IMPACT OF UTERINE CROWDING ON FARROWING PERFROMANCE, POSTNATAL PERFORMANCE, AND ADRENAL STRESS RESPONSE

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ABSTRACT

The main purpose of this study was to determine the associations between uterine crowding and embryonic and fetal survival in gestation, and postnatal performance (birth weight, postnatal growth and survival, backfat depth, and adrenocorticotrophic hormone (ACTH)-induced cortisol responses in pigs. The present research was based on the hypothesis that piglets from the less crowded (unilateral oviduct cauterized; CAUT) litters will exhibit superior postnatal performance compared to piglets from control (non-cauterized; CTRL). For this purpose, 13 unilaterally oviduct cauterized (CAUT) and 12 non-cauterized control (CTRL) sows were studied. At gestation day (D) 35 a laparotomy surgery was performed on all sows and the total number of corpora lutea and embryos present was used to calculate embryo survival. Piglet weight, gender and morphometrics were measured at birth. Piglet weights were again measured at D21, week (W) 3, W7, W12, W16, and W19 of age. Available stillborn piglets were dissected from litters of each group to assess the weights of vital organs. Average daily gain (ADG) was calculated for the lactation (ADG_{LACT}; 0-21 days of age), nursery ($ADG_{NUR;}$ W3-7 of age), finisher ($ADG_{FIN;}$ W7-19) period, and from birth to market ($ADG_{LIFETIME}$; W0-19). Backfat (P2) and loin eye depth were measured when pigs were approximately 70 kg and 110 kg live weight. Saliva samples were collected from offspring following ACTH challenges at W12 and 20 of age. Our results showed that uterine crowding in CTRL compared to CAUT resulted in a decrease in embryonic survival rate (CAUT = 66%, CTRL = 53%; P=0.04), fetal survival rate (CAUT = 59%, CTRL = 42.4%; P = 0.007), WT_{D0} (CAUT = 1.7 ± 0.3 kg, CTRL = 1.4 ± 0.2 kg; P < 0.001), and body mass index (CAUT = 0.18 ±0.01, CTRL = 0.17 ± 0.01; P = 0.04) at birth. Adrenal weight (CAUT = 0.5 ± 0.1 gm, CTRL = 0.3 ± 0.1 gm; P = 0.005) and adrenal:brain weight ratio (CAUT = 0.02 ± 0.004, CTRL = 0.01 ± 0.002; P = 0.009) in dissected stillborns (CAUT = 7, CTRL = 10) were lower in pigs from CTRL compared to CAUT sows, demonstrating that the brain sparing associated with intrauterine growth retardation markedly affected development of the adrenal gland. Pre-weaning survival (CAUT = 92.9%, CTRL = 82.1%; P = 0.01) was lower in pigs from CTRL sows. While doing overall analysis across the progeny, birth weight was most significant factor affecting ADG. Heavier birth weight pigs had higher ADG_{LACT} (P =0.01). Pigs with higher ADG_{LACT}, a potential proxy measure of post-natal catch up growth, had higher $ADG_{LIFETIME}$ (P < 0.001) after controlling for WT_{D0}. In summary,

uterine crowding negatively affected pre-natal growth and development, as manifested by decreased birth weight and body mass index. Birth weight was the most consistent factor affecting ADG throughout life, and the effects of uterine crowding *per se* were mediated through its impact on birth weight. In spite of having only one "functional" ovary in CAUT sows, the total litter weight at lactation D21 was not different between CTRL and CAUT litters. As both birth weight and piglet growth during lactation contribute to the pig's lifetime performance, the intra- uterine environment, as well as the size of the litter and lactational environment, needs critical consideration for efficient pork production.

Key words: Pig, Intrauterine crowding (IUC), Prenatal programming, Fetal development, Postnatal development, Adrenocorticotrophic hormone (ACTH), Cortisol.

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I dedicate this thesis to my parents Mukti Nath Dhakal and Radha Devi Dhakal, and to my loving husband Roshan Parajuli.

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TABLE OF CONTENTS

LIST OF TABLES

TABLE **PAGE**

LIST OF FIGURES

FIGURE **PAGE**

LIST OF ABBREVIATIONS

ACTH	Adrenocorticotrophic hormone
ADG	Average daily gain
ADG_{FIN}	Average daily gain during grow finish (Week 7-Week 19)
$ADG_{LIFETIME}$	Average daily gain from birth to market
$ADG_{NURSERY}$	Average daily gain in nursery (Week 3-Week 7)
BF	Backfat
BMI	Body mass index
CAUT	unilateral oviduct cauterized
CRL	Crown rump length
CL	Corpus luteum (corpora lutea)
CW	Composite White (breed)
CTRL	Control group (non-cauterized)
FOS_{YN}	Fostered yes/no
GC	Glucocorticoid
GNDR	Gender
GRP	Group
HRP	Horseradish peroxidase
IUC	Intrauterine crowding
IUGR	Intrauterine growth restriction
LOIN	Loin depth
LS_{POSFOS}	Litter size post foster at day 0
MEIS	Meishan (breed)
REP	Replication, consequent farrowing
TMB	Tetra-methylbenzidine
W	Week
WT	Body weight
UHO	Unilateral hysterectomy and ovariectomy

HPA	Hypothalamic-pituitary-adrenal
SO	Slow twitch oxidative
FG	Fast twitch glycolytic
FOG	Fast-twitch oxidative glycolytic
D	Day
IGF	Insulin like growth factor
GR	Glucocorticoid receptor
CRH	Corticotropic releasing hormone
DEX	Dexamethasone
EMB_TOT	Total number of embryos at gestation day 35
MT_{PRE}	Pre-weaning mortality
MT_{POST}	Post- weaning mortality

CHAPTER 1

GENERAL INTRODUCTION

Earlier research has found a direct relationship between poor fetal, or early postnatal, growth and the development of type-2 diabetes [1], cardiovascular and metabolic disease in humans [2]. In pigs, low birth weight is associated with hypertension [3], glucose intolerance [4], and an altered hypothalamic-pituitary-adrenal (HPA) axis response which may indirectly affect the stress and inflammatory response in postnatal life [5]. The concept of uterine capacity in pigs was established as early as 1968 by using a unilateral hysterectomy and ovariectomy (UHO) model, superovulation, embryo transfer, and unilateral oviductal ligation [6]. Dzuik concluded that uterine capacity is a limiting factor when the number of embryos exceeds 14. Higher numbers of embryos cause higher embryonic and fetal loss [7], and lower placental and fetal weight at D90 of gestation [8]. Unilateral oviduct ligation in sows was performed to study the effects of relative embryo crowding *in utero* on prenatal development, it was found thatD90 fetuses from non-ligated sows demonstrated brain sparing and had lower numbers of secondary muscle fibres due to decreased expression of genes related to myogenesis [9].

These studies were terminated mostly during gestation or immediately after farrowing. It was our objective however, to determine the effects of relative uterine crowding on farrowing and postnatal performance using the unilateral oviduct cauterization model to artificially decrease litter size and allow the development of litters in a more spacious uterine environment. Birth weight is widely used as a proxy measure of intra-uterine growth restriction (IUGR), and IUGR is also associated with alterations in early life adrenal function. For example, [10] used *in vitro* stimulation of adrenal cells to demonstrate that cortisol concentration responses in low birth weight offspring from UHO sows were higher in cells recovered at 3 and 7 days of age. Low birth weight piglets also had higher adrenal weights relative to body weight. Similarly, the adrenal weight and the ratio of adrenal cortex to medulla were significantly higher at 3 months of age in low birth weight piglets [5] and the adrenal cortex to medulla ratio trended higher at 12 months.

The present research was based on the hypothesis that piglets from the less crowded (unilateral oviduct cauterized; CAUT) litters will exhibit superior postnatal performance compared to piglets from control (non-cauterized; CTRL) litters. The specific objective of this project was to determine the effects of uterine crowding on: i) embryonic and fetal survivability; ii) weight, morphometrics and gender of piglets at birth; iii) the offspring's performance during lactation and the nursery and grow-finish stages of production; iv) carcass quality measured as backfat (BF) and loin (LOIN) depth (at 70 and 110 kg weight; and v) ATCH-induced cortisol responses in pigs at 12 and 20 weeks of age.

Chapter 2 of this thesis provides a review of the related literature. Research methodology and results are presented in Chapters 3 and 4. Chapter 3 discusses the effects of uterine crowding on reproductive (number of embryos and survivability at gestation D35, litter size at farrowing, birth weight, morphometrics at birth), and lactational performance (average daily gain and survivability up to D21) in cauterized and non-cauterized sows using litter as the experimental unit. Chapter 4 presents data on the offspring's performance from birth to market age and different factors affecting it, fat and loin depth, and ACTH-induced cortisol response in pigs of both groups. The concluding Chapter 5 reviews the important findings of this thesis. Chapters 3 and 4 were written as independent papers submitted for peer-reviewed publication.

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CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Over the last decade, the swine industry has made remarkable improvements in litter size, which is considered of key importance in increasing efficient pork production. Increasing the litter size corresponds to a higher number of live born pigs per sow surviving to market, resulting in higher income for the producer. Cross breeding is also widely used to increase the litter size of commercial dams [1]. Pure breed Yorkshire and Landrace females have been found to have high litter size but crossbreeding increases litter size by 0.3 piglets per litter in Yorkshire (gilt)×Landrace(boar) dams, while decreases by 0.3 with Yorkshire (boar)×Landrace(gilts). Litter size increased by 0.071 total born, 0.048 total live born, and 0.072 of weaned pigs per litter from 1977-1987 [1]. The selection of Nebraska Index line (I) for 19 generations increased litter size by 2.53/live born [2].

Recent PigCHAM^{TM} data shows that in Canada the number of total piglets born per litter is 13.01, live born per litter is 11.61 [3] and that litter size increased by one pig per litter in the last six to seven years. Now the question arises, by increasing the litter size is the profit of pork producers really increased?

Selecting on large litter size alone can create corresponding disadvantages, especially if the larger litters have lighter birth weight. Lower birth weight piglets have significant disadvantages during life compared to heavier born piglets, which include increased mortality, lower lean percent and slower growth rate. This chapter summarizes the pertinent literature pertaining to the physiology of fetal growth, uterine crowding and its effect on fetal and muscle development, postnatal development, and the hypothalamo-pituitary adrenal (HPA) axis.

