirectly affect the secretion of regulatory hormones as well as follicle development and oocyte maturation. Metabolic state contributes to embryonic survival as well as impacts the physiology of oviductal cells interfering in cell motility and secretion. Similarly, Miller et al. (1998) described how negative energy balance (weight loss) interferes with gonadotrophin secretion by altering the reproductive axis besides having effects on embryonic development. Similarly, the onset of ovarian activity in sows (PP and MP) and related estrous behavior is influenced by metabolic status during lactation, stress related to environment and weaning of piglets (Madej et al., 2005).

According to Foxcroft et al. (2009), the most consistent response to a previous catabolic state in sows is a reduction in embryonic weight, independent of embryo sex. However, in the current study, no difference in embryonic weight related to the treatment applied or the sow's catabolic state during lactation was detected. Furthermore, and as reviewed by Foxcroft et al. (2007) lactational catabolism is suggested to be related to development of immature follicles

and oocytes, and the reduced fertility when first parity sows are bred at the first post-weaning estrus. Madej et al. (2005) also described other factors such as artificial insemination, boar presence, and the role of seminal plasma which may regulate the temporal kinetics of ovulation, corpus luteum development, uterine function and steroid production in the ovary. As already discussed, genetic selection over the years improving reproductive performance has changed the biology of commercial sows which, as a consequence, sows are becoming more resistant to the negative effects of lactational catabolism due to their higher reproductive performance.

The results obtained indicate that with proper management (e.g. optimal sow-piglet separation, estrus stimulation and detection, nutrition) sow fertility can be maintained at acceptable levels when applying IS and lactational estrus induction protocols. However, a review of the literature reveals that there are no other studies comparing IS protocols, with skipping-a-heat and breeding second estrous cycle after weaning. Weaning to estrus interval is one of the main contributors to non-productive days in sows and is influenced by lactation length, parity order, litter size, season and nutrition (Poleze et al., 2006). Sows that respond to IS display estrus before weaning would have fewer non-productive days which would be an obvious advantage for pig producers.

CHAPTER 3 - FIGURES

Figure 3.1 Intermittent suckling treatments and schematic diagram of the protocol

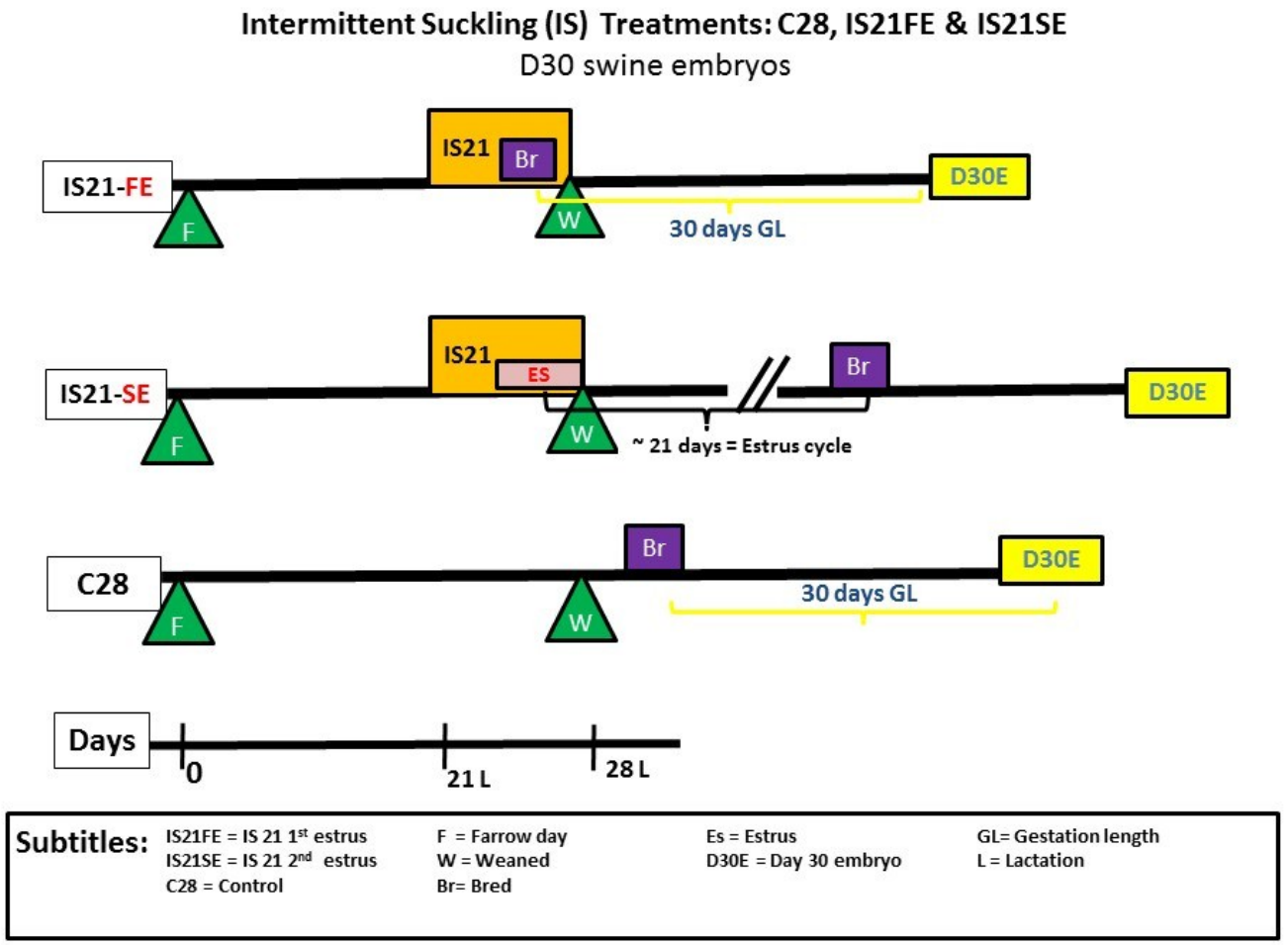
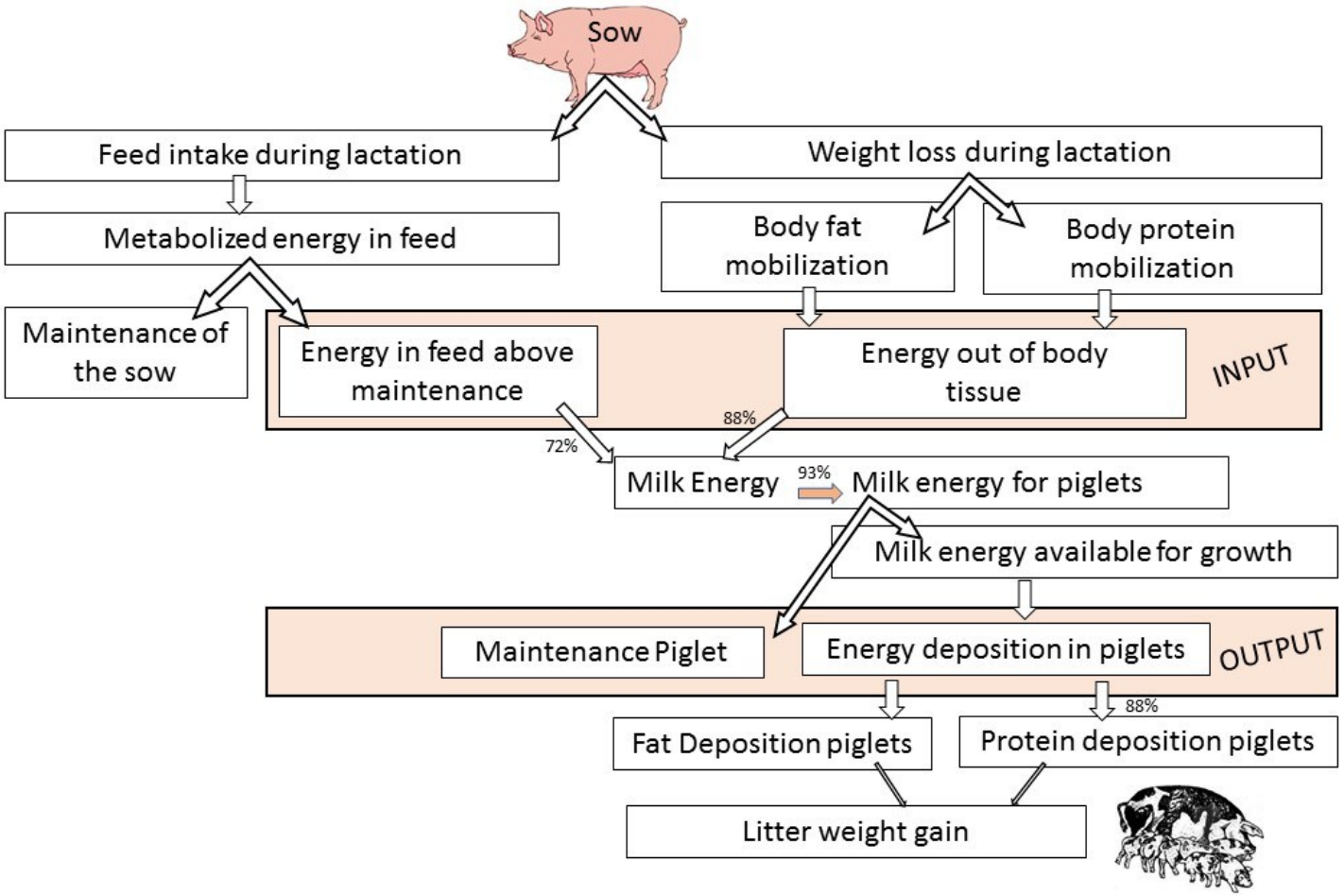


Figure 3.2 Schematic flowchart of the energy metabolism in lactating sows adapted from Bergsma et al. (2009).



CHAPTER 3 - TABLES

Table 3.1. Lactational estrus and subsequent reproductive outcomes in PP sows bred at lactational estrus (IS21FE), at the subsequent estrus following lactational estrus (IS21SE), or at normal post-weaning estrus (non-responders) (least square means ± SEM).

Treatment[#]	C28 (n=19)	IS21 (n=57)		
Lactational estrus (%)	-	61% (35/57)		
		IS21FE (n=18)	IS21SE (n=17)	NR[*] (n=22)
Weaning to estrus interval (d)	5.1 ± 0.3 [a]	-2.2 ± 0.3 [b]	-2.7 ± 0.3 [b]	5.5 ± 0.3 [a]
Day of gestation at slaughter	29.2 ± 0.5	29.8 ± 0.5	29.1 ± 0.5	29.1 ± 0.5
Breeding rate (%)	79 (15/19)	100 (18/18)	100 (17/17)	77 (17/22)
Pregnancy rate (%)	100.0[a] (15/15)	83.3[ab] (15/18)	100.0[a] (17/17)	76.5[b] (13/17)
Ovulation rate*	23.3 ± 0.8[a] (n=15)	22.7 ± 0.9[a] (n=15)	19.3 ± 0.8[b] (n=17)	22.3 ± 0.8[ab] (n=13)
Number of live embryos*	16.4 ± 1.0 (n=14)	17.5 ± 1.1 (n=10)	17.1 ± 1.1 (n=12)	18.5 ± 1.1 (n=12)
Embryonic survival (%)	72 ± 4.5[a]	78 ± 4.9[ab]	88.1 ± 4.6[b]	82.5 ± 4.7[b]
Embryonic weight (g)	1.51 ± 0.04[a]	1.33 ± 0.04[b]	1.45 ± 0.04[ab]	1.48 ± 0.04[a]
Allanto-chorioic fluid volume (ml)	229.6 ± 9.7[a]	200.4 .0 ± 11.5[b]	190.7 ± 9.8[b]	210.7± 10.5[ab]

[a,b] $P < 0.05$. [#]C28 = Control sows weaned at day 28 of lactation and bred at first post-weaning estrus; IS21 = Sows separated from their piglets for 8hrs/day from d 21 of lactation until weaning; [*]NR = Non-responders. SEM: standard error of the mean.* n values provided within the table refers to number of observation available

Table 3.2. Sow weight, weight loss and backfat parameters at farrowing, D20 of lactation and weaning (D28) and litter average daily gain (adg) from D20 of lactation to weaning (D28). (Least square means ± S.E.M.)

Parameter	**C28**	**ISR**	**ISN**
Weight at farrowing (kg)	186.5 ± 2.67 SEM	189 ± 1.9 SEM	189 ± 2.4 SEM
Weight at D20 lactation (kg)	180.6 ± 2.8 SEM	185.5 ± 2.09SEM	180 ± 2.6 SEM
Weight at weaning (kg)	176.1 ± 2.9 SEM	179.4 ± 2.1 SEM	177.2 ± 2.7 SEM
Weight loss D20 – weaning (kg)	- 4.4 ± 2.9 SEM [a]	- 6.38 ± 1.3 SEM [b]	- 3.3 ± 1.5SEM [ab]
Backfat at farrowing (mm)	18.39 ± 0.7 SEM	18.31 ± 0.5 SEM	17.86 ± 0.7 SEM
Backfat at D20 of lactation (mm)	15.75 ± 0.9 SEM	16.8 ± 0.9 SEM	15.14 ± 0.9 SEM
Backfat at weaning (mm)	14.59 ± 0.7 SEM	13.7 ± 0.67SEM	14.4± 0.53 SEM
Litter adg (g) D20 – D28	3172.38 ± 87.85 SEM [a]	2926.43 ± 76.08 SEM [b]	2817.71 ± 68.04 SEM [ab]

Within rows, values without a common superscript (a, b) differed significantly ($P < 0.05$)

Table 3.3. Estimates of energy inputs from feed and tissue mobilization, and energy outputs for ow maintenance as well as litter maintenance and growth. (Least squares means ± S.E.M.)

Item (MJ ME/d)	C28 (n=19)	IS Responders (ISR n=26)	IS Non-Responders (ISN n=22)
Farrowing to D20 of lactation			
Input energy from the sow			
ME intake from feed consumed	78.5 ± 3.4	80.4 ± 2.7	71.8 ± 3.3
Energy from tissue mobilization	5.9 ± 1.3 [ab]	3.4 ± 1.0 [b]	6.7 ± 1.2 [a]
Energy requirements for sow maintenance	21.9 ± 0.2	22.3 ± 0.2	22.0 ± 0.2
Net energy inputs from the sow	61.9 ± 3.1	61.5 ± 2.4	56.9 ± 2.9
Energy output to the litter			
Energy for litter maintenance	12.9 ± 0.4	12.3 ± 0.3	12.8 ± 0.4
Energy for litter growth	29.7 ± 1.5	27.8 ± 1.3	28.2 ± 1.4
Total output to the litter	42.7 ± 1.8	40.1 ± 1.6	40.9 ± 1.7
D20 to Weaning (D28)			
Input energy from the sow			
ME intake from feed consumed	110.5 ± 4.2 [a]	82.2 ± 3.1 [b]	88.9 ± 4.0 [b]
Energy from sow tissue mobilization	7.4 ± 3.7 [a]	14.5 ± 3.3 [b]	8.9 ± 3.5 [ab]
Energy required for sow maintenance	21.5 ± 0.3	21.8 ± 0.2	21.5 ± 0.2
Net Energy inputs from the sow	101.8 ± 5.0 [a]	73.5 ± 3.5 [b]	75.9 ± 4.6 [b]
Energy outputs			
Number of pigs nursed	11.0 ± 0.2	11.2 ± 0.2	11.4 ± 0.2
Energy for litter maintenance	20.5 ± 0.6	19.6 ± 0.5	20.0 ± 0.5
Energy for litter growth	34.3 ± 1.0 [a]	29.8 ± 0.8 [b]	31.2 ± 0.9 [ab]
Total energy output to the litter	54.6 ±1.4 [a]	49.4 ± 1.1 [b]	51.0 ± 1.2 [ab]

Within rows, values without a common superscript (a, b) differed significantly ($P < 0.05$)

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CHAPTER 4

4. Effects of applying intermittent suckling protocols in primiparous sows on embryonic quality.[1]

4.1. Introduction

As sows are in a state of anestrus during lactation (Armstrong et al., 1988), breeding typically occurs after weaning following a weaning to estrus interval of approximately 4 to 7 days. Although extending lactation length can benefit piglet welfare, this management technique can also influence sow reproductive performance, decreasing the number of piglets a sow produces per year, and impact producers' yearly profits. Intermittent suckling (IS) is a protocol implemented during lactation which involves a sow and her piglets being separated daily in order to reduce suckling during lactation, and inducing estrus (Newton et al., 1987; Gerritsen et al., 2008). Inducing lactational estrus and breeding during lactation has certain advantages, it increases lactation length and allows for weaning older piglets without significant losses in sow productivity and piglet performance as well as an increase in piglet welfare at weaning.

Despite several extensive studies related to IS protocols and sow reproductive performance (Gerritsen et al., 2005; 2008; 2009; Soede et al., 2012), the influence of IS on embryonic development has not yet been well investigated. However, it has been established that embryonic survival and development can be influenced by a number of factors including genetic makeup, maternal nutrition, uterine capacity and environmental temperature (Foxcroft et al., 2006; 2007; Vinsky et al., 2007; Wright et al., 2016). The maternal metabolic state at the time of ovulation, as well as during the prenatal period, can negatively affect the embryo

[1] Portions of this chapter have been published in: Tsoi S., Blanes M., Chen T., Langendjk P., Athorn R., Foxcroft G., Dyck M. (2016) Identification of differentially expressed genes in sexed pig embryos during post-hatching development in primiparous sows exposed to differing intermittent suckling and breeding strategies. Genomics Data 9: 30-34; and Blanes M.S., Tsoi S.C., Dyck M.K. (2016). Accurate and Phenol Free DNA Sexing of Day 30 Porcine Embryos by PCR. J Vis Exp. 2016 108:53301.

survival (Foxcroft et al., 2006). Given the high metabolic demand of lactation, it is expected that a negative energy balance of the sow during early gestation can negatively affect embryonic development. Specially, the metabolic status of the sow during breeding has been shown to not only affect embryonic gene expression, but it can also influence the sex ratio of the surviving embryos (Vinsky et al., 2006). In primiparous sows (PP) in particular, a negative energy balance is expected to be significant during lactation due to the fact that they are being bred prior to reaching full physical maturity (Bortolozzo et al., 2010). In order to negate the effects of this "second litter syndrome", in which reduced fertility is commonly observed with PP sows, breeding on the second post-weaning estrus has been shown to increase embryonic survival and litter size (Clowes et al., 1994; Patterson et al., 2007).