The search engines used for this review were ISI web of knowledge, CAB abstract, as well as

other non-scientific engines such as Google Scholar. Search terms included, but were not limited to, a combination of following: pig, prenatal programming, intra- uterine crowding, mortality, muscle development, fetus, embryo, stress, HPA axis. References were cited using EndNote Web.

2.2 Methodological Procedures to Study Uterine Overcrowding

The following different methods have been used to study overcrowding and fetal development.

2.2.1 Unilateral Hysterectomy and Ovariectomy

The UHO model is one of the most popular methods used to study the effects of uterine crowding in pigs. Christenson *et al.* used this method to study the effect of ovulation rate on litter size, postnatal survival and the development of progeny [4]. This method has been used in many other studies, for example to determine the effect of intrauterine crowding on conceptus development, embryonic mortality and the hormonal status of fetuses [5]; to study the effect of pre- and post-puberty UHO surgery in gilts on the age of puberty, uterine size and embryo development to D 30 of gestation [6]; and to study the effect of uterine crowding on the survival and development of embryos [7].

In earlier studies, one ovary and the ipsilateral uterine horn were removed from sows between eight and 12 days following estrus [4-6]. In this model, a compensatory ovarian hypertrophy in UHO sows resulted in a similar number of ovulations in both UHO and normal sows [4]. The UHO sows had the same number of embryos but only half of the uterine endometrial surface area available for conceptus development. A crowded uterus was thus created in UHO sows that had similar ovulation rates to normal sows but limited uterine space for fetal development. UHO sows had lower litter size at gestation D86 and farrowed litters with decreased birth weight and higher fetal loss. In another study, the ipsilateral uterine horn was removed at either D130 of age or D12 post puberty to see the effect in pre- and post-pubertal gilts in terms of age of puberty, uterine size and embryo development to D30 of gestation [6]. This study showed that the gilts

with one uterine horn had fewer embryos, less uterine space occupied by each embryo and less total placental surface per embryo. Length and weight of remaining uterine horn, and the age of puberty did not change when UHO was done before puberty but the length of remaining uterine horn was greater in sows when UHO was performed post puberty.

2.2.2 Oviduct Ligation/Resection

In a study by [7] a left oviductal ligature was performed on gilts to study the effect of number of embryos *in utero* on the survivability and development of embryos in ligated and normal sows. This model was also used to study the effect of uterine space on prenatal survival of pigs [8]. Town *et al.*(2005) used unilateral oviduct ligation before breeding in weaned sows to study the effect of uterine crowding on fetal and placental growth and muscle fibre development, and found placental weight and muscle fibre number decreased in fetuses from ligated sows at D30 and 90 of gestation [9].

In the unilateral oviduct ligation model, one oviduct is ligated at both ends either before [10] or after mating [8]. This allows the embryos from only one ovary to enter the uterus and doubles the space available for each embryo. This results in a more spacious intra-uterine compartment, as the number of embryos entering the uterus is approximately halved. Dziuk reported that embryo survival was not higher in ligated sows, possibly due to low ovulation rates in both ligated (7.6) and non-ligated (9.9) sows [10]. Webel *et al.* concluded that increasing uterine space does not necessarily increase embryo survival up to gestation D30, but does after gestation D30.

2.2.3 Superovulation

Superovulation involves the use of exogenous fertility drugs to stimulate the ovaries to produce more ova, which may or may not result in higher fetal numbers. To facilitate **s**uperovulation in sows, Dziuk injected 1,500 I.U. pregnant mare serum gonadotropin (PMSG) followed by 500 I.U. human chorionic gonadotropin (HCG) 96 hrs later [10]. The number of embryos at D25 did

not increase significantly in superovulated gilts, but a few of the superovulated gilts had more than 20 embryos at D25. The researchers concluded that the uterus can accommodate more fetuses than they actually carry at least up to D25.

2.2.4 Embryo Transfer/Superinduction

Embryo transfer in pigs has been widely used as a research tool. It is a process of transferring embryos from the uterus of one sow into another. In a study by Dziuk, estrus was synchronized between donor and recipient sows and each were mated on the same day [10]. The donor embryos were transferred to recipient gilts after 4 days, adding to existing embryos in the recipient sow and thus causing intra-uterine crowding in recipients.

In a study by [11]., embryos from super-ovulated gilts were added to the uteri of recipients 60 hrs after the onset of estrus in donor gilts, which ensured a higher embryo number compared to normal gilts. The embryos were either 2- or 4-cell stage when deposited into the uterine horns above the utero-tubal junction of the recipients. Embryos were transferred to both mated and non-mated gilts. Transfer of embryos to non-mated gilts, helped in determining the survivability of transferred embryos. In this method, uterine space remained same but the number of fertilized eggs increased creating a crowded uterus in the recipient gilts. From this study it was concluded that by increasing the number of embryos, the total number of fetus does not increase at D105 of gestation. However the number of pigs increased by 2.5 at gestation D25 when 11 embryos were transferred into recipients.

2.3 Reproductive Stages and Normal Fetal Development in Pigs

2.3.1 Estrous Cycle

Sows and gilts normally have a 21 day estrous cycle. The estrus cycle of pigs can be divided into follicular and luteal phases. The follicular phase is nearly 5 days long, and starts after D16 of the estrus cycle and ends at ovulation. Follicle stimulating hormone (FSH) stimulates the

development of follicles beginning soon after the ovulation of the follicles of the previous estrus cycle. The growing follicles secrete the hormone estradiol during the period when estrus (heat) is observed, which usually lasts for 24-72 hours. The follicle develops from 4-5mm to an ovulatory diameter of 8-12mm during this period. Estradiol stimulates a surge in luteinizing hormone (LH) that results in ovulation some 35 – 40 hours later. Ovulation normally occurs towards the end of the estrus period, at which time the follicles release their ova (eggs). The ovarian theca and granulosa cells that remain form the corpora lutea (CL), which secrete progesterone and this period of progesterone dominance is termed the luteal phase. The normal CL of 4-5mm develop to maturational CL of 9-11mm in diameter. Progesterone concentrations increase beginning about 24 hours after ovulation, and plateau between 6 and 10 days of the cycle. If fertilization does not occur, the level of progesterone begins to decrease rapidly after 12-14 days and becomes undetectable at D16. An increase in episodic LH secretion occurs in response to the fall in progesterone feedback and stimulates the growth of existing FSH-primed antral follicles to develop as a new group of estrogenic, pre-ovulatory follicles, again resulting in estrus and ovulation [12].

2.3.2 Ovulation Rate

Ovulation rate is of great importance, as it is one determinant of litter size. Ovulation rate differs between breeds and with age or parity of sows. Landrace and Yorkshire gilts had ovulation rates of 13.8 and 15.3 respectively [13]. Christenson studied Meishan (MEIS) and Composite White (CW) crossbred gilts and sows [14] and at puberty the ovulation rate was similar (12.3 ± 0.4 vs 12.7 ± 0.4) between the breeds, but changed with age (D220 of age: MEIS = 16.7 ± 0 .5 vs CW = 12.7 ± 0.4 CL; D280 of age: MEIS = 16.5 ± 0.7 vs CW = 13.9 ± 0.6 CL). In earlier studies, litter size also differed by parity: 9.4, 11.2 and 9.7 for first, second, and third parity, respectively [15]. However, in a recent study increased ovulation rate, did not increase the number of live fetuses, but it impaired fetal and placental development [16]. Town *et al.* also studied sows with higher ovulation (23-25) to evaluate relationships among ovulation rate, pattern of prenatal loss, and embryonic and fetal development during different stages of gestation: D20-30, 50-55 and 85-90. They did not find any relationship between ovulation rate and number of live embryos at D20-30

or 85-90 of gestation. Embryonic and fetal survival was negatively related with days of gestation. Embryo/fetal survival were 62% at D20-30, 50% at D50-55 and 49% at D85-90. Embryo/fetal survival was higher in sows of parity 2-3 compared to parity 1 and with parity more than 4 [17].

2.3.3 Fertilization, Embryonic and Fetal Development

Fertilization usually occurs in the proximal part of the oviduct and the zygote is formed. About 2 days after fertilization, the pig embryo enters the uterus. They are in the 4-8 cell stage and begin to space themselves equal distances apart in both uterine horns between D6 and D12 [18]. By D11, embryos attach to the uterine lining and implantation occurs by 18 days. The ectoderm, mesoderm, and entoderm are formed by this time, from which different tissues and organs are formed [19]. Each embryo is surrounded by a placenta which helps to protect and nourish the growing embryos [19]. Pigs have diffuse/epitheliochorial placentation with countercurrent materno-fetal blood interrelationship between fetus and mother. The interlocked blood circulation between fetal ridges and maternal ridges is separated by maternal troughs of variable depth [20]. UHO resulting in small fetus causes increased microscopic fold width, increased length of the placental epithelial bilayer per unit length of the placenta [21]. This causes the increased blood flow and helps to overcome the nutrient deficiency due to overcrowding.

2.3.4 Factors affecting Embryonic and Fetal Survivability

Several factors have been found to affect fetal development and survivability.

2.3.4.1. Sow Nutrition

The sow's nutritional status affects fetal growth, as nutrients from the dam are supplied to the fetus via the placenta. Robinson *et al.* reviewed nutritional effects on fetal growth in cattle, sheep and pigs, and concluded that during pregnancy, either in the early or later stages, maternal diet influences fetal growth [22]. This can be a direct effect by altering the supply of essential nutrients, or act indirectly by stimulating both the maternal and fetal endocrine systems

responsible for maternal nutrient utilization by the fetus. In another experiment, sows provided with limited feed (inanition) for 10 days before mating and inanition continued after mating. One-hundred percent of sows maintained their pregnancy until D14, which gradually decreased to 72% by D18, and was decreased to 33% when inanition was continued to D31 of gestation. Embryo survival rate also decreased in the restricted animals compared to animals on full feed. Inanition in animals 10 days before mating also caused a reduction in ovulation rate by 2 [23]. More recently Wu *et al.* [24] have shown that both maternal under-nutrition and over-nutrition causes impaired fetal growth. Wu *et al.* indicated that under-nutrition was associated with placental insufficiency which reduces the transfer of nutrients from mother to fetus, and results in competition among the fetuses for nutrition, causing IUGR. Placental inefficiency in the synthesis of nitric oxide (a major vasodilator) and other polyamines, causes IUGR in response to nutritional under- and over-nutrition [24]. Furthermore, sows that were restrict fed during the last week of lactation (D14-D21) had lower embryonic survivability [25].

2.3.4.2. Sow Health and Condition

The overall condition of the sow contributes to the developing fetus. A recent study in Camborough sows demonstrated the effect of body reserve (backfat) at weaning and its utilization during lactation on second parity litter size [26]. The study found that body fat reserves at farrowing and weaning, accompanied by lower weight loss during lactation improves second parity litter size. Foxcroft *et al.* studied the metabolic state of the sows in peri-ovulatory period. They found that the nutrient restriction during the peri-ovulatory period decreases embryonic survival at gestation D30 [25].

2.3.4.3. Uterine Space

The effect of uterine crowding on embryonic survival varies according to the period of gestation. A study was performed to define the stage of gestation when uterine capacity limits the embryo survival in gilts [27]. In the experiment, additional embryos were transferred to the uterus of

pregnant recipients at gestation D7 and both control and recipient animals were slaughtered at gestation D21 to D35. It was found that uterine capacity limits embryo survival between gestation D7 to D25. In another study, Chen and Dziuk found that crowding affects the prenatal survival before D17 and then between D29 and D35 [28]. Wu *et al.* also studied the effect of limited uterine space on embryo and fetal survivability by restricting the uterine space available for each embryo [29]. It was found that the restriction of uterine space to 5 cm reduced embryo survival to 8% at gestation D50. When uterine space was increased to 30cm, fetal survival increased to 52%. Town *et al.* also performed a study using 30 unilateral oviductal-ligated and 30 control sows [9]. They found that the number of viable embryos at gestation D30 and D90 was higher in non-ligated sows, and that embryo survival was higher in the ligated sows. Moreover, increasing the number of potential embryos in the uterus by super-induction decreased embryo survivability but did not change the litter size at D25 and D105 of gestation [11].

Contradictory results to above studies were reported by [30]. Meta-analysis was performed on data obtained from 78 publications pertaining to the relationship between the average number of CLs and prenatal survival rate. It was found that there was no influence of CL number on prenatal survival before D35 of gestation, but there was a negative relationship after D35.

In a study by Ashworth *et al.*, within litter variation in embryo weight and inadequately grown embryos were found by gestation D30, however, the number of inadequately grown embryos remained the same until parturition [31]. The authors concluded that inadequate intrauterine space only partly contributes to reduced fetal growth. Christenson *et al.* found that piglet birth weight decreased with increased litter size, and that higher fetal losses occurred in larger litters [4]. In this study, intact and UHO gilts were used to study the effects of ovulation rate on fetal development and postnatal survival. Laparoscopy was performed on gestation D40 to count CLs, and the pregnant females were slaughtered at D86 of gestation or allowed to farrow naturally. The results showed that fetal loss was higher in UHO compared to intact sows between gestation D86 and farrowing.

2.3.4.4. Placental Area

Pigs have a non-invasive epitheliochorial placenta that apposes the circulation of the the developing fetus to the maternal vasculature in the uterine wall to facilitate nutrient uptake, gas exchange and waste elimination via the dam's blood supply. As the placenta transfers nutrients to the fetus, it plays a vital role in fetal survival and development. Fetal losses later in gestation may be due to intra-uterine competition for the establishment of an adequate placental surface area required for nutrient exchange between the fetal and maternal circulations [11]. In other research using intact and UHO gilts to study the conceptus development, Knight *et al.* found higher placental weight and length in intact gilts, and a period of rapid growth in placental length between gestation D20 and D30. Fetal weight was significantly higher in intact gilts after gestation D40. A strong correlation between fetal weight and placental length ($r = 0.64$) and placental surface area ($r = 0.72$) indicated that intra-uterine crowding is associated with decreased endometrial surface area which causes reduced placental growth. This also caused higher fetal mortality and decreased fetal growth [5]. The study of Bazer *et al.* found that early pregnancy is dominated by the more viable embryos that are better able to compete for biochemical factors [11] required for their development . This leads to the sacrifice of less viable embryos. In another study uterine crowding was found to decrease placental volume and placental weight [32]. Due to the lack of compensatory placental development in swine, uterine crowding critically affects embryo development and survival of the embryos.

2.3.4.5. Location in Uterus

During uterine migration, embryos become randomly located in the uterus [33]. Consequently, fetal growth may eventually be influenced by their location in the uterus if nutrient supply to a given fetus is limited. Gilts were slaughtered at D0, D45, D60, D75, D90, D102, and D110 of gestation to find the relationship between intra-uterine location and fetal tissue growth at different stages of gestation. Fetal weight, fetal carcass weight, and the weight of GIT, liver, heart, lung, spleen and kidney decreased when the location of fetus changed from cranial to cervical, regardless of day of gestation [19], implying that the sows in this study had a limited

ability to supply those fetuses at the cervical end of the uterine horn.

2.3.4.6. Fetal Gender

Male and female fetuses have been found to grow differently. Male fetuses were heavier relative to their placenta compared to female fetuses from D70 of gestation [34]. This study also suggested that the sex of a neighboring fetus affects fetal size. It was found that a fetus surrounded by opposite sexed fetuses was lighter than those surrounded by same sex fetuses. By contrast, Kaminski et al. [35] transferred embryos between Yorkshire and Meishan females, and found no effect of sex on embryo development at gestation D12 nor on DNA, protein or estradiol content of the embryo.

2.4. Birth Weight and Postnatal Performance

With increasing litter size, mean piglet birth weight decreases [36]. Litter size is also dependent on parity or age of sow. First and second parity sows produce lower litter sizes compared to higher parity sows, and the number of stillborn pigs increases with higher parity. Mean birth weight of live born piglets is higher in higher parity sows compared to gilts [36]. The pre-weaning survival of piglets decreases with increasing litter size. Piglets from larger litters and of low mean birth weight had lower weaning weights compared with pigs from smaller litters of higher mean birth weight [37]. Pigs with higher birth weight had higher body weight gain during lactation and lower pre-weaning mortality (16%).However, the small birth weight piglets were able to catch up in growth with larger piglets to some extent during this period. The lighter birth weight piglets took a longer time to gain weight than their heavier counterparts after weaning, and thus took longer to reach market weight [38]. Recently, Fix *et al.* (2010) found that higher birth weight resulted in higher weaning weight and post weaning ADG in piglets [39]. In studying the effects of weaning weight on subsequent performance Wolter *et al.* [40] found that heavier pigs at weaning were heavier at birth and remained heavier throughout the life. Accelerated growth in those pigs for 14 days post weaning however, did not increase ADG in the

grow finish period. Lush *et al.* studied factors affecting birth weight from 506 litters with 3639 pigs from gilts [41]. He found that larger litter size decreased birth weight by reducing the rate of fetal growth. Breed, environmental conditions such as weather, feed type and quality, management, and seasonal variation are also responsible for affecting the birth weight [41]

2.5. Prenatal Programming and Muscle Development

2.5.1. Types of Muscle Fibre

The functional unit of muscle is the muscle fibre. Muscle fibres are classified as: type I or slow twitch oxidative (SO) fibres which help in the maintenance of posture and for endurance exercise; and type II or fast-twitch fibres that are used for more rapid bursts of activity. On the basis of contractile and metabolic activities, fast-twitch fibres are subdivided into at least two classes: fast twitch glycolytic (FG) and fast-twitch oxidative glycolytic (FOG) [42]. FG fibres have a high speed contraction in anaerobic metabolism while FOG fibres have high speed contraction in both aerobic and anaerobic metabolism

2.5.2. Muscle Fibre Development

The production of fetal muscle occurs in two phases, with formation of primary and secondary muscle fibres. Primary fibres are formed by the fusion of primary myoblasts. These primary fibres are formed during early fetal development, around gestation D38 in the pig [43]. With the completion of primary myofibre formation, secondary myofibres form on the surface of primary fibres [42, 43]. Primary fibres are used as a surface for the attachment and fusion of myoblasts forming secondary fibres. Enlargement of primary fibres during secondary fibre formation provides greater surface area for the attachment and fusion of myoblasts forming secondary fibres. Smaller primary fibres in a small fetus may result in a lower number of secondary fibres forming on their surfaces. Thus, the more the myofibril free space in primary fibre, the more secondary fibres it can accommodate. In the fetal pig, secondary muscle fibre hyperplasia begins

at approximately D50 of gestation and is completed by D90 [43]. Primary fibre number is not susceptible to environmental influences but the development of secondary muscle fibres is influenced by several factors such as nutrition, hormones, and the environment [42, 43].

2.5.3. Effect of Uterine Crowding on Muscle Fibre Development

Town *et al.* used unilateral oviductal ligation surgery to reduce the number of conceptus growing in the uterus [9]. The higher number of fetuses in the non-ligated gilts resulted in impaired fetal and placental growth. There was also a reduction in the number of secondary muscle fibres in fetuses of non-ligated gilts. Tse *et al* [44] also studied the effect of uterine crowding on expression of two myogenic regulatory factors, myogenin and myoD, and showed that uterine crowding impaired the differentiation of muscle fibres through a reduction in myogenin expression at gestation D30. IUGR also affects the proteomes of skeletal muscle responsible for the regulation of cellular signaling, immune response, oxidative defense, and intermediary metabolism, ultimately resulting in impaired development in IUGR pigs [45]**.**

2.5.4. Early nutrition and muscle development

L-carnitine supplementation to piglets during suckling results in an increased number of myofibres in the semitendinosus muscle of low birth weight, but not in midbirth weight piglets. DNA concentration and DNA:protein ratios increased in semitendinosus muscle in response to L-carnitine in low birth weight piglet. Fibre area, protein concentration and creatine kinase (CK) activity, a marker of myogenic differentiation, were not reduced demonstrating that myogenic proliferation was increased with L-carnitine feeding. The authors have described this phenomenon as compensatory growth of muscle fibres due to a third fibre generation in low birth weight piglets at W4 [46]. Further study needs to be done on the existence and function of tertiary fibres.

Increased maternal nutrition during gestation also causes increased secondary:primary ratio and

higher growth rate in pigs from D70 until they were 80kg weight [47].