Limited information on the application of IS protocols in PP sows and its impact on embryonic quality is available (please list the information (references). Therefore, building on the findings outlined in Chapter 3 of this thesis, the objective of this study was to determine the impact of IS breeding strategies on embryonic development, litter sex ratio and gene expression when it is applied to PP sows. Based on previous studies, it was hypothesised that breeding PP sows at the second estrus following the initial estrus induced during lactation (skip-a-heat) would improve embryo survival when combined with an intermittent suckling estrus induction protocol. The recent development of a porcine specific platform in our lab to assess the embryonic transcriptome (Tsoi et al., 2012) facilitates analysis of embryonic gene expression from sows bred during lactation. Previous research by our team (unpublished data) showed no difference in gene expression from IS21FE and IS21SE day 9 embryos. Therefore, for this investigation, a more advanced stage of embryo development was chosen. Development and quality of day 30 embryo (D30E) were assessed based on gene expression analysis of day 30 embryos (D30E) from sows bred during lactational estrus, bred at the subsequent estrus following their first estrus during lactation and from control sows bred after weaning from a 28 day lactation. If, as demonstrated in Chapter 3, IS can be used to induce lactational estrus in PP sows without a dramatic impact the sows' fertility parameters, the question remains whether breeding PP sows during lactation has an impact on embryonic quality and possibly litter quality. Therefore, assessing embryonic gene expression and litter sex ratios from sows bred during lactation is an important question to address.

4.2. Materials and methods

All animal procedures were conducted at the University of Alberta, Swine Research and Technology Centre (Edmonton, Alberta, CANADA) with approval of the Faculty Animal Policy and Welfare Committee – Livestock (AUP00000521). As described in Chapter 3 of this thesis, primiparous (PP) sows (Large White x Landrace, Hypor genetics; n = 76) were allocated to either a Control treatment (C28) where piglets were weaned at day (D) 28 of lactation or an intermittent suckling treatment (IS21) where all piglets were separated from the sow for 8 hours (h) each day. IS started at D21 of lactation until weaning at D28. All IS21 sows were housed together in group pens during the separation period. In a heat detection area, sows were maintained in a fenced pen and twice daily received fence-line boar exposure for 20 minutes and were bred by artificial insemination (AI) at either lactational estrus (IS21FE) or at the subsequent estrus following lactational estrus (skip-a-heat = IS21SE, Figure 4.1At approximately D30 of gestation the sows were euthanized in order to examine embryo characteristics. Embryos harvested at D30 of gestation were exposed to sex identification by PCR and gene expression analysis using a microarray platform as described in the following sections.

4.2.1 Sex typing day 30 Embryos (D30E)

A total of 615 embryos were collected individually and sex-typed for each treatment (C28 n=234, IS21FE n=173, IS21SE n=208). As a part this study, a novel DNA-based phenol-free embryo sex typing protocol was developed (Blanes et al., 2016) and is briefly described here.

4.2.2 Sample preparation - grinding embryos

A pre-chilled mortar and pestle were used for each embryo and examination gloves were changed after grinding each embryo to avoid cross contamination between samples (McCulloch et al., 2012). Each frozen embryo was placed into a mortar on dry ice. Liquid nitrogen was poured into the mortar to cover the embryo and the embryo pulverized and ground into a fine powder using a pre-chilled pestle. Then, the embryo powder was transferred to a pre-labeled sample tube, kept on dry ice, with a micro-spatula and stored at -80°C until extraction.

4.2.3 DNA preparation using modified sodium hydroxide method

Sample tubes containing the powdered embryos were transferred from -80°C storage onto dry ice and 180 µl of 50 mM NaOH (sodium hydroxide) was pipetted into pre-labeled micro-centrifuge tubes. The embryo powder (about 5-10 mg) was transferred from the sample tube into a pre-labeled micro-centrifuge tube containing 50 mM NaOH solution. Once the sample was mixed with NaOH, DNA lysate was formed. Next, micro-centrifuge tubes with DNA lysate were transferred to a pre-heated incubator at 95°C for five min. Then, 20 µl of 1M Tris–HCl were added directly into the microcentrifuge tube and mixed by gently tapping the tube. The tubes with the DNA lysate were centrifuged at 2,000 Xg for two min at room temperature to remove undissolved tissue debris. Approximately 150 µl of the clear supernatant containing the DNA lysate was transferred into 96 well plates. DNA lysate was then ready to use as a template in PCR reaction.

4.2.4 Design Sex-specific PCR primers

Accession numbers for Porcine sex determining region Y (SRY) (NM_214452.3) and zinc finger protein X-linked (ZFX) genes (XM_005673501.1) were obtained from the NCBI website www.ncbi.nlm.nih.gov. NCBI accession numbers were used to develop primers using an online primer design tool (PrimerQuest; www.idtdna.com/primerquest/home/index). Optimal primers were generated based on primer length, Tm and GC% as well as amplicon sizes (bp) (Figure 4.2 - A, B). Primers' specificity was validated using a nucleotide Blast program (Blast) against the current porcine genomic database 104 to ensure the sequences were only located on the X and Y chromosomes for the SRY and ZFX regions respectively.

Primers specific to the SRY region of the Y-chromosome SRY 5′-GGGAAAGGCTCCTCACTATTT-3′ (forward) and 5′-AGGATACATCCTCTCCTCTAC-3′ (reverse), were used. While for the ZFX region of the X-chromosome, 5′-GTGCTGCTTTGTCTTGGAATG -3′ (forward) and 5′- GAGGGAGTTAGGTCTGGATACT -3′ (reverse), were used (Figure 4.3).

4.2.5 Genomic DNA PCR condition and validation

Only 1 µl of the DNA lysate was used as a template for a 15 µl PCR reaction. For quality control purposes, for each PCR run, a negative control with no template was prepared as well as two male and female positive controls containing 0.5 ng of porcine genomic DNA from each sex.

A HOTSTART ready mix enzyme (Phire Hot Start II PCR Master Mix, Thermo Scientific™) was used and a master mix prepared by adding primers and nuclease free water. The final volumes of HOTSTART and master mix were calculated based on the total number of PCR reactions to be performed. One microliter of the DNA lysate for each sample was added into a pre-prepared PCR tube with 14 µl of the PCR master mix. The primers were added such that the final concentration of the primers from two sex-specific genes was 0.3 µM in total of 15 µl PCR reaction.

The PCR program was used in a thermal cycler was as follows: 95°C for 3 min, 35 cycles each with a 20 sec melting step 98°C, followed by a 15 sec. annealing step at 65°C, followed by a 15 sec. elongation step at 72°C. PCR conditions were developed based on the primers' annealing temperatures and validated with pilot runs prior to the study. In the final step, reactions were incubated for 1 min at 72°C and continued to incubate at 4°C until removal of the PCR tube for gel electrophoresis verification to assess amplicons and determine the sex. Amplicons were visualized on a 2% TBE agarose gel with an appropriate amount of non-toxic green fluorescent SYBR DNA gel stain added to visualize the bands. A volume of 1.5 µl of loading dye (10X) was added into the PCR tube and mixed by pipetting the PCR reaction buffer. Ten µl of each sample was loaded into the well and the agarose gel was run with appropriate voltage settings (e.g. small gel apparatus at 100V, 96-well gel apparatus at 150V until the dye band runs halfway through the gel). A Typhoon FLA 9500 laser scanner (GE Life Sciences) was used to capture the image of the gel.

4.2.6 Gene expression analysis experimental design

From the embryos collected and sex typed, female and male subsets of D30E from each treatment (C28, IS21FE, IS21SE) were selected and pooled with embryos of the same sex and of similar weight, for gene expression analysis by microarray. The experimental design for

microarray analysis is shown in Figure 4.4. In general, a reference design was used and three biological replicates (three sows) were chosen from each treatment group in order to compare embryos from Control sows (C28) to either IS21FE or IS21SE or sows. Three male and three female D30E were pooled within gender from each sow in each treatment group resulting in 24 distinct biological replicates. The details of this array study were submitted to NCBI the Gene Expression Omnibus (GEO) repository to satisfy journal publication expectations (Tsoi et al., 2016).

4.2.7 RNA extraction from D30E

At least 0.05 mg of powdered embryo (described above) was transferred into a pre-labeled 2 ml micro-centrifuge tube containing 500 µl of TRIzol® Reagent (Invitrogen-Life Technologies, ON, Canada). The mixture was agitated until the powder completely disappeared. The lysate mixture was centrifuged at 12,000 Xg for at least 1 min until all suspension particles were visually pelleted at the bottom of the tube. Approximately 400 µl of clear supernatant was transferred into a new 2 mL tube followed by the addition of an equal volume of ethanol. Total RNA was extracted using Direct-zol^{TM} RNA MiniPrep (Zymo Research, CA, USA), as per the manufacturer instructions. The quality and quantity of the total RNA was assessed using a Agilent 2200 TapeStation system with RNA ScreenTape and NanoDrop respectively. RNA samples with RIN value higher than 6 were stored at -80°C until further treatment for microarray analysis.

4.2.8 Gene expression analysis by Microarray

For this study, a porcine microarray platform manufactured by Agilent Technologies and previously described (Tsoi et al., 2012) was used to assess embryonic gene expression. The arrays contain a total of 43,795 probes to enhance detection of the genes expressed during different stages of embryonic development (Tsoi et al., 2012). Total RNA for D30E in 200 ng of sample was used for antisense RNA (aRNA) synthesis. Then labelling of the aRNA with Cy3 and Cy5 was performed according to instructions provided by Low Input Quick Amp Labeling Kit (Agilent Technologies, Inc., Santa Clara, CA, USA) for two-color processing. Microarray hybridization and washing procedures were performed according to Agilent Gene Expression Hybridization Kit 60-mer oligo microarray protocol (version 4.0) and as described

elsewhere (Zhou et al., 2014b). After array slides were dried, each slide was scanned using Axon 4200AL scanner and its embbed software Gene Pix Pro 6.0 (Molecular Device, Sunnyvale, CA, USA) for spot image analysis. Spot images were saved in the GenePix Results (GPR) format for further data analysis and deposited to NCBI with GEO ID: GSE73020.

4.2.9 Gene expression and statistical analysis

Observations were tested for treatment effect, sex-specific effects, and any significant interactions between them. Sow was the experimental unit and individual measurements were pooled according to sex within a litter prior to statistical analysis. The effects of treatment on sex ratio was statistically analyzed using the Mixed procedure of SAS 9.4 (Statistical Analysis System Institute, Inc., Cary, NC, USA). Statistical differences between treatments were analyzed using the chi-squared test. A probability of less than 0.05 was considered significant.

For microarray analysis of gene expression, a dye-swapped direct comparison design was used. First, three biological replicates from each treatment group (C28, IS21FE and IS21SE) were analyzed by comparing C28 embryos with either IS21FE or IS21SE from females and males separately. Subsequently, a reference design approach was chosen to detect differentially expressed genes (DE) between IS21FE and IS21SE from both sexes independently. Microarray data analyses were performed using FlexArray software version 1.6.3 (http://genomequebec.mcgill.ca/FlexArray) for data normalization methods using simple background subtraction, LOWESS normalization within and between arrays (Figure 4.5). Further analysis to detect differentially expressed genes was performed using embedded programs of FlexArray such as Limma (Smyth, 2005) and the Benjamini and Hochberg false discovery rate, BH-FDR (Benjamini and Hochberg, 1995) multiple comparison correction condition with additional switching on the calulation setting for false positives due to the dye effect. The threshold pararmeter setting for DE genes were considered to be significant when a fold change (FC) of ≥ 2 (or ≤ 0.5) was detected with a BH-FDR adjusted P value of ≤ 0.05 (B-H P-value).

4.2.10 Bioinformatics analysis and gene annotation

Two lists of genes (target and background sets) were chosen as a running mode from GORILLA (Eden et al., 2009) which is a free online tool for discovery and visualization of enriched GO terms in ranked gene lists (http://cbl-gorilla.cs.technion.ac.il/) to identify biological process, molecular function and cellular component. A list of 16,260 unique GS, previously identified on the array (Zhou, 2014b), was used as a background gene set during the run.

Gene annotation was performed using probe sequences to target the known sequences from NCBI BLAST program by selecting two different pig nucleotide databases (Annotated RNAs Annotation Release 105) or Scrofa 10.2 (reference Annotation Release 105) to maximize the search for positive hits with genes of interest. Sequences were considered to have significant alignments with a gene of interest when the identity match was greater than 98% with the bit score ranging from 56.5 to 111. The gene symbols were then converted to orthologous human gene symbols as described in previous publications (Tsoi et al., 2012; Zhou, 2014a,b).

4.3. Results

4.3.1 PCR sexing of D30E

The sex of each embryo was determined by identifying embryos with one PCR band as a female (520 bp) and two bands as a male (502 and 400 bp) (Figure 4.6). Neither male nor female samples yielded non-specific amplification. However, in rare cases, cross-contamination between DNA lysates of two different samples was possible and could be detected by PCR as indicated with a red star in the gel (Figure 4.7). Red star on Figure 4.7 indicates the possible sample contamination with the appearance of a very faint lower band (400bp) compared to upper band (506bp) PCR product caused by SRY Y-specific primers Assessment of the embryos' sex revealed no significant differences, so there were no effects of treatments IS21FE and IS21SE on sex ratio ($P > 0.05$, Table 4.1).

4.3.2 Differential gene expression in D30E

Microarray analysis revealed no significant differences in gene expression when comparing Control (C28) embryos with either IS21FE or IS21SE within sex (female-to-female and male-to-male). However, when comparing expression between IS21FE and IS21SE embryos using a reference design and utilizing all the Control samples as the reference setting in Flex array analysis, the volcano plot indicated that there were 26 and 4 differentially expressed (DE) genes in female and male D30E, respectively (Figure 4.8). After gene annotation, no overlapping of DE genes were found between male and female D30E resulting from the effect of two different maternal treatments between IS21FE and IS21SE. Table 4.2 shows the results of all DE genes with gene symbols and GenBank accession numbers in female and male D30E along with log2 (Fold change) and adjusted P-value.

4.3.3 Non-Differential genes in D30E

Table 4.3 shows the results of all non-DE genes in D30E with no effect seen between the two different maternal treatments between IS21FE and IS21SE. After the gene annotation process, only 430 unique genes were listed as targets for GO term analysis using GORILLA (Eden et al., 2009). The results of the biological process analysis of the unique non-DE genes from D30E (by setting the p-value threshold at 10^{-4}) is shown in Figure 4.9. Three GO terms (GO:0030048- actin filament-based movement, GO:0019222- regulation of metabolic process and GO:0090131- mesenchyme migration) were significantly lower than the threshold setting (Table 4.4).

4.4 Discussion and Conclusions

Although the application of IS protocols to induce estrus and breeding during lactation has been well studied in sows (Gerritsen et al., 2005; 2008; 2009; Soede et al., 2012), there is limited information on the application of IS protocols in PP sows and its impact on embryonic quality, particularly at a molecular level. For the current study, the objective of this study was to determine the impact of IS and the effect of breeding PP sows during lactational estrus on embryonic development, litter sex ratio and embryonic gene expression. Therefore, theses reproductive parameters were assessed through gene expression analysis of sexed D30 embryos from PP sows bred during lactational estrus (IS21SE), bred at the subsequent estrus

following their first estrus during lactation (skip-a-heat, IS21SE), and Control sows (C28) bred after weaning from a 28d lactation.

Embryonic development and phenotypic outcomes of the offspring, such as viability and birth weight, are influenced by the maternal environment (Foxcroft et al., 2009). A common response to a sow's catabolic state prior to breeding is a decrease in embryonic weight, independent of embryo gender (Foxcroft et al., 2009), and the most significant consequence of a catabolic state in sows is a reduction in embryonic weight, independent of embryo sex. Patterson et al. (2011) also reported a detrimental effect of feed restriction in late lactation on embryonic weight at D30, however they reported no effect on embryonic survival. Based on the results of Patterson et al. (2011), a follow-up study (Oliver et al., 2011) described variations in expression of embryonic and placental genes related to embryonic development. Oliver et al. (2011) reported that previous sow catabolism led to a reduction of average conceptus growth at D 9.5 of gestation in first estrus sows compared to skipped estrus sows. As such, sows bred at second estrus (IS21SE) would be expected to have better fertility rates than sows bred during lactation (IS21FE) (Clowes et al., 1994). Despite of having anticipated that embryonic gene expression among our treatment groups (IS21FE, IS21SE and C28) would be different in this study we did not detect differences in gene expression.

As stated in Chapter 3 of this thesis, ovulation rate and embryo survival between sows bred at their first estrus during lactation (IS21FE) and those bred at the subsequent estrus following lactational estrus (IS21SE) differed significantly. However, this effect of treatment on these reproductive parameters did not result in a significant difference in the number of viable embryos at D30, which would indicate embryonic survival was not influenced by treatment. Similarly, microarray analysis revealed no significant difference in gene expression when comparing C28 with either IS21FE or IS21SE embryos in either sex. The lack of variance in gene expression among treatment groups in the current study could be due to the dam selection processes, directed to increased ovulation rates and litter sizes in the modern sow, at the same time as improving post-weaning fertility (Oliver et al., 2011). As well, the limited effect on gene expression could also be explained by the similarity of the sows' metabolic states when treatments were assigned or during lactation and pregnancy (Chapter 3). Lactational catabolism is suggested to affect development of immature follicles and oocytes

and reduced fertility when first parity sows are bred at the first post-weaning estrus (Foxcroft et al., 2007). Therefore, in the current study, the effects of breeding during lactation on embryonic quality were not significant because the sows were well managed and the metabolic impact of lactation was limited. The lack of differences in gene expression could also be explained by the stage of embryo development that were assessed.

As described by Vinsky et al. (2006), the metabolic status of the sow during breeding can affect embryonic gene expression and influence sex ratio within the litter. Therefore, assessment of litter sex ratio can be an indication of altered embryonic survival and gene expression. The most sensitive and effective way of embryo sex typing is using PCR to amplify DNA with gene specific primers located on X and Y chromosomes. Pomp et al. (1995) were the first to use PCR to identify sex of D10 and D11 porcine embryos, using gene markers such as the SYR and ZFX gene. This embryo sexing protocol was later adapted by Vinsky et al. (2006), but the approach was prone to non-specific PCR products. In order to improve the reliability of the PCR sex-typing procedure, more specific PCR primers for the SYR and ZFX genes were designed and applied in the present study. The primers used in the current study did not produce non-specific PCR amplicons and are therefore considered to be more reliable. Using the re-developed embryo sexing technique in the current study, no significant difference in sex ratio in D30E was found when compared across the C28, IS21FE and IS21SE treatments, despite the fact that sows were in different physiological states in each treatment. That is, the IS21FE sows were lactating when bred, IS21SE sows had the lactational estrus skipped and were bred on the following estrus and C28 sows were bred at the first estrus after weaning.