2.5.5. Muscle Fibre and Postnatal Growth

Total number of primary and secondary muscle fibres is determined before birth and postnatal growth in muscle mass is due to hypertrophy of the existing muscle fibres [43]. Dwyer *et al.* has shown that pigs with higher muscle fibre number grow faster than pigs with lower muscle fibre number [48]. In another study, UHO sows were assessed to understand the impact of intra-uterine crowding (IUC) on the muscle development of piglets at birth [49]. It was found that IUC causes a reduction in hyperplasia of secondary and total myofibres in the semitendinosus and psoas major muscles. In another account, piglets of low birth weight had a decreased number of myofibres in the semitendinosus at slaugher compared to mid- and high birth weight piglets [50].

Rehfeldt *et al.* found that low birth weight piglets have a higher proportion of internal organs, bones and skin but lower proportion of muscle than high weight piglets [51]. Piglets with low birth weight have a lower number of muscle fibres that demonstrate accelerated hypertrophy in postnatal growth. This excessive fibre hypertrophy results in the formation of giant fibres in low birth weight pigs. Enlarged myofibres accompanied by lower fibre number is found to decrease pH, and increase the drip loss causing lower tenderness in pork meat at market age [50]. In a similar study by Gondret *et al.* low birth weight pigs had higher muscle fibre number and cross sectional area compared to high birth weight pigs [52]. These discrepancies resulted in lower feed conversion ratio, lower meat content and higher subcutaneous fat in lower birth weight pigs.

2.6. Prenatal Programming and Fat Deposition

Low weight pigs had higher backfat thickness and perirenal fat, resulting in lower carcass quality [53] [50].

2.6.1. Causes of Altered Fat Deposition

The higher fat accumulation in low birth weight pigs is due to differences in lipogenic enzyme activity, duration of fat accumulation, and physiological maturity between high and low birth weight pigs. For example, higher fat deposition in low birth weight pigs is associated with the early attainment of postnatal growth plateau, after which dietary energy is mainly used for fat deposition [53]. In addition, with higher lipogenic enzyme activity, low weight pigs had higher potential for fatty acid synthesis compared to high birth weight pigs [50].

Appetite programming may also occur in animals experiencing intrauterine growth retardation, resulting in hyperphagia and higher fat deposition in later life in low birth weight pigs. This is because environmental conditions during intra-uterine and early postnatal life affects hypothalamic neurogenesis and axonal outgrowth which have long term consequences for appetite and metabolism [54].

Altered fat deposition may also be due to the programming of the endocrine axis during prenatal life. Leptin, a hormone secreted by fat cells, plays a role at the level of the hypothalamus in controlling body fat stores through regulation of the fat-brain axis. Specifically, leptin, an appetite regulating hormone produced in peripheral tissues, interacts with its receptors in the hypothalamus to inhibit food intake [55]. IUGR due to nutrient deprivation causes leptin resistance in offspring, which inhibits satiety and leads to large amounts of triglyceride storage when food is plentiful. Leptin resistance therefore creates a competitive advantage for IUGR offspring in preparation for nutrient deprived environments by facilitating the storage of as much fat as possible when food becomes available [55].

2.7. Prenatal Programming, Asymmetrical Fetal Growth and Stress Response

The association between fetal growth and adult diseases is linked to hyperactivity or resetting of the hypothalamic- pituitary adrenal (HPA) axis in response to stress in prenatal and early postnatal life [56]. Prenatal maternal stress during pregnancy causes long-term alteration of the

HPA axis [56], behaviour, growth performance, and immune function [57] in later life. This leads to increased fetal exposure to glucocorticoid (GC). GC affects growth and development of individual fetal tissues and organ systems, pre-partum tissue maturation, and fetal adaptations involved in the transformation of a fetus from an intra- to extra-uterine life [56, 58]. GCs have a very wide range of functions during prenatal, as well as post-natal, life [59]. In prenatal life, GC induces the maturation of different tissues that are necessary for the adaptation of life immediately after birth. GC helps lungs prepare for gas exchange after birth, helps in the synthesis of different enzymes receptors and growth factors, increases the deposition of glycogen in fetal liver, and also suppresses the activity of insulin like growth factor (IGF) II. Thus, prenatal GC exposure resets various endocrine systems, including the somatotrophic and HPA axes, which in turn may contribute to the pathogenesis of adult disease.

Intrauterine crowding also leads to early activation of fetal HPA axis, resulting in elevated fetal plasma cortisol concentrations before term. This causes premature differentiation of cells and also results in an asymmetric pattern of organ growth [59]. The fetal cortisol surge normally only occurs during the late phase of gestation when tissue maturation occurs. In response to stressful conditions however, the fetal HPA axis is triggered early and causes a premature surge in fetal cortisol [59]. Prenatal cortisol surge initiates a switch in the somatotrophic axis causing the suppression of IGF. This causes fetal growth retardation and switches fetal cells from proliferation to maturation resulting in inappropriate differentiation of tissues for the age of the fetus. This leads to asymmetrical growth of the fetus.

Asymmetrical fetal growth occurs in pigs experiencing intra-uterine crowding. Restriction in the placental exchange surface may result in fetal hypoxemia. Since the brain maintains its oxygen consumption even when oxygen availability is decreased [60], it continues to develop in spite of limited nutrient supply. This result in the preferential growth of brain tissue at the expense of all other organs, a phenomenon called “brain sparing”.

2.7.1. Programming of the HPA Axis

The HPA axis relies on a complex set of interactions between the hypothalamus, pituitary gland and adrenal gland. Fetal exposure to poor circumstance as intra-uterine crowding alters the set point of the HPA axis, which leads to increased post-natal HPA axis activity and subsequently increases cortisol levels [56].

The HPA axis is controlled by a negative feedback system in which GC released into the circulation by the adrenal gland interacts with GC receptors (GRs) located in the pituitary, hypothalamus and hippocampus. Over activity of the adrenal gland within this pathway results in a negative feedback and reduced corticotrophic releasing hormone (CRH) release [61]. Kanitz E. *et al.* showed that pregnant sows subjected to a restraint stress (i.e. nose snaring) for 5 minutes daily during the last 5 weeks of gestation demonstrated significantly higher numbers of GRs in the hippocampus and decreased GRs in the hypothalamus of offspring when assessed at 1 day of age [62]. This showed that programming of HPA function involved modification of the glucocorticoid negative feedback mechanism.

2.7.2. Sources of Fetal Cortisol

Cortisol, produced by adrenal cortex in response to ACTH released from the pituitary, plays a crucial role in fetal development. Cortisol is responsible for fetal organ maturation and preparation of the fetus for the external life, and cardiovascular regulation [58]. Fetal cortisol is derived from the fetal adrenal gland and maternal cortisol. High fetal exposure to cortisol is associated with reduced fetal growth. This may occur due to increased maternal plasma cortisol concentrations, increased adrenal cortisol output by fetus, or decreased placental 11beta hydroxysteroid dehydrogenase (11βHSD2) activity [58].

2.7.2.1. Maternal Cortisol

Maternal cortisol significantly contributes to circulating fetal cortisol [63]. Klemcke found that maternal cortisol contributed 23% of total fetal cortisol at D50 of gestation, and only 6% of total fetal cortisol at D100 of gestation [63]. This confirmed that maternal cortisol enters the fetal compartment. The enzyme 11βHSD2 is a critical component of the placenta that helps the fetus to both regenerate and deactivate GC. It converts biologically active cortisol to inactive cortisone, thus regulating GC exposure to target fetal tissues [64]. This mechanism protects the fetus from high concentrations of maternal GC. In another study, McNeil *et al.* found that plasma cortisol level at D45 and D100 of gestation in the porcine fetus differed between small and average sized siblings from the same litter but maternal cortisol level was similar at both the time. This suggests that fetal cortisol production rather than maternal supply is responsible for the cortisol concentration difference. [65].

2.7.2.2. Fetal Adrenal Gland

The fetal adrenal gland, regulated by the HPA axis, is the primary source of circulating GC once it is activated in late gestation [58]. Klemcke *et al.* found that fetal HPA axis cortisol contributed 77% of the total supply of fetal cortisol at D50 of gestation, which increased to 94% by D100. This shows that the fetal adrenal gland is the major source of fetal cortisol in late gestation [63].

2.7.2.3. Factors Affecting Alteration of HPA Axis

Several factors affect the HPA axis in prenatal and postnatal conditions. Some of the factors are maternal undernutrition, and endogenous and exogenous GC exposure.

2.7.2.4. Maternal Undernutrition

Human and animal studies provide nutritional explanations for the links between low birth weight and adult diseases. By restricting the diet of pregnant dams, Navarrete *et.al* showed that undernutrition in fetal life of rats resulted in increased plasma cortisol in 40 days old pups. This may be due to programming of glucocorticoid secretion in the pups associated with prolonged maternal undernutrition resulting in adrenal hypertrophy. By contrast, subcutaneous administration of dexamethasone (DEX) led to decreased plasma corticosterone, a dominant glucocorticoid in rodents, at D40 of age [66]. It is believed that prenatal undernutrition affects the HPA axis by decreasing expression of hypothalamic and pituitary GC receptors, thereby decreasing negative feedback mechanisms and increasing plasma cortisol concentrations.

2.7.2.5. Exogenous Exposure to Glucocorticoids

The use of exogenous corticosteroids in women for maternal or fetal indications during pregnancy has raised concerns about their potential programming effects on the fetal HPA axis. In swine, repeated administration with exogenous ACTH during late gestation in sows caused adrenocortical activation with increased plasma cortisol in sows until 8 hrs post application [57]. Cortisol concentration in the umbilical vein of fetuses from ACTH treated sows were lower, reportedly due to increased conversion of cortisol to cortisone by placental 11β-HSD2 [57]. ACTH administration to gilts in mid gestation caused an increase in circulating cortisol concentrations in gilts and in their fetuses 3 hours after injection. The authors indicate this was due to the passage of maternal cortisol to the fetus during mid gestation [67]. In an experiment by Haussmann *et al.*, prenatal stress in the form of restraint, and weekly ACTH administration to pregnant sows, resulted in decreased negative feedback effect of cortisol on the fetal hypothalamus. This resulted in hyperactivity of the HPA indicated by increased secretion of CRH, pituitary gland mass, cortex to medulla ratio, ACTH receptor mRNA expression, and prolonged cortisol release in response to stress [68]. This showed that alteration of the fetus HPA occurs following exogenous exposure to glucocorticoids. Klemcke *et al.*, demonstrated that the birth weight of pigs from UHO sows was negatively related to adrenal weight, plasma cortisol

and cortisol binding globulin [69]. This indicated that the adrenal function is initiated prenatally and that stress during fetal development results in a permanent modification of HPA axis.

2.8. Hypothesis and Relevance of our Research

This study of prenatal programming using a model of uterine uncrowding is important in order to determine if the high litter sizes occurring naturally noted in modern genotypes affects postnatal performance of the offspring. This research assumes that pigs born to a relatively crowded uterus are programmed during prenatal development. In this research, a relatively non-crowded uterus was created in a group of multiparous sows by unilateral oviduct cauterization surgery. By contrast, higher numbers of embryos are present in non-cauterized control sows compared to CAUT sows. Though the CTRL sows used in this study were normal in terms of their reproductive performance in the Canadian commercial swine industry, we were interested in investigating their offspring relative to the offspring of CAUT sows that were reared in relatively less crowded uterine environments. The higher number of embryos in CTRL sows may be associated with higher embryo loss and the restricted development of surviving live embryos. We hypothesized that due to impaired fetal development, the pigs surviving to term will be lighter and smaller at birth, which may further lead to smaller pigs at market age. We also hypothesized that the post-natal HPA axis would be altered in pigs of non-cauterized sows. Thus when stimulated with exogenous ACTH, the pigs from relatively crowded uterine conditions will express a faster and higher cortisol response than progeny from non-crowded or cauterized sows.

As litter bearing species, pigs are ideally used in models studying the effects of pre-natal programming. In modern pork production, increased ovulation and embryonic survival rates have potentially created crowded uterine environments that potentially impair fetal development. Thus, the large litter sizes of modern sows provides the opportunity to study and compare the physiology of high and low birth weight piglets within litters. Moreover, there are large variations in the average litter birth weight between litters of the same size [70] which provides the opportunity to study the consequences of relatively crowded intrauterine environments as was done in this experiment. Pigs share an evolutionary resemblance with humans. Similarities

in anatomical, physiological, immunological and nutritional states between pigs and human has been found in various organ system including the cardiovascular, digestive, epidermal and renal system [71]. Therefore, research in this area is applicable to both agriculture and human health.

The relevance of this research is directly related to pork producers wanting to achieve optimal neonatal development and postnatal performance. Producing a healthy pig with greater potential for postnatal performance is very important to improve income and efficiency in the pork industry. This research may lead to a better understanding of what is needed to reduce losses due to postnatal mortality and poor growth performance. Some aspects of how uterine crowding affects prenatal survival and development is previously reported in pigs, however much still needs to be determined about the effects into postnatal life. It will also be helpful to determine, what maternal traits should be considered when selecting replacement gilts for commercial farms in order to minimize the programming effects on postnatal performance. This research may also be useful for human medicine as the ACTH challenge described herein in pigs, may prove that pigs are a good model for studying the prenatal programming of postnatal metabolic and infective diseases. Due to the immunosuppressive effects of GC, an exaggerated adrenal response may result in increased levels of disease susceptibility. This may also give the new insights into the early life programming of postnatal health in humans.

2.9. Specific Objectives

The objectives of this thesis were to determine the effect of intra-uterine crowding on: i) prenatal development, embryo and fetal survival; ii) sow performance, specifically litter birth weight and litter size; iii) piglet performance, specifically birth weight, body weight and average daily gain during lactation, and the nursery and grow-finisher stages of prodcution; iv) carcass quality, specifically backfat and loin muscle thickness between 65 and 80kg and 105-115 kg body weight; and v) the adrenal cortisol response in offspring after ACTH injection at W12 and W20 of age.

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CHAPTER 3

INTRAUTERINE CROWDING IN MULTIPAROUS SOWS ADVERSELY AFFECTS PRE-NATAL DEVELOPMENT, SURVIVAL, AND POST-NATAL LACTATIONAL PERFORMANCE

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ABSTRACT

Twelve control (CTRL) and 13 unilateral oviductal cauterized (CAUT) sows were used to determine the effects of uterine crowding on pre-natal development and post-natal lactational performance using litter as the experimental unit. Ovulation rate, number of embryos, and embryonic survival rate were recorded during a laparotomy performed at gestation D 35. Piglet weight and morphometrics were measured at birth, and pre-weaning survival and average daily gain were recorded up to 21 days of age (ADG_{LACT}). CAUT sows had fewer embryos (CAUT = 9.1 ± 3.0, CTRL = 15.3 ± 4.3; *P* = 0.001) at gestation D35 than CTRL. Uterine crowding in CTRL resulted in lowered embryo (CAUT = 66%, CTRL = 53%; *P* = 0.04) and fetal (CAUT = 59%, CTRL = 42.4%; *P* = 0.007) survival rates, higher total borns (CAUT= 7.9 ± 2.9, CTRL= 11.2 ± 3.23; *P* = 0.002) birth weight (CAUT = 1.7 ± 0.3 kg, CTRL = 1.4 ± 0.2 kg; *P* < 0.001) and body mass index (CAUT = 0.18, CTRL = 0.17; *P* = 0.04) at birth. Adrenal weight (CAUT = 0.5 gm, CTRL = 0.3 gm; *P* = 0.005) and adrenal:brain weight ratio (CAUT = 0.02, CTRL = 0.01; *P* = 0.009) in dissected stillborns (n = 17) were lower in CTRL compared to CAUT, which demonstrated brain sparing associated with intrauterine growth retardation. ADG_{LACT} (CAUT = 0.300 ± 0.04 kg/d, CTRL = 0.270 ± 0.04 kg/d; *P* = 0.01) and pre-weaning survival (CAUT = 92.9%, CTRL = 82.1%; *P* = 0.01) were significantly lower in CTRL in spite of judicious cross-fostering performed on D1 to even out litter sizes between groups. Despite having only one "functional ovary "the total litter weight at lactation D21 in CAUT sows was not different from CTRL litters. We conclude that uterine crowding negatively affected pre-natal growth and development and reduced pre-weaning piglet weight gain and survival.

(Key words: Fetal development, swine, prenatal programming, uterine crowding, intrauterine growth retardation (IUGR))

3.1 Introduction

Studies have shown that uterine capacity is a limiting factor for fetal development and litter size at term [1]. Uterine overcrowding in early gestation reduces fetal weight, impairs myogenesis and organ development [2] and may increase the incidence of cardiovascular and metabolic disease in later life [3]. In addition, uterine crowding may result in the hyperactivity or resetting of the hypothalamic-pituitary-adrenal axis, which may indirectly affect the stress and inflammatory responses in postnatal life [4]. In pigs, uterine capacity is set as early as D11 to D13 of gestation [5] but only affects litter size after gestation D30 [5, 6]. Intra-uterine crowding is a limiting factor for litter size born when the embryo number in sows exceeds 14 [7]. Most embryo losses occur during the first month of gestation, and there are separate mechanisms that control pre- and post-implantation losses [1]. For example, some sows have high embryonic survival during the first month of gestation which in combination with the high ovulation rates in modern genotypes, results in excessive numbers of fetuses surviving post-implantation and possibly to term [8]. Although post-implantation and late gestational losses occur and, act to reduce intra-uterine crowding, fetal and placental growth fails to compensate for growth restriction occurring in earlier stages of gestation. This inevitably leads to entire litters that are affected by intra-uterine growth retardation (IUGR) [9]. The competition between developing conceptus for essential biochemical factors, and surface area for nutrient exchange controls embryo survival [10] and subsequently affects fetal survival [2].

Past studies on uterine crowding using alternate methods to alter uterine capacity were terminated during gestation [2] or immediately after farrowing [11]. Our goal in the present experiment was to evaluate the effects of uterine crowding on prenatal development and survivability, birth weight, and on post-natal performance using litter as the experimental unit. We studied the offsprings from birth to W19, and report pre-natal and pre-weaning performance herein. The experiment is based on the hypothesis that piglets from the less crowded (unilateral oviductal cauterized; CAUT) litters will exhibit superior performance compared to piglets from control (non-cauterized; CTRL) litters. The specific objectives were to determine the impact of

unilateral oviductal cauterization on embryo number at gestation D35, litter size at farrowing, organ:brain weight ratios (brain sparing) in available stillborn piglets, average litter weight and body mass index at birth, pre-weaning survival and average daily gain from birth to D21 in live born piglets.

3.2 Materials and Method

The experiment was conducted in a 300 sow farrow-to-finish farm in accordance with the University of Saskatchewan's Committee for Animal Care and Supply (permit #2008005). Twenty-five PIC Line 42 (Camborough Plus) sows of parity 4-8 were selected for the experiment. Each had 10-17 totals born in their most recent farrowing and was in good health and body condition at weaning. The selected sows were randomly divided into CTRL and CAUT at weaning. Groups (GRP) were balanced and blocked by weaning week and parity. After weaning, the selected sows were kept in individual pens and fed 2.5 to 3kg feed per day. Fourteen days post-weaning, a right oviductal cauterization surgery was performed on 13 CAUT sows. No surgery or placebo treatment was performed on the 12 CRTL sows which although was a limitation of the study,was not performed in keeping with the 3R's of animal care.

Sows were weighed, removed from feed, and transferred to a single-animal pre-operative pen adjacent the surgical theatre one day before surgery. Right oviductal cautery surgery was performed on two sows each surgical day.

3.2.1 Anesthetic Procedures

The sows were moved into an induction stall and sedated using a combination of atropine (0.04mg/kg IM, ATRO SA, RAFTER8 Products, Calgary, AB), azaperone (4mg/kg, IM, Stresnil, Merial Canada, Baie D'Urfé, QC), and morphine (0.1mg/kg IM, M.O.S. 20, ICN Ltd, Montreal, QC) 30 minutes prior to induction. A 21g butterfly catheter was inserted into an ear vein and an extension set attached. All sows were induced with thiopental sodium (Thiotal,

Vetoquinol Canada, Lavaltrie, QC) using a 6mg/kg IV bolus as initial dose. If required for intubation, sows were topped up with an additional 2mg/kg thiopental sodium IV. To accommodate an anesthetic research experiment [12], sows were randomly allocated to receive immediately following induction a paralytic agent: rocuronium (0.6 mg/kg IV, Zemuron, Organon Canada, Scarborough, ON) or succinyl choline (1.0 mg/kg IV, Quelicin, Abbott Canada, St. Laurent, QC), or an equivalent volume of saline placebo. Forty-five seconds later, sows were intubated with a 14 mm endotracheal tube with the assistance of a laryngoscope and flexible stylette. Anesthesia was maintained with isoflurane (Isoflo, Abbott Canada) using a closed-circle anesthetic circuit and positive pressure ventilation. Prophylactic oxytetracycline hydrochloride (20mg/kg IM, Biomycin 200, Boehringer Ingelheim, Burlington, ON) was administered at induction.