The effects of sex on the differences in offspring outcomes are important factors to consider during embryo development. Despite limited assessment of how sex differences influences offspring responses in most studies, molecular and phenotypic outcomes of adverse *in utero* conditions, such as a low energy diet or uterine hypoxia, often have a greater impact on the development and survival of males compared to female offspring (Vinsky et al., 2006). By categorizing the sex of surviving embryos, Vinsky et al. (2006) provided the first evidence for selective pressure against the survival of female embryos in sows subjected to restricted feed intake at critical stages of follicular development (during lactation). In contrast, Oliver et al. (2011) also reported no statistical differences in the number of female and male embryos.

From our study, maternal estrus and breeding during lactation or after weaning does not appear to have influence on the embryonic sex determination during development. On the other hand, we have to consider that we did not observe significant difference in the sows' metabolic status, leading us to the conclusion that metabolic stage of sows during lactational induced estrus could play an important role in altering litter sex ratios.

Overall, the results of the present study suggest the application of IS protocols to induce estrus and breed PP sows during lactation does not influence the embryo development nor does it have an effect on embryonic gene expression at D30 of development. Furthermore, breeding during lactational estrus or skipping estrus does not seem to impact embryonic development either. Therefore, I must reject the hypothesis that breeding PP sows at the second estrus following the initial estrus induced during lactation (skip-a-heat) improves embryo survival when combined with an IS estrus induction protocol. In addition to these findings, this study emphasizes the importance of maternal management and metabolic state in early swine embryonic development and embryo quality. Given that metabolic state appears to be the underlying factor that affects the reproductive response and performance of lactating sows the ability to manage the sow at this level is a major consideration It is well established that regardless of the breeding management system being applied, if the sow's metabolic status is compromised, then embryo quality, development and survival will be reduced. Therefore a key aspect consideration in the successful of IS protocols needs to be good management of the sows' body condition and nutritional requirements.

CHAPTER 4 - FIGURES

Figure 4.1 Intermittent suckling treatments

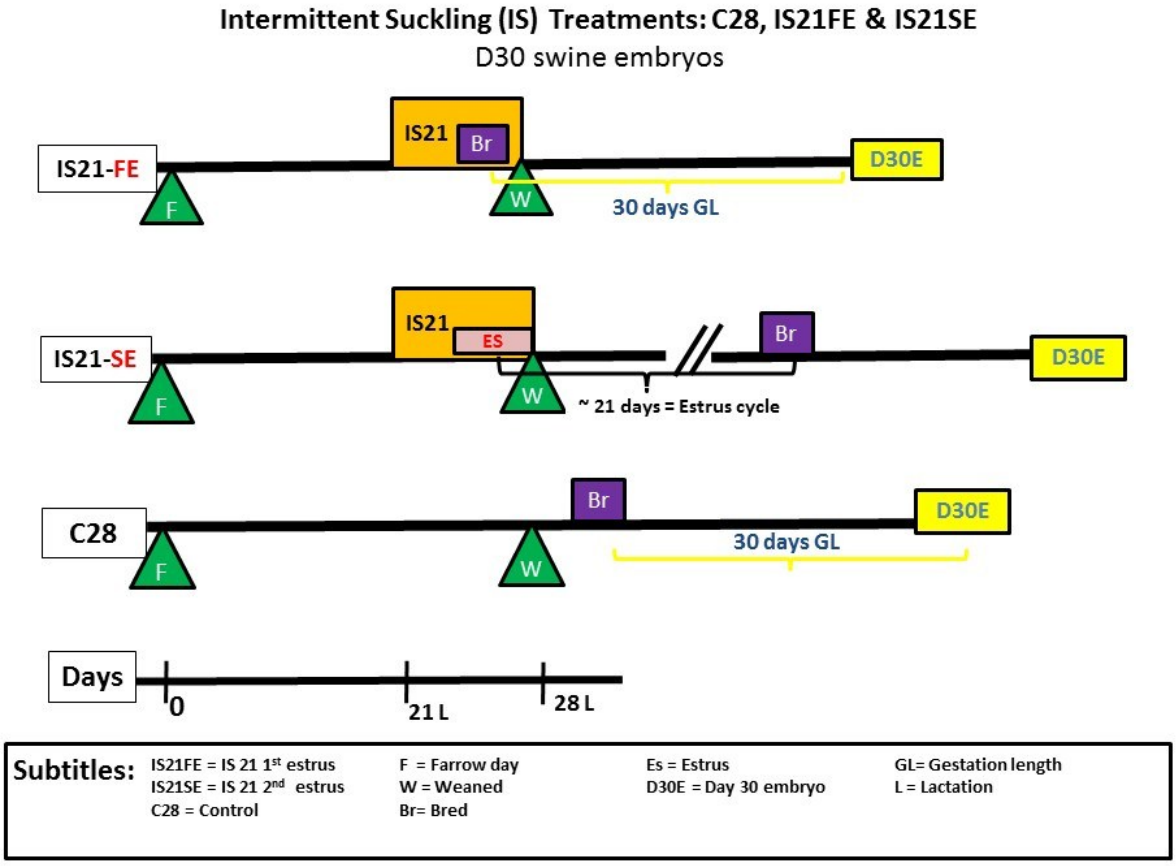
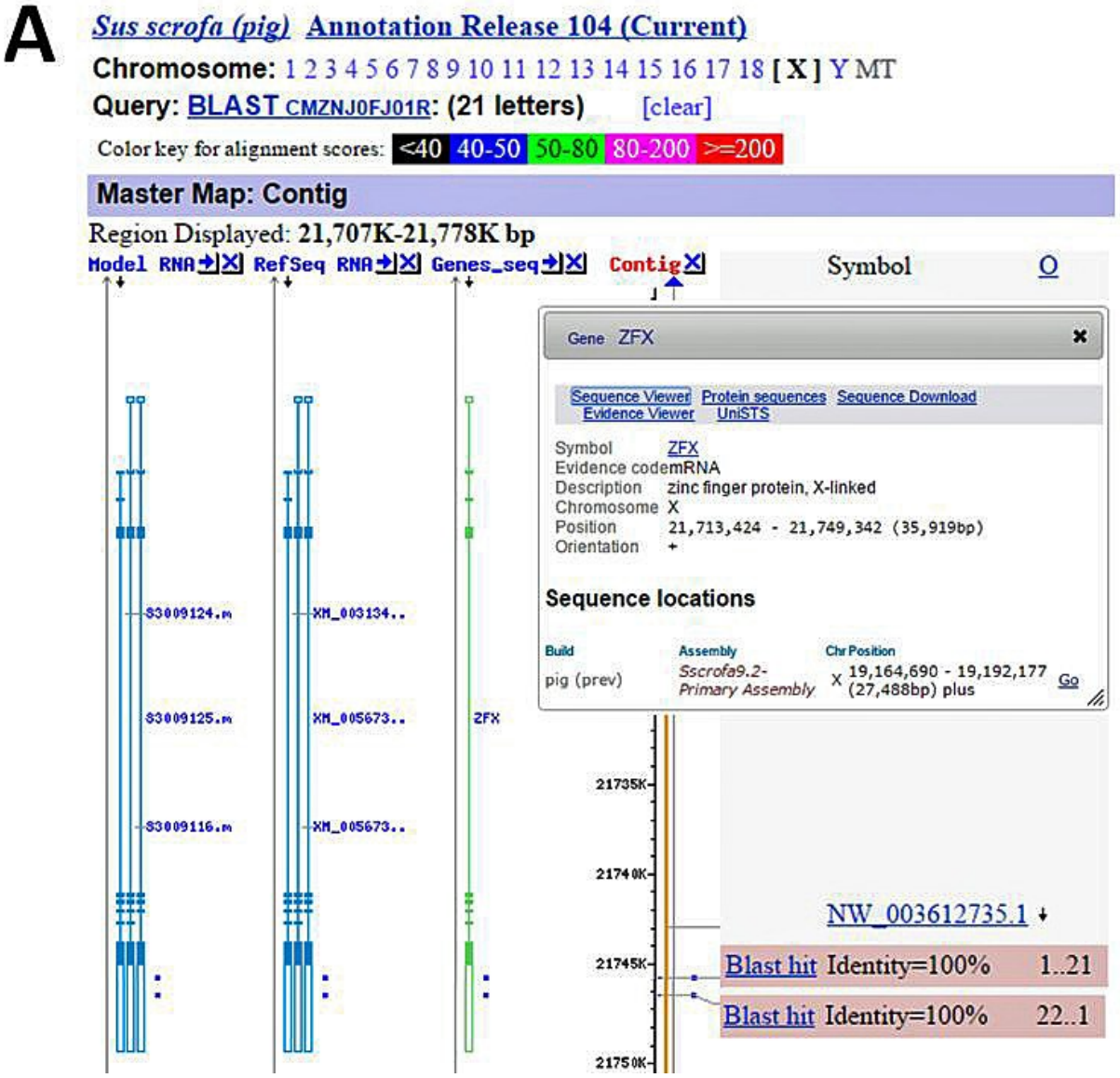


Figure 4.2 A and B. Mapping of porcine sex-specific primers on Chromosome X and Y. **A)** Location of the forward and reversed primers specified on the ZFX gene. **B)** Location of the forward and reversed primers specified on the SRY gene.



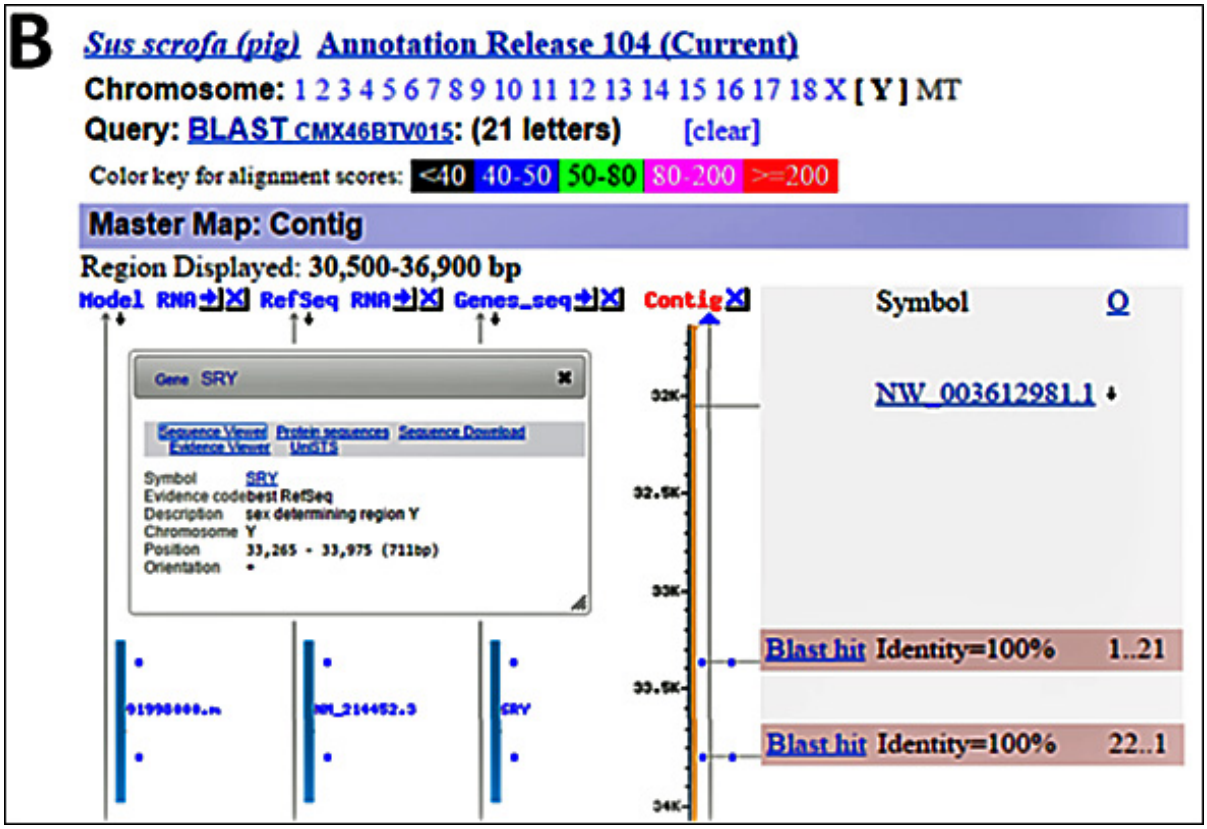

B
Sus scrofa (pig) Annotation Release 104 (Current)
Chromosome: 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 X [Y] MT
Query: BLAST CMX46BTV015: (21 letters) [clear]
Color key for alignment scores: <40 40-50 50-80 80-200 >=200
Master Map: Contig
Region Displayed: 30,500-36,900 bp
Model RNA
RefSeq RNA
Genes_seq
Contig
Gene SRY
Sequence Viewer
Protein sequences
Sequence Download
Evidence Viewer
UniSTS
Symbol SRY
Evidence code best RefSeq
Description sex determining region Y
Chromosome Y
Position 33,265 - 33,975 (711bp)
Orientation +
Symbol
Q
NW_003612981.1
32K
32.5K
33K
33.5K
34K
Blast hit Identity=100% 1..21
Blast hit Identity=100% 22..1

Figure 4.3. Primers sequences information specific to the SRY region of the Y-chromosome and the ZFX region of the X-chromosome, annealing temperature and the amplicon length from PrimerQuest® Design Tool.

Parameter Set: General PCR (Primers only)
Sequence Name: SRY
Amplicon Length: 400

		Start	Stop	Length	Tm	GC%
Forward	GGGAAAGGCTCCTCACTATTT (Sense)	91	112	21	62	47.6
Reverse	AGGGATACATCCTCTCCTCTAC (AntiSense)	469	491	22	62	50

Sequence Name: ZFX
Amplicon Length: 506

		Start	Stop	Length	Tm	GC%
Forward	GTGCTGCTTTGTCTTGGAATG (Sense)	3291	3312	21	62	47.6
Reverse	GAGGGAGTTAGGTCTGGATACT (AntiSense)	3775	3797	22	62	50

Figure 4.4. Experimental design of microarray analysis. Biological replicates of Control (C28) were compared to either IS21FE or IS21SE. Three males and three females D30E were pooled separately from each sow in each treatment group ending up with 18 distinct biological replicates.

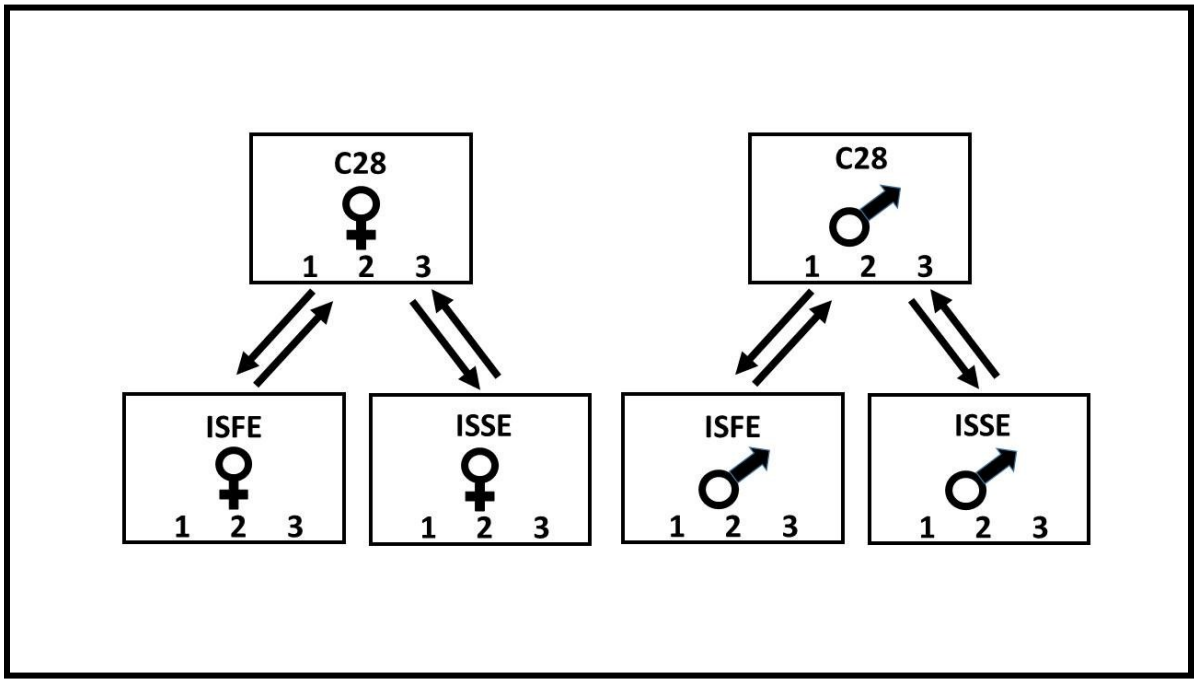


Figure 4.5. Box plot of M-values of expression before and after normalization process using simple background subtraction, LOWESS normalization within and between arrays.