3.2.2 Right Oviductal Cautery Surgery

Anesthetized sows were positioned in dorsal recumbency. The ventral abdomen was clipped, surgically prepared and draped for aseptic surgery. A 12g teat cannula was placed into the abdomen at the umbilicus and the abdomen insufflated with CO_2 to a pressure of 10 to 15 mm Hg. When the abdominal wall was distended, a 1 cm incision was made through the skin at the umbilicus, and a trochar-cannula assembly passed through the abdominal wall. A 10 mm diameter 30^{o}telescope was inserted to allow visual observation of the abdominal cavity, ovaries and uterine horns. One cm skin incisions were made 15 cm caudal and 10 cm lateral to the umbilicus on either side of the midline to allow placement of 11 mm diameter instrument cannulas. An atraumatic right-angle grasping forceps was used to locate and manipulate the right oviduct. The ovary was located by following the round ligament following it in a hand-over-hand fashion. Using a bipolar cautery instrument, the right oviduct was coagulated then severed with an endolaparoscopic scissors. A 0.5 to 1.0 cm section of oviduct was removed and placed in 10% buffered formalin for subsequent confirmatory histopathologic examination. At the end of the surgery all instruments were withdrawn and cannulas removed following the evacuation of carbon dioxide from the abdomen. Each incision site was closed using a simple continuous suture using 1 PDS II, (Ethicon Inc., Markham, ON) placed in the linea alba or the external

rectus fascia followed by subcuticular skin sutures (2/0 Monocryl, Ethicon Inc.). Flunixin meglumine (2.2mg/kg IM, Banamine, Intervet-Schering Plough Animal Health Canada, Kirkland, QC) was administered during surgery for pain control.

3.2.3 Post operative Procedures

Following surgery, anaesthetized sows were returned to their preoperative holding pen and placed on a rubber mat. The room temperature was increased to 25 ^{o}C to help prevent hypothermia. The sows were transferred to comfort pens in the gestation barn the day following surgery and offered food. The animal's recovery was assessed daily for 5 days, and appropriate intervention taken as required. Pain was assessed every 24 hours by palpation of the incision site, and additional flunixin meglumine was administered when necessary.

3.2.4 Breeding Procedures

All sows were vaccinated for parvovirus, leptospirosis and erysipelas (Farrowsure B, Pfizer Animal Health, Kirkland, QC) 1-2 weeks prior to anticipate breeding. Sows were kept in individual pens and heat synchronized using altrenogest (20 mg/sow/day, Regu–Mate, Intervet-Schering Plough Animal Health, Kirkland, QC) fed orally for 14 days. Animals were artificially inseminated using pooled PIC337 semen once daily for up to 3 consecutive days beginning on their first day of standing estrus after the withdrawal of Regu-Mate. Sows were confirmed pregnant at gestation D 25 using real-time ultrasound (Agroscan A7, ECM, France). Non-pregnant sows (CAUT = 8, CTRL = 9) were rebred on their next observed estrus. At gestation D50, P2 backfat (BF_{D50}) was measured in sows using the Renco Lean-Meater (**Renco** Corp., Minneapolis, MN) on the right lateral flank, 5cm off midline over the last rib.

3.2.5 Embryo Counting Surgery

Embryo count surgeries were performed between D32 and D39 of gestation. Preoperative**,** anesthetic, and pre-surgical procedures were identical to those described above. Sows were positioned in dorsal recumbency and an area bordered by the sternum, inguinal canal, and mammary chain was surgically prepared. When anesthetized, a 20-30 cm incision was made through skin and underlying tissue along the ventral midline, caudal to the umbilicus. The tip of one uterine horn was located and gently drawn towards the incision and exteriorized. Starting at one uterine tip, the uterus was palpated and the probe of a real-time ultrasound (Shimatzu SDU 350 XL, Universal Medical Systems, Bedford, NY) inserted within a sterile sleeve, was placed on the uterus directly over each embryo. Embryos with visible heart beat were considered viable. This procedure was repeated on the opposite uterine horn until all embryos were counted. The number of CL's was counted on each ovary, and the embryonic survival rate calculated. The uterus was rinsed with sterile saline, and returned to the abdomen. The linea alba was closed with a simple continuous suture pattern using 1 PDS II. The subcutaneous tissue was opposed with simple continuous suture using 2/0 Monocryl. A subcuticular suture using 2/0 Monocryl was used to close the skin. An adhesive skin drape (Opsite Incise Drape, Smith & Nephrew, Hull, UK) was applied to the skin over the incision site to prevent external contamination of the incision site. For pain control two 100 mcg/hr fentanyl patches (RAN-Fentanyl Transdermal System, Ranbaxy, Mississauga, ON) were applied to the upper medial aspect of each hind leg.

3.2.6 Farrowing Procedures

One week prior to farrowing sows were vaccinated for *E. coli* (2 ml Litterguard, Pfizer Animal Health, Kirkland, QC) and moved to an all-in-all-out farrowing room. P2 backfat (BF_{D110}) was re-measured as previously described. At 8 am and 2 pm on gestation D 115 sows were induced with 87.5 ug/sow cloprostenol (Planate, Intervet-Schering Plough Animal Health) administered in the external abdominal oblique muscle if they had not farrowed naturally. Farrowing was observed closely and oxytocin (20 USP IM, Vetoquinol, Lavaltrie, QC) was used between piglet expulsions when necessary in order to prevent stillborn delivery. As soon after birth as possible,

the piglets were individually identified by ear notch. Within 24 hours of birth, litter size, gender, crown-rump length (CRL), heart, girth circumference and individual weight of all the live and stillborn piglets were taken. Body mass index (BMI_{D0}) was calculated by dividing birth weight (WT_{D0}; kg) by CRL squared (cm^2) as described by [4]. When possible, some piglets from each litter were cross fostered to a sow in the opposing group allowing the potential discrimination of gestational versus the lactational environmental effects. Cross-fostering between groups was possible only when sows in both groups farrowed on the same day. Some piglets were cross-fostered to non-trial sows and piglets from non-trial sows were fostered to some trial sows. This was done to ensure that there were roughly similar number and size of piglets nursing sows of both groups. All fostering was performed within the same farrowing week and approximately 20% of the piglets were cross-fostered between CAUT and CTRL groups. Creep feeding was provided to the piglets one week prior to weaning as per standard barn protocol.

3.2.7 Stillborn Dissection

All stillborn piglets (n = 17) were dissected and the weight of the carcass, brain, spleen, liver, adrenal gland, small intestine (duodenum, jejunum, ileum) and drained heart were measured. The buoyancy of lungs was tested by placing in water to confirm stillborn delivery.

3.2.8 Day 21 Weight and Weaning

Piglets were weighed at 21 days of age and the average daily gain from birth was calculated (ADG_{LACT}). The D21 total litter weight was calculated for each biological litter by multiplying the number of pigs alive at D21 by their body weight. Piglets were weaned at 3.5 weeks of age. The date and reason of pre-weaning mortality and treatment was recorded. The pre-weaning survival rate of piglets at D21 was calculated as percentage of live born in their biological litter.

3.2.9 Statistical Analysis:

Data analyses were performed using SPSS inclusion 17 (Chicago, USA). Descriptive analysis comparing GRP differences was performed using either Student's t-test or Mann-Whitney U test for parametric and non-parametric variables, whichever was suitable. GRP differences in organ mass and organ:brain weight ratios in stillborn piglets was analyzed using Mann-Whitney U test.

Separate regression models were developed to estimate the effects of GRP or the total number of embryos at gestation D35 (EMB_TOT) on three broad outcomes of fetal development including average litter WT_{D0}, BMI, MALE%; and on two outcomes of lactational performance including ADG_{LACT} and pre-weaning piglet survivability. The models used litter as the experimental unit. To help overcome potential bias associate with cross-fostering piglets, two variables were created and offered in the ADG_{LACT} model. The first was "weighted mean of litter size on day 0" ($D0LS_{WTMEAN}$)which accurately reflected the average litter size of piglets suckling biological and surrogate sows. The second variable was "fostered off percentage" (FO_{PC}), which reflected the number of pigs that were fostered from biological to surrogate sows. For the pre-weaning survival model, survival rate was categorized as ≥90% (SURV ≥ 90), or < 90% based on the natural distribution of the data. EMB_TOT was categorized as ≥10 or <10 in this model, due to sparcity of data but was a continuous variable for all other models. For all models, parity was categorized (PAR_CAT) as either parity 3-5 or parity 6-9 based on the distribution of sows in the dataset. Univariate analysis was used to identify significant variables for inclusion in the full models. Biologically relevant but non-intervening variables were selected for the full model if $P < 0.2$. Final models were developed using stepwise backward selection and variables were retained in final model if $P < 0.05$. The final linear regression models were checked for the assumption of linearity, normality and homoscedasticity.

3.3 Results

3.3.1 Descriptive Analysis

The results of the embryo counting surgery revealed CTRL sows had more crowded uteruses compared to CAUT. Embryo numbers were approximately 68% higher in CTRL sows at gestation D35. The ovulation rate per available ovary (ovary that provides ova for fertilization) was 109% higher in CTRL compared to CAUT (Figure 1). Embryo survival rate was significantly increased in CAUT sows at gestation D35. Similarly, the survival rate of fetuses to term (D115) was also significantly increased in CAUT (Table 1). The most substantial loss occurred before D35 (Table 3.1). Litter sizes were significantly higher in CTRL, whereas average litter birth weight (WT_{D0}), crown rump length (CRL), girth circumference, and BMI_{D0} were significantly higher in CAUT sows (Table 3.1). The proportion of males in each litter did not differ by group. Pre-weaning survival as a percentage of piglets born alive was significantly higher in CAUT. Although born alive per litter was ~2.5 pigs/litter lower in CAUT sows, the number of piglets weaned was lower by only ~1.2 pigs, which was not statistically different from CTRL sows. Piglet weight at D21 and ADG_{LACT} was higher in CAUT compared to CTRL. In spite of CTRL sows having ovulation rates (available ovary) more than twice that of CAUT, there was no statistical GRP difference in total litter weight at D21 between GRP. This was due to a combination of factors including lower survival, lower birth weight and lower post-natal growth rate (Figure 3.1).