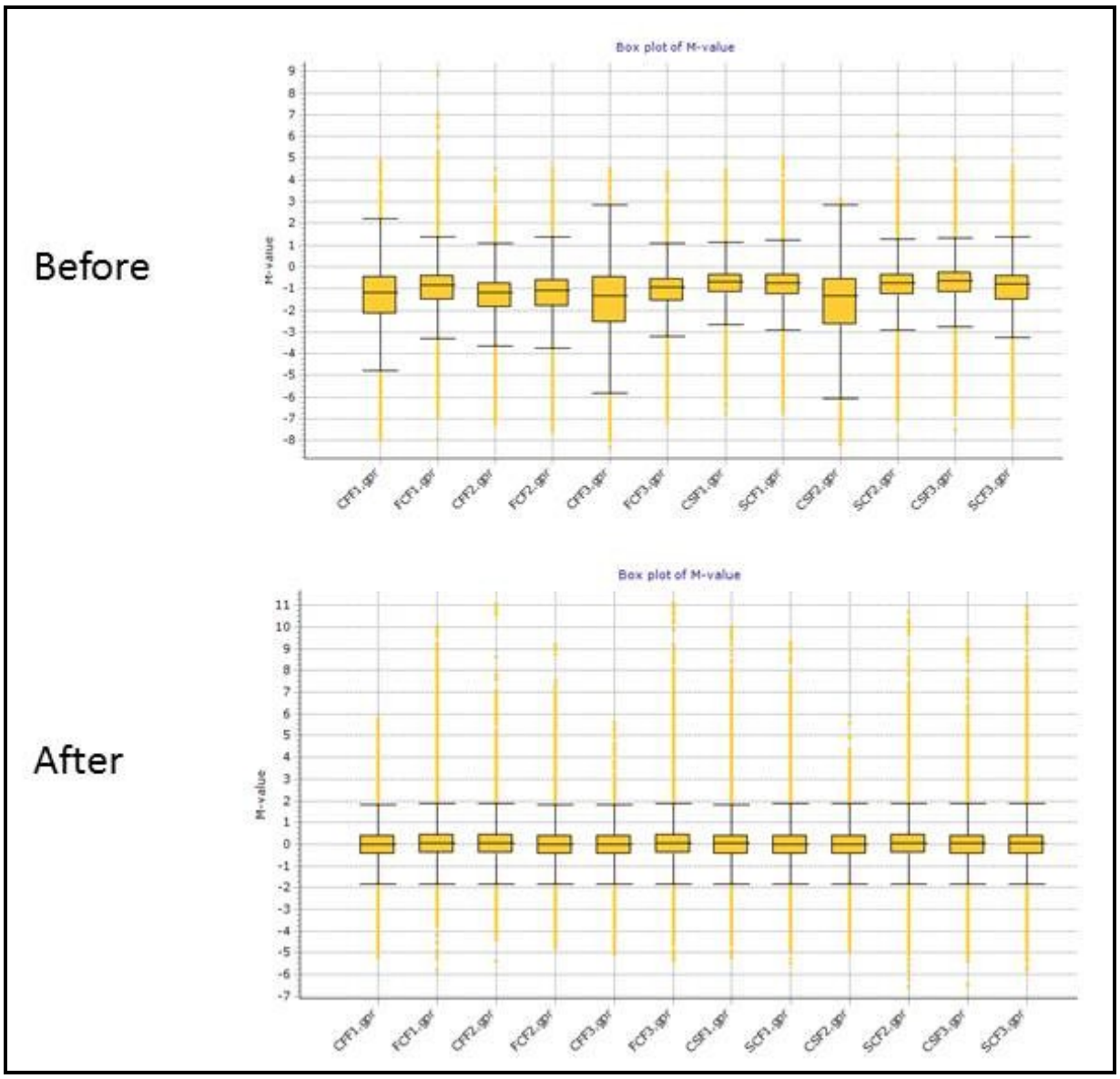


Figure 4.6. The sex of each embryo was determined by identifying embryo with one band as a female and two bands as a male.

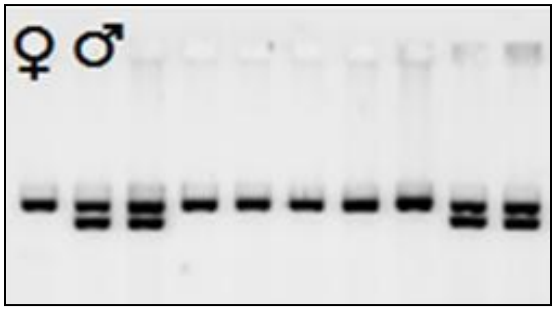


Figure 4.7. PCR amplification of porcine sex-specific genes from D30 embryos. 2% TBE agarose gel stained with SYBR® Safe DNA gel stain with the known positive controls are indicated with male and female symbols. Two bands indicated as males and a single band as females. Red star indicates the possible sample contamination in the well with the appearance of a very faint lower band (400bp) compared to upper band (506bp) PCR product caused by SRY Y-specific primers. The red arrow indicates the unknown sex identification from the sample with no PCR product.

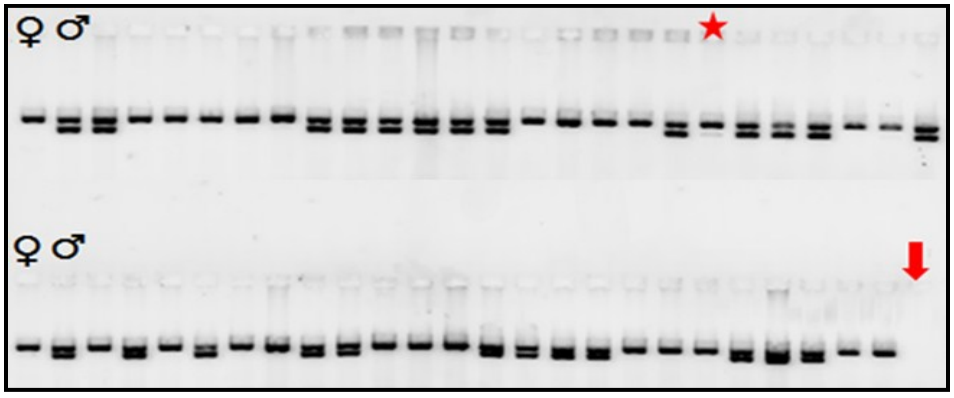


Figure 4.8. Volcano plot from Flexarray analysis between IS21FE and IS21SE treatment. The large red diamonds = significant spots, FC = Fold change threshold, Adj P-val = Adjusted p-value threshold, Black spots influenced by dye effect.

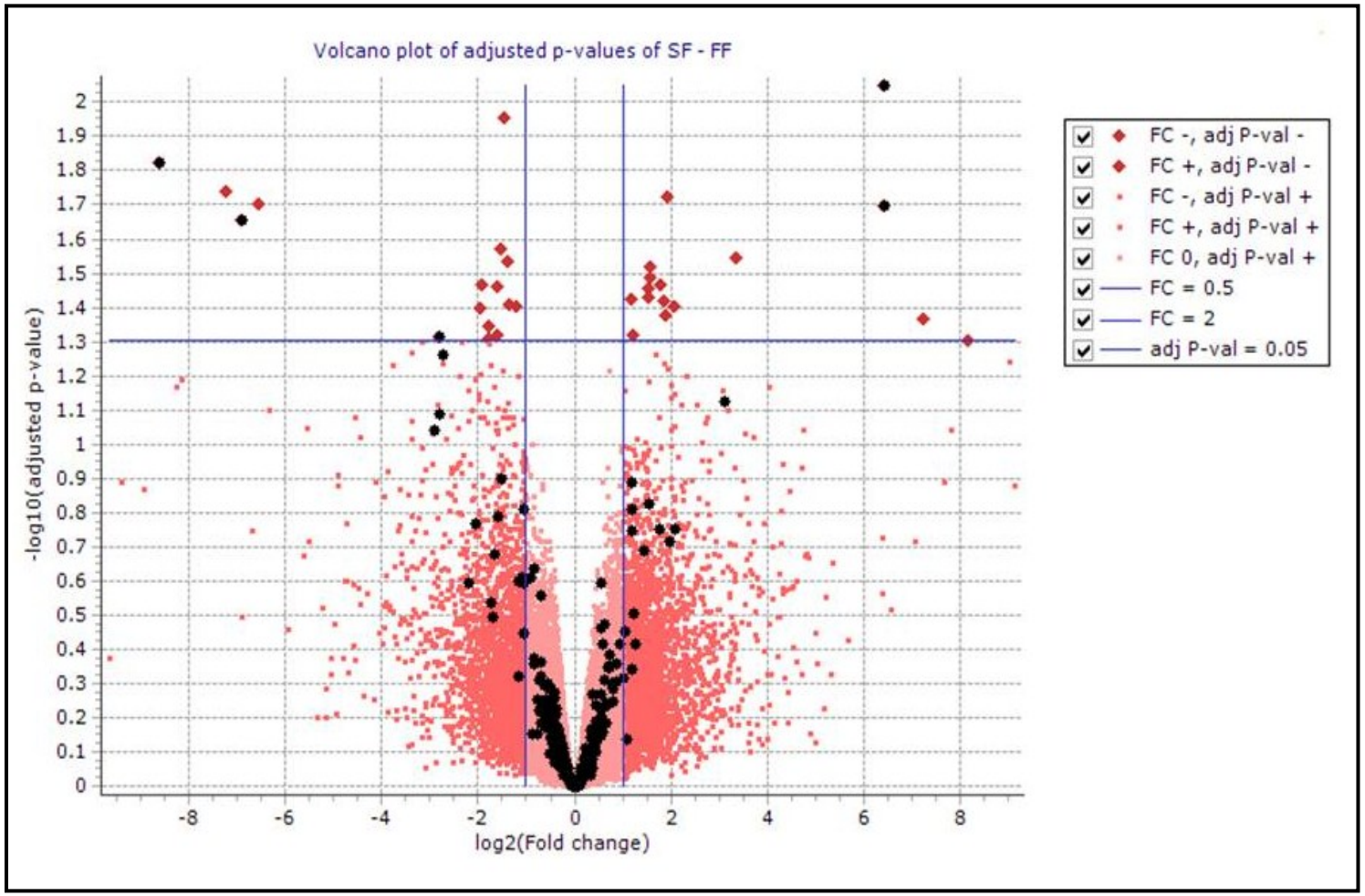
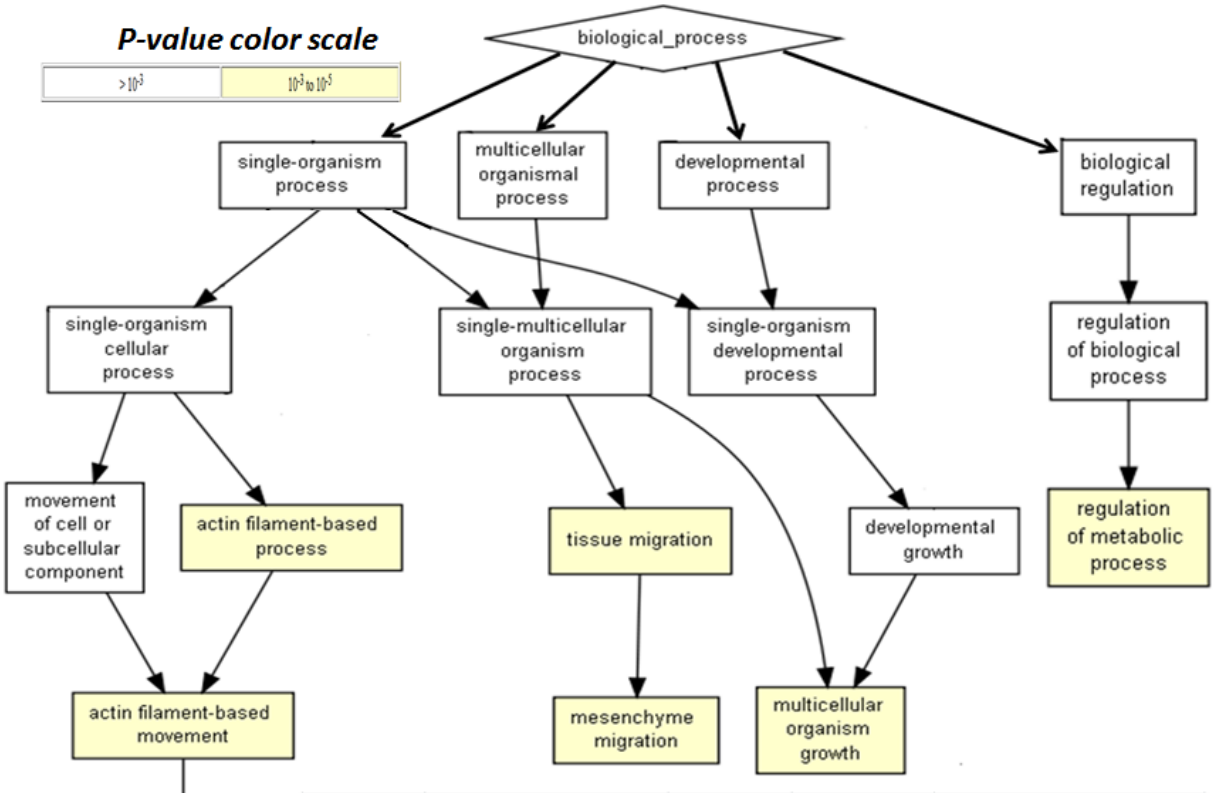


Figure 4.9. GOrilla analysis of non-DE genes from the D30E. N is the total number of genes; B is the total number of genes associated with a specific GO term; n is the flexible cutoff, i.e. the automatically determined number of genes in the 'target set' and b is the number of genes in the 'target set' that are associated with a specific GO term. Enrichment is defined as (b/n)/(B/N). 'P-value' is the enrichment p-value computed according to the mHG or HG model. This p-value is not corrected for multiple testing of 13256 GO terms. 'FDR q-value' is the correction of the above P-value for multiple testing using the Benjamini and Hochberg (1995) method.



GO Term	Description	P-value	FDR q-value	Enrichment (N, B, n, b)
GO:0030048	actin filament-based movement	3.64E-05	4.83E-01	5.41 (13814,57,403,9)
GO:0019222	regulation of metabolic process	7.42E-05	4.92E-01	1.25 (13814,5003,403,183)
GO:0090131	mesenchyme migration	9.65E-05	4.26E-01	25.71 (13814,4,403,3)
GO:0090130	tissue migration	7.90E-04	9.52E-01	14.69 (13814,7,403,3)
GO:0070252	actin-mediated cell contraction	8.57E-04	8.11E-01	5.27 (13814,7,403,6)
GO:0030029	actin filament-based process	8.64E-04	7.63E-01	2.31 (13814,7,403,18)
GO:0035264	multicellular organism growth	9.51E-04	7.41E-01	3.92 (13814,7,403,8)

CHAPTER 4 - TABLES

Table 4.1 Percentage of female, male embryos.

Treat	Female	Male	unknown sex	Total
CON (14 sows)	123(52.8%)*	110 (47%)*	1 (0.02%)*	234
IS21FE (10 sows)	91 (53.51%)*	80 (46.4%)*	2 (0.09%)*	173
IS21SE (13 sows)	111 (53%)*	97 (46.7%)*	0%*	208
Grand Total	**326**	**289**		**615**

* p value >0.05, no sex difference among sows within the same treatment group.

Table 4.2 DE genes with gene symbols and GenBank accession numbers in female and male D30E along with log2 (Fold change) and adjusted p-value. **(Appendix 1)**

https://drive.google.com/open?id=0B6WCqRHpqzrPRE9hbUhiYllXZUE

Table 4.3 Non-differentially expressed genes (DE) in D30E with gene symbols and GenBank accession numbers along with p-value and fold change below 1.5 between two treatment groups IS21FE and IS21SE. **(Appendix 2)**

https://drive.google.com/open?id=0B6WCqRHpqzrPMmV0M1gxcnhuRTA

Table 4.4 A list of associated genes within each GO term. **(Appendix 3)**
https://drive.google.com/open?id=0B6WCqRHpqzrPZ2EzcG1lTE1UaXc

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CHAPTER 5

5.1 General discussion

High reproductive efficiency is an essential component of successful swine production. To achieve this, producers must identify and incorporate breeding management programs that allow them to maximize conception and farrowing rates, as well as produce optimal litter sizes. In order for intermittent suckling (IS) to be successful as a breeding management tool, it must address several reproductive factors. Initially IS must stimulate the underlying endocrine and physiological mechanisms that initiate reproductive cyclicity during lactation. As a part of this, the lactating sow must not only show estrus behavior and ovulate, but it is also important that the developing follicles and oocytes produced are of good quality in order to generate viable embryos and large litters of healthy piglets.

In the studies presented in this thesis, we assessed the application of IS in primiparous (PP) sows with these reproductive factors and their interactions in mind. One must also consider that PP sows are unique members of the breeding herd, because during lactation they experience significant metabolic challenges, which may compromise subsequent reproduction (Foxcroft et al., 2007). As a result, PP sows were generally expected to respond differently to IS when compared to multiparous sows. In PP sows, during the last week of lactation, catabolism increases and can reduce embryonic survival and development to D30 of gestation in the following litter (Foxcroft, 1996). Considering this physiological response, one would assume that PP sows would benefit from skipping a heat and breeding at the subsequent estrus after application of an IS protocol. Given this background, the overall objectives of the studies presented here were to initially determine the ability of IS protocols to induce lactational estrus in PP sows and the relative impact of IS on their reproductive efficiency when bred during lactation. The next objective was to assess the impact of applying IS in these young sows on embryonic development and gene expression, as well as litter-sex ratio and to determine if breeding at the subsequent estrus following their first estrus during lactation (skip-a-heat) would affect these parameters.

5.1.1 Induction of lactational estrus

In our study, 61% of PP sows exposed to IS protocols showed estrus during lactation. Previous IS work showed that only 23% of the PP sows had estrus induced during lactation (Soede et al., 2012). The percentage of sows with lactational estrus was greatly affected by the parity of sow with 23% (8/26) of first parity (PP) sows showing estrus, while 85% (22/26) of second parity and 61 to 65% of older parity sows showing lactational estrus. As well, in a recently published study with PP sows exposed to an IS regime initiated at D28 of lactation, 72% (21/29) of sows showed estrus during lactation (Chen et al., 2017). In our study, the 39% of sows that that did not respond to IS mobilized more tissue that the 61% of sows that responded to IS and showed lactational estrus, indicating that the non-reponders were more metabolically challenged. Similarly, Chen and colleages (2017) determined that IS sows that did not ovulate during lactation had a greater negative energy balance that those that did ovulate. Chen and colleages (2017) also found that IS sows which did not show lactational estrus exhibited a different pattern of LH secretion than those that did, with less pulsality and reduced LH surges. They attributed these differences in endocrine responses and estrus behavior to energy balance. Therefore, maternal metabolic status and nutritional management during lactation should be considered to play important roles in the response of sows to IS protocols. This was shown by the IS non responders which were more metabolic challenged at the time of IS, which affected their ability to return to estrus

Although some recent IS studies have shown similar responses by sows to IS, there has been dramatic variation in the response to IS reported over the past decades (Langendijk et al., 2007; Gerritsen et al., 2008b; 2009). The variation in response observed may be related to genetic selection strategies that have been applied to reduce weaning-to-estrus intervals and produce the lines of robust modern breeding sows that exist today. Weaning-to-estrus interval (WEI) is one of the main contributors to non-productive days (Poleze et al., 2006) and studies have shown that WEI can be influenced by factors such as breed and parity (Vesseur et al., 1994). As a result, modern sows have been selected for a reduced WEI which may explain why they have responded differently to IS than sows have in past studies (Gerritsen et al., 2008b). With this in mind, it should be noted that commercial swine breeding organizations will have different goals may not have selected for breeding sows in the same way. This difference in

selection goals could result in genetic lines of breeding sows that respond differently to factors such as metabolic status and nursing stimuli. That is, some lines of sows may be more prone to exhibiting lactational estrus than others. The role of sow genotype in the response to these factors is an area that may require further investigation. If genetic and physiological differences contribute to the variation in sows' responses IS, it may be possible to select for a genetic line of sows that respond well to IS and are more likely to express lactational estrus.