The carcass weight of stillborns did not statistically differ between the GRP but the BMI of CAUT stillborns was statistically higher than CTRL (Table 3.2). The adrenal weight and adrenal:brain ratio were significantly lower , and the liver weight and liver:brain ratio trended lower in CTRL stillborns (Table 3.2). There was no statistical difference in brain weight between GRP (Table 3.2).

3.3.2. Effect of Uterine Crowding on Fetal Development

Average litter WT_{D0} was unconditionally associated with GRP (P<0.001) and total embryo (EMB_TOT) at gestation D35 (P = 0.003). In separate models, WT_{D0} was regressed with GRP and EMB_TOT. CAUT sows had significantly higher average litter WT_{D0} (P < 0.001, β = 0.308, 95%CI = 0.149, 0.466) than CTRL sows. EMB_TOT was negatively associated with average WT_{D0}: for each additional embryo, WT_{D0} decreased by 0.029 kg (P = 0.003, β = -0.029, 95%CI (-0.048, -0.01)).

Average litter body mass index (BMI) was unconditionally associated with GRP (P = 0.042) and EMB_TOT (P = 0.008). BMI was significantly higher in CAUT compared CTRL sows (P = 0.042, β = 0.012, 95%CI = 0, 0.023). EMB_TOT had a negative effect on BMI: each additional embryo decreased BMI by 0.002 kg/cm^2 (P = 0.008, CI = -0.003, -0.001).

3.3.3. Factors Affecting Male Percentage (MALE%) in Litter

MALE% in litter was not affected by GRP or EMB_TOT. MALE% was unconditionally associated with sow backfat at gestation D50 (BF_{D50}; P = 0.023) and at D110 (BF_{D110}; P = 0.149). Since BF_{D50} and BF_{D110} were poorly correlated (r = 0.22), both were regressed simultaneously with MALE%. MALE% was significantly associated with BF_{D50} suggesting a relationship between sow body condition (a proxy measure for fitness) and the birth of higher percentage of male piglets. For each one mm increase in BF_{D50}, MALE% increased by 1.7% (P = 0.023, β = 1.732, 95%, CI = 0.258, 3.207).

3.3.4. Factors Affecting Average Daily Gain (ADGLACT) and Survivability from Birth to D21

ADG_{LACT} was unconditionally associated with GRP (P = 0.06), $D0LS_{WTMEAN}$ (P = 0.05), and MALE% (P = 0.02) which were considered in the full regression model. ADG_{LACT} was 32

gm/day (P = 0.006, β = 0.032, 95% CI = 0.01, 0.058) higher in CAUT compared to CTRL litters. When ADG_{LACT} was regressed with EMB_TOT category (categorized as ≥10 or <10 for this model), MALE%, and $D0LS_{WTMEAN;}$ only $D0LS_{WTMEAN}$ was significantly related to ADG_{LACT}. ADG_{LACT} decreased 7 gm/day (P = 0.05, β = -0.007, 95% CI = -0.014, 0) for each additional piglet in the litter at D0.

Having greater than 90% survival to D21within a litter (SURV ≥ 90) was unconditionally related to GRP (P=0.009) and EMB_TOT category (P = 0.08; categorized as ≥10 or <10 for this model). The odds of CAUT litters having SURV ≥ 90 was 6.5 (P = 0.009, OR = 6.5, 95%CI = 1.61, 24.46) times higher than CTRL litters.

3.4 Discussion

The overall objective of this study was to determine if early gestational litter size and intrauterine crowding affects fetal development and postnatal lactational performance in pigs. A relatively less crowded uterus was created in CAUT using laparoscopic unilateral oviduct cauterization surgery. The surgery blocked gamete transport and fertilization from one ovary, thus reducing embryo numbers and intra-uterine competition and allowing the development of litters in a spacious or less crowded uterine environment. Our central hypothesis is that uterine crowding in gestation is detrimental to pre-natal development and results in inferior post-natal performance. The results of this present study are in accordance with studies of Town *et al.* in which a relatively more spacious uterus was created using unilateral oviduct ligation surgery to study the effects of intra-uterine crowding on prenatal development measured at gestation D30 and D90 [2]. The number of embryos in ligated (9.3) and non-ligated (15.1) sows at gestation D30 in Town *et al.* study were very similar to our study.

Our results indicate that uterine crowding adversely affects embryo survival to D35 which corresponds with results of Town *et al.* [2]. Early pregnancy is dominated by the more viable embryos that are better able to compete for biochemical factors in the uterus required for their development [10]. This leads to the sacrifice of less viable embryos. Fenton *et al.*[6] found this

mechanism active between D7 to D25 of gestation, while Chen and Dziuk [13] concluded that crowding affects the prenatal survival before D17 and then between D29 and D35. In our study, embryo survival rate to gestation D35 and fetal survival rate to term were significantly higher in CAUT sows. The number of embryos in CTRL sows at D35 was slightly higher than CAUT. Embryo losses prior to D35 were considerably higher than embryo losses between D35 and term regardless of GRP. Fetal loses later in gestation may be due to intra-uterine competition for the establishment of an adequate placental surface area required for nutrient exchange between fetal and maternal circulation [10].

Tissue accretion (hyperplasia) and differentiation *in utero* are regulated by a number of hormones and when in balance, are associated with normal fetal growth. However, abnormality in any of these endocrine processes will result in an altered pattern of fetal growth [14]. In particular, decreased thyroxin and increased cortisol alter patterns of tissue accretion and differentiation in skeletal muscle, skin, lung, liver, kidney and gut which leads to an asymmetrical type of IUGR [14]. Some evidence of asymmetrical intrauterine growth retardation and brain sparing was found in stillborn piglets of CTRL sows in this present experiment. Adrenal and liver weights, and adrenal:brain and liver:brain ratios were decreased in CTRL litters, as was BMI. Because our primary goal was to farrow live piglets and monitor their postnatal performance, only the organs from available stillborns were used to evaluate the effect of crowding on organ development. Our sample size was small (n = 17) which impeded our ability to find significant group differences in other organs if they existed. Similar brain sparing of liver, thymus, muscle, pancreas, adrenal gland, skeleton and spleen was found in IUGR piglets at birth [15]. In addition, Town *et al.* found higher brain:liver and brain:total secondary muscle fibre ratio in non-ligated control (relatively overcrowded) sows [2] using the similar unilateral oviductal ligation model.

The restriction in placental exchange surface may result in fetal hypoxemia. Since the brain maintains its oxygen consumption even when oxygen availability is decreased [16] it continues to develop in spite of limited nutrient supply. This results in the preferential growth of brain tissue at the expense of all other organs. Intrauterine crowding also leads to early activation of fetal HPA axis resulting in elevated cortisol level before term. This causes premature

differentiation of cells and also results in asymmetric pattern of organ growth [17]. The lower BMI of stillborns in our study is in agreement with findings of Chen and Dziuk [13] in which the fetal weight and length were restricted after D35 in spite of the availability of uterine space.

Two contradictory models have been developed for the maternal prediction of secondary sex ratio: the Trivers and Willard hypothesis, and local resource competition model. Trivers and Willard hypothesis predicts that offspring of dominant mothers will be biased toward males, and offspring of subordinate mothers towards females [18]. By contrast, the local resource competition model has an opposite prediction in that low ranking females produce a greater proportion of male offspring than do higher ranking females, and high ranking females may sometime produce more females than males [19]. We attempted to determine if sex ratio was associated with uterine crowding, which if occurring in early gestation, may place selective pressure on either male or female pigs, in a similar manner to which a submissive sow in a highly competitive environment may experience restricted nutrient consumption. While our data set is small and the study did not evaluate social rank *per se,* we found no relationship between uterine crowding and sex ratio. MALE% however, was positively related with sow backfat at gestation D50, a proxy measure of sow body condition. This suggests that older sows (P3 and above) in good body condition are more likely to give birth to a higher proportion of male compared to female piglets which is consistent with the Trivers and Willard model.

Pre-weaning survivability is negatively related to birth weight. In our study, uterine crowding decreased pre-weaning survival in CTRL litters compared to CAUT. This result is in accordance with Fix *et al.*, which demonstrated decreased pre-weaning mortality with increasing birth weight [20]. There is an inverse relationship between mean weight of litter and litter size [15, 21-23]. In our study, CTRL sows had approximately 3 more pigs/ litter than CAUT, and were 300 gram/piglet lower in birth weight. Seventy-five percent of pre-weaning mortality occurs within 7 days of the life, with a higher death risk associated with low birth weight piglets [23]. Since piglets of CTRL sows had lower birth weights, higher death risk was not unexpected. Average daily gain from birth until weaning however was higher in CAUT than CTRL litters and was directly related to higher birth weight and lower embryo numbers during gestation. This is in agreement with the finding of Quiniou *et al.* which found a direct relationship between birth

weight and average daily gain to weaning [23]. Higher birth weight piglets grow more rapidly and have higher average daily gain.

The decision to cross-foster a proportion of piglets in some litters was controversial, but was made to ensure all piglets grew to their genetic potential. If we had not cross-fostered piglets between groups in an attempt to equalize litter sizes and suckling environments at birth, the litter size of CAUT would be less than CTRL. This would have introduced suckling bias favoring the CAUT litters in terms of D21 weight. However, by cross fostering between the two litters, we might have introduced more variation in terms of different suckling environments among pigs of same litter. This is a clear limitation of this study that we attempted to overcome by including $D0LS_{WTMEAN}$ and FO_{PC} as variables offered in the regression analysis.

In our experiment, the accumulative effects of uterine crowding are clearly evident. Litters from CAUT sows had higher embryo and fetal survival, birth weight and body mass index. In spite of having only one "functional" ovary, the total litter weight at lactation D21 of CAUT litters was not statistically different from CTRL litters that had ovulation rates over 2 fold higher. Although there are generally financial advantages of weaning more rather than fewer heavier pigs (albeit both are important) this research demonstrates that uterine crowding is clearly detrimental to fetal growth and development. Thus, the economic advantages of larger litter sizes may not be as great as seen at first glance. This highlights a dilemma facing the pork industry: should producers continue to seek larger and larger litter sizes knowing that fetal development, survivability, birth weight, post-natal growth and weaning weight will be adversely affected?