In relation to IS regimes, previous research done by Kluivers-Poodt et al. (2010) has shown that sows submitted to a 12 h separation IS regime exhibit cortisol secretion patterns similar to sows that were abruptly weaned. In this context, the IS 12h regimes did not have a negative effect on the sow welfare neither on the peri–ovulatory processes (Kluivers-Poodt et al., 2010). In our trial, cortisol levels were not measured but 61% of the PP sows submitted to the IS protocol showed lactational estrus. As well, sows bred during lactation (IS21FE group) had pregnancy rates numerically lower than Control sows or sows breed at the subsequent estrus following lactational estrus (IS21SE). This trend towards a difference in pregnancy rate could be related to stress may indicate a physiologically relevant response, as explained by Newton et al. (1987). In his studies, Newton and colleagues described that PP sows, in response to litter separation and boar exposure, showed high concentration of cortisol and elevated progesterone which prevented PP sows from cycling during lactation. Likewise, Newton et al. (1987) described that a lack of ovarian follicular development and estradiol secretion may prevent expression of estrus in PP sows during lactation, despite elevated concentrations of FSH and LH in serum. Given this role of stress in the reproductive process and animal welfare, it is a factor that needs to be considered and further studied in relation to protocols to induce lactational estrus like IS.

5.1.2 Sow reproductive performance and embryonic quality

As discussed in this thesis, if IS can successfully induce lactational estrus, not only is it important for the sow to exhibit estrus behavior and to ovulate, but it is also important that the follicles that develop and oocytes produced are of good quality in order to generate viable embryos and large healthy litters. Therefore, it is also important to consider the effects of IS and lactational estrus induction on the downstream indicators of sow reproductive performance (e.g. pregnancy rate, embryo survival, litter size).

In the present study, the reproductive results obtained were not entirely anticipated. Based on previous research, it was assumed that breeding PP sows at the subsequent estrus following their first estrus during lactation (skip-a-heat; IS21SE) would improve reproductive outcomes (e.g. increased pregnancy rate and embryo survival) when combined with an IS estrus induction protocol. It was assumed that embryonic development in sows bred during lactation (IS21FE) would be negatively impacted compared to the IS21SE sows. Unexpectedly, it was found that embryo survival among IS21FE and IS21SE sows did not differ, nor was there a difference in the number of viable embryos. This result was also reflected in pregnancy rates which were not different for the IS21FE, IS21SE or Control sows. Our results are supported by Gerritsen et al. (2008a) and Chen et al. (2017), who found that sows bred during lactation had similar pregnancy rates and litter sizes to those bred after weaning. However, neither of these studies included skip-a-heat treatment and a review of the literature reveals no other studies comparing conventional IS protocols with IS combined with skip-a-heat breeding.

Despite the fact that the number of surviving embryo and pregnancy rates were not impacted by our treatments, placental weight was reduced in both the IS21FE and IS21SE sows compared to Control sows. As well, embryonic weights of litters from ISFE21 sows was lower than Control sows. This observation raised the question whether there may be a negative impact of IS protocols on embryonic development and quality which was evaluated through the gene expression and sex ratio studies presented in Chapter 4. However, our assessment of gene expression for D30 embryos from across the treatment groups (ISFE21, ISSE21, Control) revealed no differentially expressed genes for either sex of embryo. Additionally, there was no impact on litter-sex ratio for any treatment groups. Although past research has shown that a severally negative energy balance in weaned sows at the time of breeding can affect embryonic gene expression, as well as influence the sex ratio of surviving embryos (Vinsky et al., 2006), it should be noted the sows in that study were in poor metabolic state and had become very catabolic. In our study, the lack of impact on gene expression and sex-ratio may be explained by the similarity of the sows' metabolic states when the treatments were assigned as well as during lactation and pregnancy. Nevertheless, it has been established previously that embryonic survival and development can be influenced by a number of factors including genetic makeup, maternal nutrition, uterine capacity and environmental temperature (Foxcroft

et al., 2006; 2007; Vinsky et al., 2007; Wright et al., 2016). All of these factors must be considered and managed to optimize reproductive performance.

Overall, the limited effect of the treatments on sow reproductive performance and embryo quality in our research can likely be attributed to the good condition of the sows in the study. As the data shows, sow weights did not differ at farrowing, D20 of lactation and at weaning regardless of treatment. Generally, this would suggest that sow weight and body condition may be an indicator of how the sow responds to IS and that with proper management, sow fertility can be maintained with the use of IS and lactational estrus induction.

5.2 Future research perspectives

At the Tenth International Conference on Pig Reproduction (June 11-14-2017, University of Missouri, Columbia, Missouri) there were several on-going studies presented on this topic, including a paper by Van Wettere and colleagues from Australia entitled "Controlling lactation oestrus: the final frontier for breeding herd management" (van Wettere et al., 2017). All of these studies looked at optimizing protocols to induce lactational estrus for contemporary pig breeding management systems. In fact, at the Tenth International lactational estrus so that they can be applied in commercial pig production. As described earlier, two areas we identified from our research that need to be studied further include the effects of metabolic status and genotype on IS protocols. A better understanding of the impact of metabolic status on the response to IS will allow producers to determine how best to manage lactating sows to improve response to IS and increase the potential for effective induction of lactational estrus. As well, if genetic lines of sows can be identified and/or selected that are more prone to exhibiting lactation estrus these lines of sows could be incorporated into production systems using IS protocols.

The development of cystic ovaries is another issue to be considering when an IS regime is being implemented. Gerritsen et al. (2008a) described the presence of cystic ovaries in sows that did not respond to IS. In their study, an LH surge was not present or was very minor in sows presenting with cystic ovaries. The authors indicated that IS with a sow-piglet separation of 6 h might not be enough to stimulate an LH surge and suggested that with such a combination there is a higher risk of disrupting the ovulation process and producing cystic

follicles. These findings are supported by a recent report that patterns of LH secretion differed between IS sows which did not show lactational estrus those that did (Chen et al, 2017). In the present study, we did not assess for the presence of ovarian cysts by ultrasound, but their presence could have interfered with our treatment effects and interpretation of responses to IS.

As discussed, another area that warrants further research is the role of stress and stress responses during IS periods. The process of weaning piglets, as well as the daily separation of sows and piglets associated with IS are both stressfull. The effect of stress on reproduction is influenced by the duration of the stress responses (Einarsson et al. (2008). In pigs the effect of brief or acute stress is sometimes stimulatory (e.g.induction of estrus after transportation in gilts, Kraeling et al. 2015), other times, prolonged or chronic stress frequently results in inhibition of reproduction which is most cases detrimental for sow reproduction (Einarsson et al. (2008).

Reproductive processes such as ovulation, expression of estrus behavior and implantation of the embryo are the most sensitive reproductive pathways influenced by stress, since they are directly controlled by the neuroendocrine system. In relation to IS regimes, previous research done by Kluivers-Poodt et al.(2010) has shown that sows submitted to a 12h separation IS regime exhibit cortisol secretion patterns similar with sows that were abruptly weaned. Therefore, IS 12h regimes did not seem to have a negative effect on the sow welfare neither on the peri – ovulatory processes (Kluivers-Poodt et al., 2010).

Beyond the work being done to understand the underlying physiology associated with protocols used for the induction of estrus during lactation, there is a need for further research related to the implementation of protocols such as IS in commercial environments. For pig producers, the ability to induce lactational estrus and mate sows during lactation provides more flexibility to the production system, as sow matings are uncoupled from weaning. The IS protocols form the basis for new breeding management systems that extend lactation periods, improve piglet welfare and potentially reduce the confinement of sows during the sow-piglet separation periods. However, for commercial production it is unclear how large scale implementation of IS protocols would occur. How would producers manage pig flow and barn space allocations to accommodate the sow-piglet separation periods need for IS? As well, how would producers organize their production streams to manage sows that do not respond to the

induction of lactational estrus and need to be bred following weaning? Would 100 percent of sows need to be responsive to induction of lactational estrus too make its commercial implementation feasible? In order to address these questions and others, additional research is needed to refine estrus induction protocols such as IS and to establish a strategy that works in a wide range of commercial production systems.

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Appendices

Appendix 1

Male SE xFE

	limma (simple)	limma (simple)	FDR											
	SE - FE	SE - FE	SE - FE											
Probe ID	**log2(Fold change)**	**Symmetrical raw fold change**	**Adjusted p-value**	**Chromosome location**	**Gene symbol**	**Accession ID**	**Description**							
EMPV1_03017	2.606863	6.091776	0.03015836	7	GTPBP2	XM_003128412.4	PREDICTED: Sus scrofa GTP binding protein 2 (GTPBP2), transcript variant X1, mRNA							
EMPV1_06451	-1.5621	-2.95295	0.00440503	2	IFITM2	NM_001246214.1	Sus scrofa interferon induced transmembrane protein 2 (IFITM2), mRNA							
EMPV1_24886	1.051954	2.073336	0.01179447	4	SCAMP3	NM_001185169.1	Sus scrofa secretory carrier membrane protein 3 (SCAMP3), mRNA							
EMPV1_05880	-2.76386	-6.7921	0.03379318	unknown	unknown	unknown	unknown							

Appendix 2

Table 4.3 Non-differentially expressed genes (DE) in D30E with gene symbols and GenBank accession numbers along with p-value and fold change below 1.5 between two treatment groups IS21FE and IS21SE.

Accession	Gene_Symbol (pig)	Description	Gene Symbol (combined with human)
XM_003123164	LOC100513975	PREDICTED: Sus scrofa e3 ubiquitin-protein ligase MARCH2-like (LOC100513975), mRNA	MARCH2
ENSSSCG00000013601	2-Mar	ENSSSCG00000013601	MARCH2
100154081		ENSSSCG00000005424	PTCD3
17:61528009-61528272	NA	Novel Transcribed Region; evidence: embryonic ESTs	7SK
ENSSSCG00000020303	7SK	ENSSSCG00000020303	7SK
ENSSSCG00000009523	A2LD1	ENSSSCG00000009523	A2LD1
XM_003121743	LOC100517743	PREDICTED: Sus scrofa alpha- and gamma-adaptin-binding protein p34-like (LOC100517743), mRNA	AAGAB
XM_003131809	ABR	PREDICTED: Sus scrofa active BCR-related gene, transcript variant 1 (ABR), mRNA	ABR
ENSSSCG00000014873	ACER3	ENSSSCG00000014873	ACER3
ENSSSCG00000011477	ACOX2	ENSSSCG00000011477	ACOX2
ENSSSCG00000010190	ACTA1	ENSSSCG00000010190	ACTA1
NM_001170517	ACTC1	Sus scrofa actin, alpha, cardiac muscle 1 (ACTC1), mRNA	ACTC1
XM_003125028	LOC100520667	PREDICTED: Sus scrofa actin, gamma-enteric smooth muscle-like (LOC100520667), mRNA	ACTG2
XM_003122477	LOC100516662	PREDICTED: Sus scrofa alpha-actinin-3-like, transcript variant 1 (LOC100516662), mRNA	ACTN3
XM_003127120	LOC100517284	PREDICTED: Sus scrofa alpha-actinin-4-like, transcript variant 1 (LOC100517284), mRNA	ACTN4
ENSSSCG00000009645	ADAMDEC1	ENSSSCG00000009645	ADAMDEC1
100521434		Novel Transcribed Region; evidence: embryonic ESTs	ADAMTS2
NM_001206384	ADM2	Sus scrofa adrenomedullin 2 (ADM2), mRNA	ADM2
XM_003132926	LOC100520333	PREDICTED: Sus scrofa alpha-ketoglutarate-dependent dioxygenase alkB homolog 2-like (LOC100520333), mRNA	ALKBH2
ENSSSCG00000008295	ALMS1	ENSSSCG00000008295	ALMS1
ENSSSCG00000004392	AMD1	ENSSSCG00000004392	AMD1
NM_214195	AMY2	Sus scrofa amylase, alpha 2B (pancreatic) (AMY2), mRNA	AMY2
XM_003127927	ANKRD13C	PREDICTED: Sus scrofa ankyrin repeat domain 13C (ANKRD13C), mRNA	ANKRD13C

XM_003127009	ANKRD27	PREDICTED: Sus scrofa ankyrin repeat domain 27 (VPS9 domain) (ANKRD27), mRNA	ANKRD27
XM_003123791	ANKRD32	PREDICTED: Sus scrofa ankyrin repeat domain 32 (ANKRD32), partial mRNA	ANKRD32
XM_003129790.1	LOC100516989	PREDICTED: Sus scrofa ankyrin repeat domain-containing protein 49-like (LOC100516989), mRNA	ANKRD49
XM_003124633	ANKS3	PREDICTED: Sus scrofa ankyrin repeat and sterile alpha motif domain containing 3 (ANKS3), mRNA	ANKS3
ENSSSCG00000009978	AP1B1	ENSSSCG00000009978	AP1B1
ENSSSCG00000012836	AP2A2	ENSSSCG00000012836	AP2A2
ENSSSCG00000014207	APC	ENSSSCG00000014207	APC
ENSSSCG00000006355	APOA2	ENSSSCG00000006355	APOA2
NM_001048072	ARF4	Sus scrofa ADP-ribosylation factor 4 (ARF4), mRNA	ARF4
ENSSSCG00000009536	ARGLU1	ENSSSCG00000009536	ARGLU1
XM_001927201	ARGLU1	PREDICTED: Sus scrofa arginine and glutamate rich 1 (ARGLU1), mRNA	ARGLU1
100520047		Novel Transcribed Region; evidence: embryonic ESTs	ARHGAP12
XM_003358013	LOC100628007	PREDICTED: Sus scrofa rho GTPase-activating protein 27-like (LOC100628007), partial mRNA	ARHGAP27
100523324		Novel Transcribed Region; evidence: embryonic ESTs	ARHGEF12
XM_003359196	ARID4B	PREDICTED: Sus scrofa AT rich interactive domain 4B (RBP1-like) (ARID4B), partial mRNA	ARID4B
ENSSSCG00000006220	ARMC1	ENSSSCG00000006220	ARMC1
ENSSSCG00000012508	ARMCX6	ENSSSCG00000012508	ARMCX6
ENSSSCG00000005958	ASAP1	ENSSSCG00000005958	ASAP1
5:97776303-97793667		Novel Transcribed Region; evidence: embryonic ESTs	ATP2B1
100516284		Novel Transcribed Region; evidence: embryonic ESTs	BARX2
ENSSSCG00000015882	BAZ2B	ENSSSCG00000015882	BAZ2B
ENSSSCG00000000769	BID	ENSSSCG00000000769	BID
8:147991654-148031534		Novel Transcribed Region; evidence: embryonic ESTs	BMP2K
ENSSSCG00000005749	BRD3	ENSSSCG00000005749	BRD3
XM_001924488	BZW1	PREDICTED: Sus scrofa basic leucine zipper and W2 domains 1, transcript variant 1 (BZW1), mRNA	BZW1
ENSSSCG00000014574	C11orf17	ENSSSCG00000014574	C11orf17
XM_003129766	LOC100512671	PREDICTED: Sus scrofa ester hydrolase C11orf54 homolog (LOC100512671), mRNA	C11orf54
ENSSSCG00000001919	C15orf60	ENSSSCG00000001919	C15orf60
XM_003123615	LOC100513912	PREDICTED: Sus scrofa multiple myeloma tumor-associated protein 2 homolog, transcript variant 2 (LOC100513912), mRNA	C1orf35

NM_001142829	C1QTNF2	Sus scrofa C1q and tumor necrosis factor related protein 2 (C1QTNF2), mRNA	C1QTNF2
ENSSSCG00000007492	C20orf108	ENSSSCG00000007492	C20orf108
100524719		Novel Transcribed Region; evidence: embryonic ESTs	C20ORF29
ENSSSCG00000007496	C20orf43	ENSSSCG00000007496	C20orf43
XM_003133928	LOC100525791	PREDICTED: Sus scrofa uncharacterized protein C5orf34 homolog (LOC100525791), mRNA	C5orf34
XM_003133993	LOC100525133	PREDICTED: Sus scrofa UPF0542 protein C5orf43-like (LOC100525133), mRNA	C5orf43
XM_003360631	LOC100622328	PREDICTED: Sus scrofa glutaredoxin-like protein YDR286C homolog, transcript variant 1 (LOC100622328), mRNA	C5orf63
XM_001927358	LOC100157036	PREDICTED: Sus scrofa UPF0364 protein C6orf211 homolog (LOC100157036), mRNA	C6orf211
ENSSSCG00000006080	C8orf47	ENSSSCG00000006080	C8orf47
XM_003353600	LOC100625952	PREDICTED: Sus scrofa transmembrane protein C9orf5-like (LOC100625952), mRNA	C9orf5
100513906		Novel Transcribed Region; evidence: embryonic ESTs	C9ORF80
ENSSSCG00000003711	CABYR	ENSSSCG00000003711	CABYR
ENSSSCG00000017543	CALCOCO2	ENSSSCG00000017543	CALCOCO2
396,838,100,154,056,000,000,000		Novel Transcribed Region; evidence: embryonic ESTs	CALM1
XM_001927085	LOC100153608	PREDICTED: Sus scrofa carbonyl reductase family member 4-like (LOC100153608), mRNA	CBR4
XM_003133050	LOC100517429	PREDICTED: Sus scrofa cell division cycle and apoptosis regulator protein 1-like (LOC100517429), mRNA	CCAR1
XM_003359040	LOC100627354	PREDICTED: Sus scrofa coiled-coil domain-containing protein 25-like (LOC100627354), mRNA	CCDC25
ENSSSCG00000017988	CCDC42	ENSSSCG00000017988	CCDC42
100516494		Novel Transcribed Region; evidence: embryonic ESTs	CCDC61
XM_003125116	LOC100525284	PREDICTED: Sus scrofa hypothetical protein LOC100525284 (LOC100525284), mRNA	CCDC72
100518207		Novel Transcribed Region; evidence: embryonic ESTs	CCDC90B
ENSSSCG00000017024	CCNG1	ENSSSCG00000017024	CCNG1
XM_003129097	CCNI	PREDICTED: Sus scrofa cyclin I, transcript variant 1 (CCNI), mRNA	CCNI
XM_003130801	LOC100525480	PREDICTED: Sus scrofa cyclin-Y-like (LOC100525480), mRNA	CCNY
100157776		Novel Transcribed Region; evidence: embryonic ESTs	CCT2
ENSSSCG00000008303	CCT7	ENSSSCG00000008303	CCT7

XM_003124281	LOC100513979	PREDICTED: Sus scrofa vacuolar fusion protein CCZ1 homolog (LOC100513979), mRNA	CCZ1
18:59601836-59696078		Novel Transcribed Region; evidence: embryonic ESTs	CDK13
XM_003135045	LOC100520819	PREDICTED: Sus scrofa cyclin-dependent kinase 16-like, transcript variant 3 (LOC100520819), mRNA	CDK16
100523273		Novel Transcribed Region; evidence: embryonic ESTs	CDK3
100736772		Novel Transcribed Region; evidence: embryonic ESTs	CENPH
ENSSSCG00000015496	CENPL	ENSSSCG00000015496	CENPL
XM_003132765	CHAF1B	PREDICTED: Sus scrofa chromatin assembly factor 1, subunit B (p60) (CHAF1B), mRNA	CHAF1B
100154153		Novel Transcribed Region; evidence: embryonic ESTs	CHD1L
100516767		Novel Transcribed Region; evidence: embryonic ESTs	CHMP4B
ENSSSCG00000014934	CHORDC1	ENSSSCG00000014934	CHORDC1
ENSSSCG00000001858	CIB1	ENSSSCG00000001858	CIB1
100157558		Novel Transcribed Region; evidence: embryonic ESTs	CIZ1
100157558		Novel Transcribed Region; evidence: embryonic ESTs	CIZ1
ENSSSCG00000005640	CIZ1	ENSSSCG00000005640	CIZ1
100153752		Novel Transcribed Region; evidence: embryonic ESTs	CLDN10
ENSSSCG00000000650	CLEC1B	ENSSSCG00000000650	CLEC1B
ENSSSCG00000016095	CLK1	ENSSSCG00000016095	CLK1
100624678		Novel Transcribed Region; evidence: embryonic ESTs	CLN8
ENSSSCG00000003628	CLSPN	ENSSSCG00000003628	CLSPN
ENSSSCG00000008202	CNNM4	ENSSSCG00000008202	CNNM4
ENSSSCG00000016553	COPG2	ENSSSCG00000016553	COPG2
ENSSSCG00000018051	COPS3	ENSSSCG00000018051	COPS3
ENSSSCG00000003339	CPSF3L	ENSSSCG00000003339	CPSF3L
ENSSSCG00000005671	CRAT	ENSSSCG00000005671	CRAT
ENSSSCG00000004609	CRIP1	ENSSSCG00000004609	CRIP1
NM_213842	CSF3	Sus scrofa colony stimulating factor 3 (granulocyte) (CSF3), mRNA	CSF3
100511866		Novel Transcribed Region; evidence: embryonic ESTs	CSNK1G3
NM_001044602	CST3	Sus scrofa cystatin C (CST3), mRNA	CST3
XR_130384.1	LOC100621302	PREDICTED: Sus scrofa hypothetical LOC100621302 (LOC100621302), miscRNA	CSTF3
100523778		Novel Transcribed Region; evidence: embryonic ESTs	CTNNA1
XM_003122495	CTSF	PREDICTED: Sus scrofa cathepsin F (CTSF), mRNA	CTSF
ENSSSCG00000012429	CXorf26	ENSSSCG00000012429	CXorf26

ENSSSCG00000015946	CYBRD1	ENSSSCG00000015946	CYBRD1
ENSSSCG00000005913	CYC1	ENSSSCG00000005913	CYC1
ENSSSCG00000007486	CYP24A1	ENSSSCG00000007486	CYP24A1
100519746		Novel Transcribed Region; evidence: embryonic ESTs	DAB2
ENSSSCG00000013243	DDB2	ENSSSCG00000013243	DDB2
NM_213854	DDC	Sus scrofa dopa decarboxylase (aromatic L-amino acid decarboxylase) (DDC), mRNA	DDC
100153560		Novel Transcribed Region; evidence: embryonic ESTs	DDX18
14:78054376-78078401		Novel Transcribed Region; evidence: embryonic ESTs	DDX21
100521835		Novel Transcribed Region; evidence: embryonic ESTs	DDX26B
ENSSSCG00000010246	DDX50	ENSSSCG00000010246	DDX50
ENSSSCG00000009876	DDX54	ENSSSCG00000009876	DDX54
XM_003132903	LOC100523515	PREDICTED: Sus scrofa ATP-dependent RNA helicase DDX54-like (LOC100523515), mRNA	DDX54
ENSSSCG00000016932	DEPDC1B	ENSSSCG00000016932	DEPDC1B
NM_214051	DGAT	Sus scrofa diacylglycerol acyltransferase (DGAT), mRNA	DGAT
ENSSSCG00000014863	DGAT2	ENSSSCG00000014863	DGAT2
NM_001160080	DGAT2	Sus scrofa diacylglycerol O-acyltransferase 2 (DGAT2), mRNA	DGAT2
XR_130423	LOC100525912	PREDICTED: Sus scrofa dihydrofolate reductase-like (LOC100525912), miscRNA	DHFR
ENSSSCG00000009220	DMP1	ENSSSCG00000009220	DMP1
100037301		Novel Transcribed Region; evidence: embryonic ESTs	DNMT3A2
ENSSSCG00000008298	DUSP11	ENSSSCG00000008298	DUSP11
7:16765928-16779079		Novel Transcribed Region; evidence: embryonic ESTs	E2F3
XM_003130959	LOC100516290	PREDICTED: Sus scrofa emopamil-binding protein-like, transcript variant 1 (LOC100516290), mRNA	EBPL
100522859		ENSSSCG00000014351	ECSCR
XM_003354006.1	LOC100624769	PREDICTED: Sus scrofa elongation factor 2-like (LOC100624769), mRNA	EEF2
ENSSSCG00000006981	EFHA2	ENSSSCG00000006981	EFHA2
ENSSSCG00000006532	EFNA4	ENSSSCG00000006532	EFNA4
100515608		Novel Transcribed Region; evidence: embryonic ESTs	EIF3L
6:63739623-63748851		Novel Transcribed Region; evidence: embryonic ESTs	ENO1
XM_003123481	EPS15L1	PREDICTED: Sus scrofa epidermal growth factor receptor pathway substrate 15-like 1 (EPS15L1), mRNA	EPS15L1
XM_003359282	ERCC6	PREDICTED: Sus scrofa excision repair cross-complementing rodent repair deficiency, complementation group 6 (ERCC6), mRNA	ERCC6

ENSSSCG00000008877	ETFDH	ENSSSCG00000008877	ETFDH
ENSSSCG00000016696	EVX1	ENSSSCG00000016696	EVX1
XM_003128453	LOC100524876	PREDICTED: Sus scrofa protein FAM103A1-like, transcript variant 2 (LOC100524876), mRNA	FAM103A1
ENSSSCG00000014159	FAM172A	ENSSSCG00000014159	FAM172A
ENSSSCG00000011045	FAM188A	ENSSSCG00000011045	FAM188A
ENSSSCG00000016865	FBXO4	ENSSSCG00000016865	FBXO4
ENSSSCG00000009702	FBXO8	ENSSSCG00000009702	FBXO8
ENSSSCG00000013885	FCHO1	ENSSSCG00000013885	FCHO1
XM_003132576	FETUIN	PREDICTED: Sus scrofa FETUIN protein (FETUIN), mRNA	FETUIN
NM_001099924	FGFR2	Sus scrofa fibroblast growth factor receptor 2 (FGFR2), mRNA	FGFR2
XM_003353685	FNBP1	PREDICTED: Sus scrofa formin binding protein 1 (FNBP1), partial mRNA	FNBP1
ENSSSCG00000005902	FOXH1	ENSSSCG00000005902	FOXH1
100739684		ENSSSCG00000007576	FOXK1
ENSSSCG00000005051	GCH1	ENSSSCG00000005051	GCH1
ENSSSCG00000007373	GDAP1L1	ENSSSCG00000007373	GDAP1L1
100512472		Novel Transcribed Region; evidence: embryonic ESTs	GINS3
ENSSSCG00000016056	GLS	ENSSSCG00000016056	GLS
NM_214312.2	GNAS	Sus scrofa GNAS complex locus (GNAS), transcript variant 1, mRNA	GNAS
100624347		Novel Transcribed Region; evidence: embryonic ESTs	GPD1L
ENSSSCG00000003371	GPR153	ENSSSCG00000003371	GPR153
ENSSSCG00000015631	GRB10	ENSSSCG00000015631	GRB10
XM_003125383	GREB1	PREDICTED: Sus scrofa growth regulation by estrogen in breast cancer 1 (GREB1), mRNA	GREB1
ENSSSCG00000006821	GSTM3	ENSSSCG00000006821	GSTM3
ENSSSCG00000006821	GSTM3	ENSSSCG00000006821	GSTM3
ENSSSCG00000006926	GTF2B	ENSSSCG00000006926	GTF2B
100622317		Novel Transcribed Region; evidence: embryonic ESTs	HAT1
ENSSSCG00000008685	HAUS3	ENSSSCG00000008685	HAUS3
XM_003135470	LOC100518818	PREDICTED: Sus scrofa HAUS augmin-like complex subunit 7-like (LOC100518818), mRNA	HAUS7
XM_003134109	LOC100522045	PREDICTED: Sus scrofa hepatitis A virus cellular receptor 2-like (LOC100522045), mRNA	HAVCR2
100512023		Novel Transcribed Region; evidence: embryonic ESTs	HECA
ENSSSCG00000005371	HEMGN	ENSSSCG00000005371	HEMGN
XM_003356575	LOC100623449	PREDICTED: Sus scrofa histone H2A type 1-F-like (LOC100623449), mRNA	HIST1H2AH

XM_001928587	LOC100156557	PREDICTED: Sus scrofa histone H3.1-like (LOC100156557), mRNA	HIST2H3C
NM_001097412	HMBS	Sus scrofa hydroxymethylbilane synthase (HMBS), mRNA	HMBS
ENSSSCG00000017213	HN1	ENSSSCG00000017213	HN1
NM_001048069	HN1	Sus scrofa hematological and neurological expressed 1 (HN1), mRNA	HN1
ENSSSCG00000014022	HNRNPH1	ENSSSCG00000014022	HNRNPH1
100625284		Novel Transcribed Region; evidence: embryonic ESTs	HP1BP3
ENSSSCG00000002686	HSD17B2	ENSSSCG00000002686	HSD17B2
XM_003355737	LOC100626806	PREDICTED: Sus scrofa estradiol 17-beta-dehydrogenase 2-like (LOC100626806), mRNA	HSD17B2
100,155,418,100,738,000		ENSSSCG00000006337	HSD17B7
ENSSSCG00000001701	HSP90AB1	ENSSSCG00000001701	HSP90AB1
ENSSSCG00000003940	HYI	ENSSSCG00000003940	HYI
XM_001924870	IBTK	PREDICTED: Sus scrofa inhibitor of Bruton agammaglobulinemia tyrosine kinase (IBTK), mRNA	IBTK
XM_001924870	IBTK	PREDICTED: Sus scrofa inhibitor of Bruton agammaglobulinemia tyrosine kinase (IBTK), mRNA	IBTK
13:216984861-216994632		Novel Transcribed Region; evidence: embryonic ESTs	ICOSLG
ENSSSCG00000001077	ID4	ENSSSCG00000001077	ID4
100525850		ENSSSCG00000012775	IDH3G
ENSSSCG00000013766	IL27RA	ENSSSCG00000013766	IL27RA
XM_003122624	INCENP	PREDICTED: Sus scrofa inner centromere protein antigens 135/155kDa (INCENP), mRNA	INCENP
ENSSSCG00000012795	IRAK1	ENSSSCG00000012795	IRAK1
ENSSSCG00000000799	IRAK4	ENSSSCG00000000799	IRAK4
100,517,053,100,737,000,000,000,000		Novel Transcribed Region; evidence: embryonic ESTs	ITGA3
ENSSSCG00000005215	JAK2	ENSSSCG00000005215	JAK2
XM_003128194	JARID2	PREDICTED: Sus scrofa jumonji, AT rich interactive domain 2 (JARID2), mRNA	JARID2
XM_003359349	LOC100037948	PREDICTED: Sus scrofa Kv channel interacting protein 2 (LOC100037948), mRNA	KCNIP2
XM_001928648	LOC100157149	PREDICTED: Sus scrofa UPF0469 protein KIAA0907-like (LOC100157149), mRNA	KIAA0907
ENSSSCG00000010309	KIAA0913	ENSSSCG00000010309	KIAA0913
ENSSSCG00000017108	KIAA0947	ENSSSCG00000017108	KIAA0947
ENSSSCG00000006367	KLHDC9	ENSSSCG00000006367	KLHDC9
ENSSSCG00000004569	LACTB	ENSSSCG00000004569	LACTB
ENSSSCG00000015291	LEMD1	ENSSSCG00000015291	LEMD1

XM_003128944	LIMCH1	PREDICTED: Sus scrofa LIM and calponin homology domains 1, transcript variant 1 (LIMCH1), mRNA	LIMCH1
100738579		Novel Transcribed Region; evidence: embryonic ESTs	LIMD1
ENSSSCG00000016412	LMBR1	ENSSSCG00000016412	LMBR1
ENSSSCG00000008009	LMF1	ENSSSCG00000008009	LMF1
XM_003126471	LOC100515609	PREDICTED: Sus scrofa low-density lipoprotein receptor-related protein 6-like (LOC100515609), mRNA	LRP6
ENSSSCG00000001486	LRRC1	ENSSSCG00000001486	LRRC1
NM_214362	LTF	Sus scrofa lactotransferrin (LTF), mRNA	LTF
4:82710960-82765981		Novel Transcribed Region; evidence: embryonic ESTs	LYN
ENSSSCG00000009890	MAPKAPK5	ENSSSCG00000009890	MAPKAPK5
XM_003354467	LOC100624302	PREDICTED: Sus scrofa DNA replication licensing factor MCM7-like (LOC100624302), mRNA	MCM7
XM_003131490	LOC100511100	PREDICTED: Sus scrofa mediator of RNA polymerase II transcription subunit 24-like, transcript variant 2 (LOC100511100), mRNA	MED24
XM_003125256	LOC100513790	PREDICTED: Sus scrofa protein MEMO1-like (LOC100513790), mRNA	MEMO1
ENSSSCG00000008515	MEMO1	ENSSSCG00000008515	MEMO1
ENSSSCG00000011190	METTL6	ENSSSCG00000011190	METTL6
XM_003125459	LOC100513654	PREDICTED: Sus scrofa major facilitator superfamily domain-containing protein 3-like (LOC100513654), mRNA	MFSD3
ENSSSCG00000014017	MGAT4B	ENSSSCG00000014017	MGAT4B
XM_003131921	LOC100515409	PREDICTED: Sus scrofa misshapen-like kinase 1-like (LOC100515409), mRNA	MINK1
XM_003134608	MKRN1	PREDICTED: Sus scrofa makorin ring finger protein 1, transcript variant 1 (MKRN1), mRNA	MKRN1
100156510		Novel Transcribed Region; evidence: embryonic ESTs	MLXIP
100626072		Novel Transcribed Region; evidence: embryonic ESTs	MMP25
ENSSSCG00000013470	MRPS33	ENSSSCG00000013470	MRPS33
XM_001928036.3	MRS2	PREDICTED: Sus scrofa MRS2 magnesium homeostasis factor homolog (S. cerevisiae) (MRS2), mRNA	MRS2
NM_001195357	MSH2	Sus scrofa mutS homolog 2, colon cancer, nonpolyposis type 1 (Ecoli) (MSH2), mRNA	MSH2
XM_001924836	MTBP	PREDICTED: Sus scrofa Mdm2, transformed 3T3 cell double minute 2, p53 binding protein (mouse) binding protein, 104kDa (MTBP), mRNA	MTBP
XM_003128370	LOC100152609	PREDICTED: Sus scrofa mitochondrial carrier homolog 1,	MTCH1

		transcript variant 2 (LOC100152609), mRNA	
ENSSSCG00000004076	MTRF1L	ENSSSCG00000004076	MTRF1L
ENSSSCG00000012973	MUS81	ENSSSCG00000012973	MUS81
ENSSSCG00000009473	MYCBP2	ENSSSCG00000009473	MYCBP2
ENSSSCG00000011251	MYD88	ENSSSCG00000011251	MYD88
XM_003133553	MYO1B	PREDICTED: Sus scrofa myosin IB (MYO1B), partial mRNA	MYO1B
397085		Novel Transcribed Region; evidence: embryonic ESTs	MYO6
100511137		Novel Transcribed Region; evidence: embryonic ESTs	MYSM1
NM_001144842.1	NDUFB8	Sus scrofa NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 8, 19kDa (NDUFB8), nuclear gene encoding mitochondrial protein, mRNA	NDUFB8
XM_003122435	LOC100521945	PREDICTED: Sus scrofa NADH dehydrogenase [ubiquinone] iron-sulfur protein 8, mitochondrial-like, transcript variant 2 (LOC100521945), mRNA	NDUFS8
ENSSSCG00000009714	NEK1	ENSSSCG00000009714	NEK1
ENSSSCG00000009714	NEK1	ENSSSCG00000009714	NEK1
ENSSSCG00000015597	NENF	ENSSSCG00000015597	NENF
XM_003355771	NFAT5	PREDICTED: Sus scrofa nuclear factor of activated T-cells 5, tonicity-responsive, transcript variant 3 (NFAT5), mRNA	NFAT5
ENSSSCG00000013477	NFIC	ENSSSCG00000013477	NFIC
XM_001928870	NFIL3	PREDICTED: Sus scrofa nuclear factor, interleukin 3 regulated, transcript variant 1 (NFIL3), mRNA	NFIL3
ENSSSCG00000009168	NFKB1	ENSSSCG00000009168	NFKB1
100152239		Novel Transcribed Region; evidence: embryonic ESTs	NHS
1:28871320-29126558		Novel Transcribed Region; evidence: embryonic ESTs	NHSL1
XM_003359151	LOC100152826	PREDICTED: Sus scrofa nipsnap homolog 1, transcript variant 2 (LOC100152826), mRNA	NIPSNAP1
XM_003132134	NKTR	PREDICTED: Sus scrofa natural killer-tumor recognition sequence (NKTR), mRNA	NKTR
XM_003132134	NKTR	PREDICTED: Sus scrofa natural killer-tumor recognition sequence (NKTR), mRNA	NKTR
ENSSSCG00000011347	NME6	ENSSSCG00000011347	NME6
ENSSSCG00000017339	NMT1	ENSSSCG00000017339	NMT1
ENSSSCG00000017513	NPEPPS	ENSSSCG00000017513	NPEPPS
ENSSSCG00000003211	NR1H2	ENSSSCG00000003211	NR1H2
XM_003134338	LOC100513186	PREDICTED: Sus scrofa NSFL1 cofactor p47-like, transcript variant 2 (LOC100513186), mRNA	NSFL1C

ENSSSCG00000011041	NSUN6	ENSSSCG00000011041	NSUN6
ENSSSCG00000017221	NT5C	ENSSSCG00000017221	NT5C
ENSSSCG00000017425	NT5C3L	ENSSSCG00000017425	NT5C3L
ENSSSCG00000012403	OGT	ENSSSCG00000012403	OGT
XM_003128262	LOC100154493	PREDICTED: Sus scrofa olfactory receptor 2G3-like (LOC100154493), mRNA	OR2H1
XM_001928290	LOC100155347	PREDICTED: Sus scrofa olfactory receptor 2G3-like (LOC100155347), mRNA	OR2H1
XM_003124145	LOC100525686	PREDICTED: Sus scrofa olfactory receptor 5AL1-like (LOC100525686), mRNA	OR5AR1
ENSSSCG00000014458	OR5D18	ENSSSCG00000014458	OR5D18
XM_003354530	LOC100626532	PREDICTED: Sus scrofa olfactory receptor 7A10-like (LOC100626532), mRNA	OR7E37P
XM_003126654	LOC100517469	PREDICTED: Sus scrofa olfactory receptor 8S1-like (LOC100517469), mRNA	OR8S1
ENSSSCG00000011215	OXSM	ENSSSCG00000011215	OXSM
XM_003134313	LOC100520054	PREDICTED: Sus scrofa pantothenate kinase 2, mitochondrial-like (LOC100520054), mRNA	PANK2
ENSSSCG00000012926	PC	ENSSSCG00000012926	PC
ENSSSCG00000000274	PCBP2	ENSSSCG00000000274	PCBP2
ENSSSCG00000009557	PCID2	ENSSSCG00000009557	PCID2
ENSSSCG00000007433	PCIF1	ENSSSCG00000007433	PCIF1
XM_003357310	LOC100627878	PREDICTED: Sus scrofa proprotein convertase subtilisin/kexin type 7-like (LOC100627878), mRNA	PCSK7
100518582		Novel Transcribed Region; evidence: embryonic ESTs	PDIA5
100156908		ENSSSCG00000009654	PEAR1
NM_213907	PECAM1	Sus scrofa platelet/endothelial cell adhesion molecule (PECAM1), mRNA	PECAM1
NM_214365	PEMT	Sus scrofa phosphatidylethanolamine N-methyltransferase (PEMT), nuclear gene encoding mitochondrial protein, mRNA	PEMT
ENSSSCG00000018055	PEMT	ENSSSCG00000018055	PEMT
ENSSSCG00000014215	PGGT1B	ENSSSCG00000014215	PGGT1B
ENSSSCG00000003099	PGLYRP1	ENSSSCG00000003099	PGLYRP1
100622686		Novel Transcribed Region; evidence: embryonic ESTs	PHACTR4
ENSSSCG00000008348	PLEK	ENSSSCG00000008348	PLEK
ENSSSCG00000007031	POLB	ENSSSCG00000007031	POLB
XM_003127364.2	POLD1	PREDICTED: Sus scrofa polymerase (DNA directed), delta 1, catalytic subunit 125kDa (POLD1), mRNA	POLD1
XM_003126162	LOC100524561	PREDICTED: Sus scrofa platelet basic protein-like (LOC100524561), mRNA	PPBP

100625026		Novel Transcribed Region; evidence: embryonic ESTs	PPIL4
XM_003355087	PPOX	PREDICTED: Sus scrofa protoporphyrinogen oxidase (PPOX), mRNA	PPOX
XM_003130114	PPP1R15B	PREDICTED: Sus scrofa protein phosphatase 1, regulatory (inhibitor) subunit 15B (PPP1R15B), mRNA	PPP1R15B
ENSSSCG00000016376	PPP1R7	ENSSSCG00000016376	PPP1R7
XM_003133809	LOC100511842	PREDICTED: Sus scrofa protein phosphatase 1 regulatory subunit 7-like (LOC100511842), mRNA	PPP1R7
XR_130678	LOC100518559	PREDICTED: Sus scrofa PR domain zinc finger protein 2-like (LOC100518559), miscRNA	PRDM2
XM_003123356	LOC100512521	PREDICTED: Sus scrofa peroxiredoxin-2-like (LOC100512521), mRNA	PRDX2
XM_001927806.1	PRPF38B	PREDICTED: Sus scrofa PRP38 pre-mRNA processing factor 38 (yeast) domain containing B (PRPF38B), mRNA	PRPF38B
ENSSSCG00000004997	PRPF39	ENSSSCG00000004997	PRPF39
XM_001927120	PRPF39	PREDICTED: Sus scrofa PRP39 pre-mRNA processing factor 39 homolog (Scerevisiae) (PRPF39), mRNA	PRPF39
100511912		Novel Transcribed Region; evidence: embryonic ESTs	PRRC2B
ENSSSCG00000007034	PSD3	ENSSSCG00000007034	PSD3
ENSSSCG00000013239	PSMC3	ENSSSCG00000013239	PSMC3
XM_003124322	LOC100522862	PREDICTED: Sus scrofa pentatricopeptide repeat-containing protein 1-like (LOC100522862), mRNA	PTCD1
ENSSSCG00000007469	PTPN1	ENSSSCG00000007469	PTPN1
ENSSSCG00000005705	QRFP	ENSSSCG00000005705	QRFP
100513782		Novel Transcribed Region; evidence: embryonic ESTs	RAB11B
ENSSSCG00000014043	RAB24	ENSSSCG00000014043	RAB24
100517651		Novel Transcribed Region; evidence: embryonic ESTs	RACGAP1
ENSSSCG00000001948	RALGAPA1	ENSSSCG00000001948	RALGAPA1
100157085		ENSSSCG00000007274	RALY
396710		Novel Transcribed Region; evidence: embryonic ESTs	RARB
XM_003132944	LOC100513761	PREDICTED: Sus scrofa ras-like protein family member 10A-like (LOC100513761), mRNA	RASL10A
ENSSSCG00000007830	RBBP6	ENSSSCG00000007830	RBBP6
ENSSSCG00000009479	RBM26	ENSSSCG00000009479	RBM26
XM_003359964	RBM39	PREDICTED: Sus scrofa RNA binding motif protein 39 (RBM39), mRNA	RBM39
XM_003358447.1	LOC100626851	PREDICTED: Sus scrofa RNA-binding protein 6-like (LOC100626851), mRNA	RBM6

XM_003133427	LOC100525434	PREDICTED: Sus scrofa RNA-binding motif, single-stranded-interacting protein 1-like (LOC100525434), mRNA	RBMS1
XM_003128890	RBPJ	PREDICTED: Sus scrofa recombination signal binding protein for immunoglobulin kappa J region, transcript variant 1 (RBPJ), mRNA	RBPJ
ENSSSCG00000008760	RBPJ	ENSSSCG00000008760	RBPJ
XM_001928924	RCSD1	PREDICTED: Sus scrofa RCSD domain containing 1 (RCSD1), mRNA	RCSD1
XM_003355554	LOC100621847	PREDICTED: Sus scrofa ATP-dependent DNA helicase Q1-like (LOC100621847), mRNA	RECQL
XM_001925355	LOC100152494	PREDICTED: Sus scrofa RILP-like protein 1-like (LOC100152494), mRNA	RILPL1
XM_001927068	LOC100155709	PREDICTED: Sus scrofa ras and Rab interactor 3-like (LOC100155709), mRNA	RIN3
XM_003131935	LOC100518689	PREDICTED: Sus scrofa chromatin complexes subunit BAP18-like, transcript variant 1 (LOC100518689), mRNA	RNASEK-C17ORF49
ENSSSCG00000009910	RNF10	ENSSSCG00000009910	RNF10
ENSSSCG00000015077	RNF214	ENSSSCG00000015077	RNF214
XM_003132451	LOC100514874	PREDICTED: Sus scrofa RING-box protein 2-like, transcript variant 1 (LOC100514874), mRNA	RNF7
ENSSSCG00000006856	RNPC3	ENSSSCG00000006856	RNPC3
ENSSSCG00000005595	RPL35	ENSSSCG00000005595	RPL35
XM_001924771	RPP30	PREDICTED: Sus scrofa ribonuclease P/MRP 30kDa subunit (RPP30), mRNA	RPP30
ENSSSCG00000009019	RPS3A	ENSSSCG00000009019	RPS3A
ENSSSCG00000002820	RSPRY1	ENSSSCG00000002820	RSPRY1
ENSSSCG00000011620	RUVBL1	ENSSSCG00000011620	RUVBL1
ENSSSCG00000010260	SAR1A	ENSSSCG00000010260	SAR1A
ENSSSCG00000012963	SART1	ENSSSCG00000012963	SART1
100524281		Novel Transcribed Region; evidence: embryonic ESTs	SCARA5
NM_001105287	SCG5	Sus scrofa secretogranin V (7B2 protein) (SCG5), mRNA	SCG5
ENSSSCG00000006283	SCYL3	ENSSSCG00000006283	SCYL3
ENSSSCG00000004382	SEC63	ENSSSCG00000004382	SEC63
XM_001928367.2	SECISBP2	PREDICTED: Sus scrofa SECIS binding protein 2 (SECISBP2), mRNA	SECISBP2
ENSSSCG00000015704	SEPHS1	ENSSSCG00000015704	SEPHS1
XM_003353167	LOC100523935	PREDICTED: Sus scrofa protein SERAC1-like (LOC100523935), mRNA	SERAC1
XM_003134027	LOC100517502	PREDICTED: Sus scrofa small EDRK-rich factor 1-like (LOC100517502), mRNA	SERF1B

XM_003130986	LOC100524214	PREDICTED: Sus scrofa stress-associated endoplasmic reticulum protein 2-like (LOC100524214), mRNA	SERP2
396,686,100,738,211		ENSSSCG00000002487	SERPINA3-2
XM_001928602	SERPINA3-3	PREDICTED: Sus scrofa alpha-1-antichymotrypsin 3 (SERPINA3-3), mRNA	SERPINA3-3
ENSSSCG00000002481	SERPINA5	ENSSSCG00000002481	SERPINA5
ENSSSCG00000011740	SERPINI1	ENSSSCG00000011740	SERPINI1
XM_003355914	LOC100624989	PREDICTED: Sus scrofa SERTA domain-containing protein 1-like, transcript variant 1 (LOC100624989), mRNA	SERTAD1
ENSSSCG00000009395	SETDB2	ENSSSCG00000009395	SETDB2
ENSSSCG00000016075	SF3B1	ENSSSCG00000016075	SF3B1
ENSSSCG00000003791	SFRS11	ENSSSCG00000003791	SFRS11
ENSSSCG00000017583	SGCA	ENSSSCG00000017583	SGCA
ENSSSCG00000005914	SHARPIN	ENSSSCG00000005914	SHARPIN
100522965		Novel Transcribed Region; evidence: embryonic ESTs	SHROOM2
XM_003355818	LOC100520614	PREDICTED: Sus scrofa e3 ubiquitin-protein ligase SIAH1-like, transcript variant 2 (LOC100520614), mRNA	SIAH1
ENSSSCG00000006751	SIKE1	ENSSSCG00000006751	SIKE1
7:26674362-26716913		ENSSSCG00000001341	SLA-11
ENSSSCG00000015949	SLC25A12	ENSSSCG00000015949	SLC25A12
XM_003357479	LOC100621546	PREDICTED: Sus scrofa solute carrier family 25 member 40-like (LOC100621546), mRNA	SLC25A40
ENSSSCG00000014367	SLC35A4	ENSSSCG00000014367	SLC35A4
XM_003129295	LOC100524695	PREDICTED: Sus scrofa zinc transporter ZIP8-like (LOC100524695), mRNA	SLC39A8
ENSSSCG00000007157	SLC4A11	ENSSSCG00000007157	SLC4A11
100524044		Novel Transcribed Region; evidence: embryonic ESTs	SLC4A7
Ssc.47513	LOC100525144	Organic solute transporter subunit beta-like	SLC51B
397458		Novel Transcribed Region; evidence: embryonic ESTs	SLC9A1
XM_003353338	SLTM	PREDICTED: Sus scrofa SAFB-like, transcription modulator, transcript variant 2 (SLTM), mRNA	SLTM
XM_003129318	SMARCAD1	PREDICTED: Sus scrofa SWI/SNF-related, matrix-associated actin-dependent regulator of chromatin, subfamily a, containing DEAD/H box 1 (SMARCAD1), mRNA	SMARCAD1
ENSSSCG00000010070	SMARCB1	ENSSSCG00000010070	SMARCB1
ENSSSCG00000005255	SMC5	ENSSSCG00000005255	SMC5
ENSSSCG00000016569	SMO	ENSSSCG00000016569	SMO

XR_130822	LOC100518980	PREDICTED: Sus scrofa sphingomyelin phosphodiesterase-like (LOC100518980), miscRNA	SMPD1
ENSSSCG00000019243	SNORA18	ENSSSCG00000019243	SNORA18
ENSSSCG00000018225	SNORA18	ENSSSCG00000018225	SNORA18
ENSSSCG00000019648	SNORA25	ENSSSCG00000019648	SNORA25
ENSSSCG00000019977	SNORA32	ENSSSCG00000019977	SNORA32
ENSSSCG00000018972	SNORA33	ENSSSCG00000018972	SNORA33
ENSSSCG00000019064	SNORD113	ENSSSCG00000019064	SNORD113
ENSSSCG00000018470	SNORD14	ENSSSCG00000018470	SNORD14
ENSSSCG00000019644	SNORD47	ENSSSCG00000019644	SNORD47
NM_001177913	SPATA5	Sus scrofa spermatogenesis associated 5 (SPATA5), mRNA	SPATA5
ENSSSCG00000002424	SPATA7	ENSSSCG00000002424	SPATA7
XM_001927757.2	SPOCK3	PREDICTED: Sus scrofa sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican) 3, transcript variant 1 (SPOCK3), mRNA	SPOCK3
XM_003131559	LOC100524522	PREDICTED: Sus scrofa speckle-type POZ protein-like (LOC100524522), mRNA	SPOP
100518625		Novel Transcribed Region; evidence: embryonic ESTs	SRGAP2
NM_001044587	SRSF11	Sus scrofa serine/arginine-rich splicing factor 11 (SRSF11), mRNA	SRSF11
XM_001927434	SRSF5	PREDICTED: Sus scrofa serine/arginine-rich splicing factor 5, transcript variant 2 (SRSF5), mRNA	SRSF5
XR_130497	LOC100522988	PREDICTED: Sus scrofa hypothetical LOC100522988 (LOC100522988), miscRNA	ST3GAL5
ENSSSCG00000006779	ST7L	ENSSSCG00000006779	ST7L
XM_001927009	STAM2	PREDICTED: Sus scrofa signal transducing adaptor molecule (SH3 domain and ITAM motif) 2 (STAM2), partial mRNA	STAM2
NM_001201385	STARD3NL	Sus scrofa STARD3 N-terminal like (STARD3NL), mRNA	STARD3NL
100620912		Novel Transcribed Region; evidence: embryonic ESTs	STEAP3
100171399		Novel Transcribed Region; evidence: embryonic ESTs	STIM1
ENSSSCG00000009511	STK24	ENSSSCG00000009511	STK24
100624126		Novel Transcribed Region; evidence: embryonic ESTs	STRAP
ENSSSCG00000009747	STX2	ENSSSCG00000009747	STX2
ENSSSCG00000004113	STXBP5	ENSSSCG00000004113	STXBP5
100523215		Novel Transcribed Region; evidence: embryonic ESTs	TACC1
XM_003128804	LOC100513802	PREDICTED: Sus scrofa transforming acidic coiled-coil-containing protein 3-like (LOC100513802), mRNA	TACC3
ENSSSCG00000014945	TAF1D	ENSSSCG00000014945	TAF1D

XM_003355305	LOC100625790	PREDICTED: Sus scrofa hypothetical protein LOC100625790 (LOC100625790), partial mRNA	TBC1D22A
ENSSSCG00000015891	TBR1	ENSSSCG00000015891	TBR1
XM_003135283	LOC100514086	PREDICTED: Sus scrofa transcription elongation factor A protein-like 4-like (LOC100514086), mRNA	TCEAL4
XM_003124072	LOC100511121	PREDICTED: Sus scrofa transcription elongation regulator 1-like (LOC100511121), mRNA	TCERG1
2:158451903-158486259		Novel Transcribed Region; evidence: embryonic ESTs	TCOF1
2:30353874-30399028		Novel Transcribed Region; evidence: embryonic ESTs	TCP11L1
XM_003359085	TCTN2	PREDICTED: Sus scrofa tectonic family member 2 (TCTN2), mRNA	TCTN2
100216478		Novel Transcribed Region; evidence: embryonic ESTs	TEF1
ENSSSCG00000000254	TENC1	ENSSSCG00000000254	TENC1
100513361		Novel Transcribed Region; evidence: embryonic ESTs	TESK2
ENSSSCG00000004602	TEX9	ENSSSCG00000004602	TEX9
100513065		Novel Transcribed Region; evidence: embryonic ESTs	TJP2
ENSSSCG00000011901	TMEM39A	ENSSSCG00000011901	TMEM39A
ENSSSCG00000010854	TMEM63A	ENSSSCG00000010854	TMEM63A
100,152,579,100,520,000		Novel Transcribed Region; evidence: embryonic ESTs	TP53BP1
414388		ENSSSCG00000006556	TPM3
ENSSSCG00000007952	TRAP1	ENSSSCG00000007952	TRAP1
100152751		ENSSSCG00000003751	TRIP4
ENSSSCG00000013040	TRPT1	ENSSSCG00000013040	TRPT1
XM_001928419	TRUB2	PREDICTED: Sus scrofa TruB pseudouridine (psi) synthase homolog 2 (E. coli) (TRUB2), mRNA	TRUB2
100136903		ENSSSCG00000007483	TSHZ2
XM_003134097	LOC100519063	PREDICTED: Sus scrofa tetratricopeptide repeat protein 1-like (LOC100519063), mRNA	TTC1
ENSSSCG00000011767	TTC14	ENSSSCG00000011767	TTC14
ENSSSCG00000003875	TTC39A	ENSSSCG00000003875	TTC39A
ENSSSCG00000005725	TTF1	ENSSSCG00000005725	TTF1
ENSSSCG00000016201	TTLL4	ENSSSCG00000016201	TTLL4
XM_003359573	LOC100519519	PREDICTED: Sus scrofa titin-like (LOC100519519), mRNA	TTN
XM_003359575	LOC100620353	PREDICTED: Sus scrofa titin-like (LOC100620353), partial mRNA	TTN
ENSSSCG00000007803	TUFM	ENSSSCG00000007803	TUFM
ENSSSCG00000011409	TUSC4	ENSSSCG00000011409	TUSC4
100516854		Novel Transcribed Region; evidence: embryonic ESTs	TYW1
ENSSSCG00000019769	U1	ENSSSCG00000019769	U1

ENSSSCG00000019204	U1	ENSSSCG00000019204	U1
ENSSSCG00000018550	U1	ENSSSCG00000018550	U1
ENSSSCG00000018430	U1	ENSSSCG00000018430	U1
ENSSSCG00000018416	U1	ENSSSCG00000018416	U1
ENSSSCG00000019019	U1	ENSSSCG00000019019	U1
ENSSSCG00000018843	U1	ENSSSCG00000018843	U1
ENSSSCG00000019522	U1	ENSSSCG00000019522	U1
ENSSSCG00000018928	U2	ENSSSCG00000018928	U2
ENSSSCG00000018129	U6	ENSSSCG00000018129	U6
ENSSSCG00000019377	U6	ENSSSCG00000019377	U6
XM_001929469	UBE2Q1	PREDICTED: Sus scrofa ubiquitin-conjugating enzyme E2Q family member 1 (UBE2Q1), mRNA	UBE2Q1
ENSSSCG00000004832	UBE3A	ENSSSCG00000004832	UBE3A
XM_003360637	LOC100623268	PREDICTED: Sus scrofa ubiquitin-like protein 7-like (LOC100623268), mRNA	UBL7
ENSSSCG00000013510	UBXN6	ENSSSCG00000013510	UBXN6
XM_003126693	UHRF1BP1L	PREDICTED: Sus scrofa UHRF1 binding protein 1-like (UHRF1BP1L), mRNA	UHRF1BP1L
ENSSSCG00000005201	UHRF2	ENSSSCG00000005201	UHRF2
ENSSSCG00000011356	UQCRC1	ENSSSCG00000011356	UQCRC1
ENSSSCG00000011356	UQCRC1	ENSSSCG00000011356	UQCRC1
100511469		Novel Transcribed Region; evidence: embryonic ESTs	USP12
XM_001925613	USP40	PREDICTED: Sus scrofa ubiquitin specific peptidase 40 (USP40), partial mRNA	USP40
XM_003134231	LOC100525492	PREDICTED: Sus scrofa voltage-dependent anion-selective channel protein 3-like, transcript variant 1 (LOC100525492), mRNA	VDAC3
ENSSSCG00000009376	VPS36	ENSSSCG00000009376	VPS36
100520868		Novel Transcribed Region; evidence: embryonic ESTs	WHSC1L1
XM_001925977	WSB2	PREDICTED: Sus scrofa WD repeat and SOCS box containing 2 (WSB2), mRNA	WSB2
ENSSSCG00000004050	WTAP	ENSSSCG00000004050	WTAP
100,518,028,100,738,000		Novel Transcribed Region; evidence: embryonic ESTs	ZBTB44
XR_130474	LOC100516601	PREDICTED: Sus scrofa hypothetical LOC100516601 (LOC100516601), miscRNA	ZNF263
XM_001927940	ZNF268	PREDICTED: Sus scrofa zinc finger protein 268 (ZNF268), mRNA	ZNF268
XM_003356105	ZNF606	PREDICTED: Sus scrofa zinc finger protein 606 (ZNF606), mRNA	ZNF606
XM_003133853	LOC100521757	PREDICTED: Sus scrofa zinc finger protein 622-like (LOC100521757), mRNA	ZNF622

XM_001926232	ZNF711	PREDICTED: Sus scrofa zinc finger protein 711, transcript variant 1 (ZNF711), mRNA	ZNF711
ENSSSCG00000012689	ZNF75D	ENSSSCG00000012689	ZNF75D
XM_003356928.1	LOC100511574	PREDICTED: Sus scrofa zinc finger protein 208-like (LOC100511574), partial mRNA	ZNF85
XM_001927707	LOC100153815	PREDICTED: Sus scrofa box C/D snoRNA protein 1-like (LOC100153815), mRNA	ZNHIT6
7:23889169-23902259		Novel Transcribed Region; evidence: embryonic ESTs	ZSCAN16
XR_131395	LOC100621871	PREDICTED: Sus scrofa hypothetical LOC100621871, transcript variant 2 (LOC100621871), miscRNA	
XM_003122663	LOC100524972	PREDICTED: Sus scrofa membrane-spanning 4-domains subfamily A member 6E-like, transcript variant 1 (LOC100524972), mRNA	
17:19411732-19411806	NA	ENSSSCG00000020259	
LOC100154801		Novel Transcribed Region; evidence: embryonic ESTs	
100513979		ENSSSCG00000007588	
XM_003126398	LOC100511327	PREDICTED: Sus scrofa uncharacterized protein C12orf35-like (LOC100511327), mRNA	
100152873		ENSSSCG00000001148	
LOC100154313		Novel Transcribed Region; evidence: embryonic ESTs	
LOC100517702		Novel Transcribed Region; evidence: embryonic ESTs	
XM_001929049	LOC100154005	PREDICTED: Sus scrofa actin-related protein 8, transcript variant 1 (LOC100154005), mRNA	
XM_003353344	LOC100620654	PREDICTED: Sus scrofa SAFB-like transcription modulator-like (LOC100620654), mRNA	
LOC100152503		Novel Transcribed Region; evidence: embryonic ESTs	
XM_003124411	LOC100525863	PREDICTED: Sus scrofa abhydrolase domain-containing protein 11-like, transcript variant 1 (LOC100525863), mRNA	
XM_003133050	LOC100517429	PREDICTED: Sus scrofa cell division cycle and apoptosis regulator protein 1-like (LOC100517429), mRNA	
XM_003360637	LOC100623268	PREDICTED: Sus scrofa ubiquitin-like protein 7-like (LOC100623268), mRNA	
LOC100513977		Novel Transcribed Region; evidence: embryonic ESTs	
LOC100521800		Novel Transcribed Region; evidence: embryonic ESTs	
LOC100624908		Novel Transcribed Region; evidence: embryonic ESTs	
XM_001929049	LOC100154005	PREDICTED: Sus scrofa actin-related protein 8, transcript variant 1 (LOC100154005), mRNA	
100515933		ENSSSCG00000016962	

LOC100156587		Novel Transcribed Region; evidence: embryonic ESTs	
XM_003121137.1	LOC100514202	PREDICTED: Sus scrofa peptide chain release factor 1-like, mitochondrial-like (LOC100514202), mRNA	
LOC100517422		ENSSSCG00000015330	
LOC100515460		Novel Transcribed Region; evidence: embryonic ESTs	
LOC100523309		Novel Transcribed Region; evidence: embryonic ESTs	
LOC100626089		Novel Transcribed Region; evidence: embryonic ESTs	
XM_003126893	LOC100513797	PREDICTED: Sus scrofa transducin-like enhancer protein 2-like (LOC100513797), mRNA	
XM_003357702	LOC100516824	PREDICTED: Sus scrofa iron-sulfur cluster assembly 1 homolog, mitochondrial-like (LOC100516824), mRNA	

GS
GS
EIF3L
TRUB2
BZW1
ADAMDEC1
FETUIN
RPS3A
RARB
TP53BP1
ZSCAN16
TCOF1
PRDX2
ZBTB44
U6
STIM1
GINS3
CIZ1
DAB2
SERPINA3-3
EEF2
NDUFB8
APOA2
CCNI
CCDC25
TACC1
HSP90AB1
SRGAP2
STAM2
PHACTR4
U1
IRAK4
SIKE1
CCNG1
CST3
ST3GAL5
RNF7
DDB2
ARGLU1
PEMT
MMP25
ACTG2
ACTA1

CYBRD1
KCNIP2
RPP30
MTCH1
ACTC1
TCP11L1
HIST2H3C
SF3B1
SLC39A8
MGAT4B
TMEM63A
RBM26
LYN
CALM1
EVX1
PCID2
PEAR1
BMP2K
C20orf43
NFIC
C5orf43
DDX21
CYP24A1
MINK1
GSTM3
HSD17B2
GPR153
HMBS
ADAMTS2
PCBP2
HNRNPH1
GPD1L
7SK
NDUFS8
VDAC3
SRSF5
C8orf47
SMARCAD1
PPBP
CENPL
HEMGN
COPS3
IBTK
CCAR1
RACGAP1
SRSF11
SFRS11
UBXN6
GREB1
ENO1
PTCD3
SEC63
LRP6
CTSF
RSPRY1
NENF
SLA-11
RAB11B
TRAP1
LIMD1
TYW1
HN1
NFIL3
TCEAL4
OR2H1
UBE2Q1
PSMC3
PRPF39
MYCBP2
C1orf35

CCDC90B
RBM39
RUVBL1
HYI
RBMS1
NKTR
ARF4
TTC1
TUFM
SERPINA5
MRS2
AMD1
BRD3
ERCC6
SMARCB1
RNF214
SERAC1
DUSP11
HECA
ARID4B
PDIA5
PTCD1
ATP2B1
MSH2
JARID2
TPM3
CCZ1
NT5C3L
CYC1
USP12
ANKRD27
CHD1L
UQCRC1
TCTN2
SLC25A12
CCNY
SERPINA3-2
LIMCH1
SPOCK3
RNASEK-C17ORF49
ANKRD49
WTAP
OR7E37P
LRRC1
ARMC1
RPL35
C20orf108
CLEC1B
CRIP1
ZNF606
BID
MAPKAPK5
LMBR1
TMEM39A
ALKBH2
KIAA0907
C11orf17
RBBP6
TRIP4
DDX54
CALCOCO2
TUSC4
SCARA5
TESK2
SLC25A40
ID4
CCT2
SCYL3
ARHGEF12
RAB24

EFHA2
WHSC1L1
SAR1A
SECISBP2
USP40
ACOX2
UBL7
SPOP
RASL10A
CRAT
TTC14
RNF10
MCM7
KLHDC9
DDX18
PPP1R7
MYO6
RBM6
TTN
KIAA0947
LMF1
SLC51B
ZNF268
CLDN10
NMT1
TAF1D
OR5D18
NFAT5
CBR4
C11orf54
ANKRD32
EBPL
SLTM
PANK2
PTPN1
ABR
ZNF711
ST7L
SPATA7
MLXIP
TJP2
FNBP1
HAUS3
MTRF1L
UHRF1BP1L
NME6
GNAS
OGT
SNORD47
TACC3
AAGAB
Mar-02
SERP2
UBE3A
DDX26B
CENPH
APC
SCG5
TEX9
GRB10
RECQL
POLB
UHRF2
RALGAPA1
MYSM1
HSD17B7
C5orf34
RNPC3
POLD1
CCDC42

TTF1
CLSPN
HAT1
DDX50
SERF1B
C15orf60
CTNNA1
SETDB2
COPG2
PPIL4
PRPF38B
SNORA32
ZNF263
ACER3
SNORA25
RBPJ
ZNF622
SMPD1
CSTF3
METTL6
VPS36
OXSM
ALMS1
DHFR
E2F3
CLK1
HAVCR2
RCSD1
HAUS7
FAM172A
U2
IL27RA
AMY2
TCERG1
SART1
TENC1
PC
JAK2
C9ORF80
AP2A2
MFSD3
DGAT2
SERPINI1
IDH3G
SEPHS1
NHSL1
MUS81
SNORA18
DNMT3A2
SERTAD1
HIST1H2AH
RALY
STXBP5
PRDM2
SNORA33
GLS
ARMCX6
ICOSLG
NPEPPS
PLEK
ITGA3
C6orf211
CSNK1G3
LACTB
DDC
AP1B1
MKRN1
MED24
STK24
SLC9A1

CDK3
STRAP
CCT7
ACTN4
EFNA4
ETFDH
ZNHIT6
FAM188A
OR8S1
ARHGAP12
CDK13
STARD3NL
FOXH1
RILPL1
ECSCR
SMC5
ANKS3
SHARPIN
DGAT
NSUN6
DEPDC1B
TTC39A
BAZ2B
NR1H2
SIAH1
SLC4A7
EPS15L1
TSHZ2
NT5C
NFKB1
BARX2
ARHGAP27
PECAM1
PPOX
ADM2
FBXO4
CHORDC1
CDK16
CCDC72
TRPT1
PSD3
GTF2B
PRRC2B
C20ORF29
GDAP1L1
SLC35A4
C5orf63
FBXO8
NSFL1C
C1QTNF2
STX2
TEF1
GCH1
SNORD14
PGLYRP1
IRAK1
ZNF85
DMP1
TBC1D22A
PGGT1B
INCENP
CPSF3L
MYO1B
A2LD1
ANKRD13C
FCHO1
MYD88
ACTN3
STEAP3
FOXK1

MRPS33
NEK1
CHAF1B
FGFR2
MEMO1
HP1BP3
CIB1
LTF
ZNF75D
CNNM4
WSB2
CXorf26
C9orf5
FAM103A1
SHROOM2
CLN8
CABYR
SMO
OR5AR1
PPP1R15B
PCIF1
RIN3
CCDC61
SNORD113
SLC4A11
SPATA5
MTBP
TBR1
ASAP1
QRFP
NIPSNAP1
CSF3
PCSK7
TTLL4
NHS
SGCA
CHMP4B
KIAA0913

Appendix 3

Table 4.4 A list of associated genes within each GO term.

GO Term	Description	Genes
GO:0030048 actinin,	actin filament-based movement	[ACTA1 - actin, alpha 1, skeletal muscle, TPM3 - tropomyosin 3, MYO1B - myosin ib, ACTN4 - alpha 4, ACTC1 - actin, alpha, cardiac muscle 1, SLC9A1 - solute carrier family 9, subfamily a (nhe1, cation proton antiporter 1), member 1, MYO6 - myosin vi, ACTN3 - actinin, alpha 3, TTN - titin]
GO:0019222	regulation of metabolic process	[SMARCAD1 - swi/snf-related, matrix-associated actin-dependent regulator of chromatin, subfamily a, containing dead/h box 1, BMP2K - bmp2 inducible kinase, MED24 - mediator complex subunit 24, WTAP - wilms tumor 1 associated protein, HAT1 - histone acetyltransferase 1, LMF1 - lipase maturation factor 1, RBM39 - rna binding motif protein 39, TAF1D - tata box binding protein (tbp)-associated factor, rna polymerase i, d, 41kda, TSHZ2 - teashirt zinc finger homeobox 2, ARID4B - at rich interactive domain 4b (rbp1-like), APOA2 - apolipoprotein a-ii, WHSC1L1 - wolf-hirschhorn syndrome candidate 1-like 1, ARHGAP27 - rho gtpase activating protein 27, RACGAP1 - rac gtpase activating protein 1, CHAF1B - chromatin assembly factor 1, subunit b (p60), SPOCK3 - sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican) 3, STXBP5 - syntaxin binding protein 5 (tomosyn), PC - pyruvate carboxylase, ABR - active bcr-related, PPP1R15B - protein phosphatase 1, regulatory subunit 15b, GCH1 - gtp cyclohydrolase 1, SERPINA5 - serpin peptidase inhibitor, clade a (alpha-1 antiproteinase, antitrypsin), member 5, APC - adenomatous polyposis coli, GTF2B - general transcription factor iib, ENO1 - enolase 1, (alpha), PANK2 - pantothenate kinase 2, SCARA5 - scavenger receptor class a, member 5 (putative), MAPKAPK5 - mitogen- activated protein kinase-activated protein kinase 5, NPRL2 - nitrogen permease regulator-like 2 (s. cerevisiae), TTF1 - transcription termination factor, rna polymerase i, SECISBP2 - secis binding protein 2, TTN - titin, BID - bh3 interacting domain death agonist, ARF4 - adp-ribosylation factor 4, TCERG1 - transcription elongation regulator 1, BARX2 - barx homeobox 2, ANKRD13C - ankyrin repeat domain 13c, ACTA1 - actin, alpha 1, skeletal muscle, ZNF85 - zinc finger protein 85, PSMC3 - proteasome (prosome, macropain) 26s subunit, atpase, 3, RALGAPA1 - ral gtpase activating protein, alpha subunit 1 (catalytic), PRDX2 - peroxiredoxin 2, ZSCAN16 - zinc finger and scan domain containing 16, ACTC1 - actin, alpha, cardiac muscle 1, ZNF75D - zinc finger protein 75d, ANKRD49 - ankyrin repeat domain 49, MYO6 - myosin vi, PLEK - pleckstrin, ACTG2 - actin, gamma 2, smooth muscle, enteric, FGFR2 - fibroblast growth factor receptor 2, EEF2 - eukaryotic translation elongation factor 2, MCM7 - minichromosome maintenance complex component 7, RIN3 - ras and rab interactor 3, ANKRD27 - ankyrin repeat domain 27 (vps9 domain), MYD88 - myeloid differentiation primary response 88, ACTN4 - actinin, alpha 4, ARGLU1 - arginine and glutamate rich 1, ZNF622 - zinc finger protein 622, ACTN3 - actinin, alpha 3, CCNY - cyclin y, TP53BP1 - tumor protein p53 binding protein 1, ITGA3 - integrin, alpha 3 (antigen cd49c, alpha 3 subunit of vla-3 receptor), VPS36 - vacuolar protein sorting 36 homolog (s. cerevisiae), NSFL1C - nsfl1 (p97)
GO:0090131 gamma 2,	mesenchyme migration	[ACTA1 - actin, alpha 1, skeletal muscle, ACTC1 - actin, alpha, cardiac muscle 1, ACTG2 - actin, smooth muscle, enteric]
GO:0090130 gamma 2,	tissue migration	[ACTA1 - actin, alpha 1, skeletal muscle, ACTC1 - actin, alpha, cardiac muscle 1, ACTG2 - actin, smooth muscle, enteric]
GO:0070252 muscle 1,	actin-mediated cell contraction	[ACTA1 - actin, alpha 1, skeletal muscle, TPM3 - tropomyosin 3, ACTC1 - actin, alpha, cardiac SLC9A1 - solute carrier family 9, subfamily a (nhe1, cation proton antiporter 1), member 1, ACTN3 - actinin, alpha 3, TTN - titin]
GO:0030029 myosin ib,	actin filament-based process	[MINK1 - misshapen-like kinase 1, TPM3 - tropomyosin 3, ACTN4 - actinin, alpha 4, MYO1B - SLC9A1 - solute carrier family 9, subfamily a (nhe1, cation proton antiporter 1), member 1, SRGAP2 - slit- robo rho gtpase activating protein 2, ACTN3 - actinin, alpha 3, TTN - titin, PTPN1 - protein tyrosine phosphatase, non-receptor type 1, RACGAP1 - rac gtpase activating protein 1, LIMCH1 - lim and calponin homology domains 1, PHACTR4 - phosphatase and actin regulator 4, ACTA1 - actin, alpha 1, skeletal muscle, ABR - active bcr-related, ACTC1 - actin, alpha, cardiac muscle 1, TESK2 - testis-specific kinase 2, MYO6 - myosin vi, PLEK - pleckstrin]
GO:0035264 repair	multicellular organism growth	[FGFR2 - fibroblast growth factor receptor 2, RARB - retinoic acid receptor, beta, ERCC6 - excision cross-complementing rodent repair deficiency, complementation group 6, TENC1 - tensin like c1 domain containing phosphatase (tensin 2), SMO - smoothened, frizzled family receptor, GNAS - gnas complex locus, NEK1 - nima-related kinase 1, RBBP6 - retinoblastoma binding protein 6]

Genes: For each GO term you can see the list of associated genes that appear in the optimal top of the list. Each gene name is specified by gene symbol followed by a short description of the gene