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Table 3.1. Effect of unilateral cauterization surgery on ovulation rate, number of embryo and prenatal survival, and on average litter weight and morphology at birth, and pre-weaning survival and growth rate (mean ± SD).

Variable	CAUT	CTRL	*P* value
Reproductive performance			
No. sows	13	12	
Total ovulation (2 ovaries)	23.9±4.9	27±4.1	0.15
Ovulation (available ovary)	12.9±3.5	27±4.1	<.001
No. embryos at D35	9.1±3.0	15.3±4.3	0.001
Embryo survival to D35 (%) (available ovary)	66±20.4	53±16.7	0.04
No. fetuses at term	7.1±2.2	10.6±2.2	.002
Fetal survival to term (available ovary) (%)	59±16.7	42.4±10.5	.006
Fetal survival from D35 to term (%)	93.6±15.38	83.1±13.8	.13
Farrowing performance			
No. sows	19	21	
Total born/L	7.9±2.9	11.2±3.23	0.002
Live born/L	7.6±2.9	10.4±3.04	0.006
Birth wt ($WT_{D0;}$ kg)	1.7±0.3	1.4±0.2	<.001
D0 CRL (cm)	30.3±1.9	28.4±2.3	0.009
Girth circumference at birth (cm)	25.6±1.6	24.1±1.5	0.004
D0 BMI	0.18±0.01	0.17±0.01	0.04
MALE%	56.2±18.0	56.4±13.1	ns
Post foster litter size at D0	10.1±1.7	11.0±1.8	ns
Lactational performance			
No. surviving pigs at D21 per biological litter	7.0±2.6	8.2±2.3	ns
Pre-weaning survival (% of pigs born alive)	92.9±11.6	82.1±12.5	0.01
D21 average piglet body weight (kg)	8.0±0.8	7.0±1.0	0.001
D21 total litter weight (kg)	55.5±22	56.5±14.6	ns
ADG_{LACT} (kg/d)	0.300±0.04	0.270±0.04	0.016

Legend: ns = not significant, CRL = crown rump length, BMI = body mass index, ADG = average daily gain, D0 = day 0

Table 3.2. Body organ weights and organ:brain weight ratios (mean±SD) in stillborn piglets from CTRL and CAUT sows.

Variable	CAUT	CTRL	*P*-value
No. of Stillborn	7	10	
Weight (gm)			
Stillborn	1643.9±321.9	1397.0±312.8	0.241
Heart	13.4±2.4	12.1±2.7	0.769
Liver	64.1±24.2	52.1±11.9	0.063
Spleen	2.0±0.4	1.6±0.7	0.265
Small intestine	51.4±13.5	47.9±12.9	0.77
Brain	34.6±3.3	33.4±2.5	0.462
Adrenal	0.5±0.1	0.3±0.1	0.005
Organ and Brain weight ratio			
Heart:Brain	0.4±0.0	0.4±0.1	0.77
Liver:Brain	1.8±0.6	1.5±0.3	0.064
Spleen:Brain	0.06±0.01	0.05±0.02	0.329
Small intestine:Brain	1.5±0.3	1.4±0.3	0.845
Adrenal:brain	0.02±0.004	0.01±0.002	0.009
No. of STB	7	9*	
D0 CRL	31.6±3.0	32.30±1.6	0.958
D0 BMI	0.16±0.02	0.14±0.02	0.023
D0 Girth circumference	24.2±1.8	23.02±2.2	0.396

Legend: CRL = crown rump length, BMI = body mass index

*The measurement from one stillborn missing

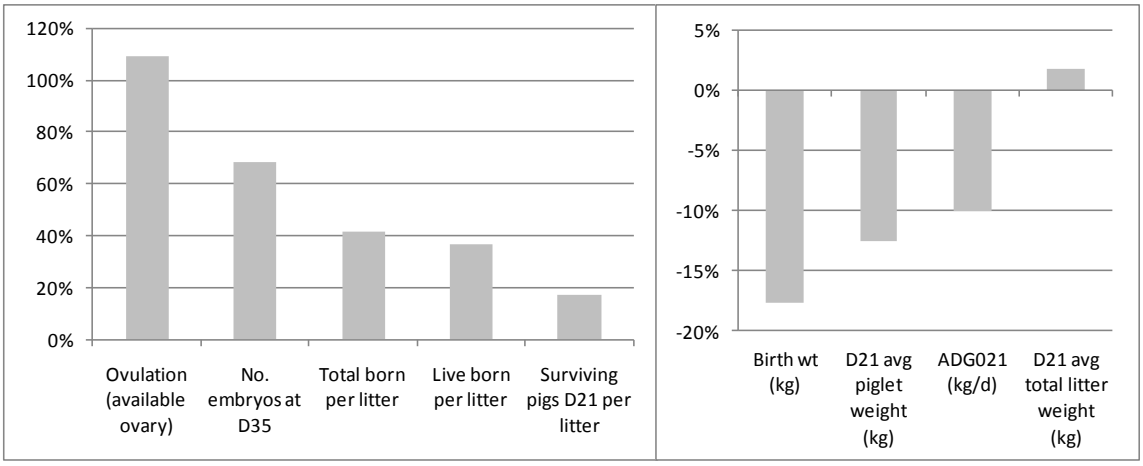

Figure 3.1. Percentage difference in the litter sizes (left) and weights (right) of unilateral oviductal cauterized (n=13, CAUT) and non-cauterized control (n = 12, CTRL) litters at different stages of gestation and lactation. Proportionate difference is calculated by: ((CTRL-CAUT)/CAUT)*100

CHAPTER 4

INTRAUTERINE CROWDING IN MULTIPAROUS SOWS ADVERSELY AFFECTS BIRTH WEIGHT, GROWTH PERFORMANCE AND ACTH INDUCED CORTISOL RESPONSE

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ABSTRACT

The offspring of unilaterally cauterized (CAUT) and non-cauterized control (CTRL) sows were used to study the effects of uterine crowding on postnatal average daily gain (ADG), backfat and loin depth, mortality, and adrenocorticotrophic hormone (ACTH) induced cortisol response. At gestation D35, the total number of embryos was significantly decreased in CAUT sows (CAUT = 9.1 ± 3.0, CTRL = 15.3 ± 4.3; *P* = 0.001). At birth, the piglet weight (WT_{D0}), gender, and morphometrics were recorded. Offspring were weighed at D21 of age, weaning, W7, 12, 16, and 19 of age. Average daily gain (ADG) was calculated for the lactation period (ADG_{LACT}; 0-21days), nursery (ADG_{NUR}, 3-7W), finisher (ADG_{FIN}, 7-19W), and birth to market ($ADG_{LIFETIME}$, 0-19W). P2 backfat (BF) and loin eye (LOIN) depth were measured at 70 and 110 kg live weight. Saliva samples were collected from offspring following 5 IU/kg ACTH challenge at W12 and W20 of age. Offspring from CAUT sows were significantly heavier than CTRL at birth, D21, W3 and W7 ($P < 0.05$ for all), and trended higher at W19 ($P = 0.09$). ADG_{LACT} and ADG_{NUR} were significantly higher in CAUT pigs ($P < 0.001$ for both), as was $ADG_{LIFETIME}$ (*P* = 0.04). Pigs with higher ADG_{LACT}, a potential proxy measure of post-natal catch up growth, had higher $ADG_{LIFETIME}$ (*P* < 0.001) after controlling for WT_{D0}. WT_{D0} however, was the most consistent driver of ADG. BF was thicker in CAUT pigs at 110 kg (*P* < 0.001). CAUT pigs had significantly higher salivary cortisol concentration 60 minutes after ACTH administration at W20 (*P* = 0.03) but not at any other time point or at W12. We conclude that the detrimental effects of intrauterine crowding on postnatal growth are primarily mediated through decreased birth weight, but age-dependent effects of fat deposition and the responsiveness of the HPA axis occur. Moreover, poor performance during lactation contributes to the poor lifetime growth rates of low birth weight pigs.

Key words: Pig, Intrauterine crowding (IUC), intrauterine growth retardation (IUGR), growth, adrenocorticotrophic hormone (ACTH), cortisol.

4.1 Introduction

Earlier research in human and animals has shown the importance of normal fetal development in the prevention of metabolic diseases later in life. In humans, poor fetal and early postnatal growth is associated with the development of type-2 diabetes [1], cardiovascular and metabolic diseases in adult life. In pigs, low birth weight or body mass index are associated with altered metabolism, fat deposition [2] and altered hypothalamic-pituitary-adrenal axis response which may indirectly affect the stress and inflammatory responses in postnatal life [3]. Intrauterine growth restriction (IUGR) is associated with impaired fetal and placental growth, and reduces the number of secondary myofibres in pigs [4]. In swine, piglets of lighter birth weight had lighter body weight in later life [5]. Moreover, piglets of lighter birth weight grew slower and were fatter at slaughter [6]. This finding was associated with decreased myofibre hyperplasia and increased myofibre hypertrophy and resulted in an earlier plateau of myofibre growth in low compared to high birth weight pigs [6]. Regarding the effect of birth weight on backfat depth, contradictory results are reported. No difference was noted at slaughter [7], whereas low birth weight pigs had higher backfat depth compared to high birth weight pigs [2] when measured at 12 months of age, suggesting an age-dependent effects.

IUGR is also associated with altered early life adrenal function. Following *in vitro* stimulation of adrenal cells, low birth weight offspring from unilaterally hysterectomized ovariectomized (UHO) sows demonstrated higher total plasma cortisol concentration at 3 and 7 days of age [8]. Low birth weight piglets also had higher adrenal weights relative to their body weight. Similarly, adrenal weight and the ratio of adrenal cortex to medulla were significantly higher at 3 months of age in low compared to high birth weight piglets and adrenal cortex to medulla ratio trended higher at 12 months [3].

Past studies investigating the biological effects of intrauterine crowding (IUC) used alternate methods to alter uterine capacity and were terminated during gestation [4] or immediately after farrowing [9]. Our goal was to evaluate the effects of IUC on postnatal performance up to market age, as well as on backfat (BF) and loin depth (LOIN), and ACTH stimulated cortisol response in early and later finishing stages. This experiment was based on the hypothesis that

piglets from the less crowded (unilateral oviduct cauterized; CAUT) litters will exhibit superior performance compared to piglets from control (non-cauterized; CTRL) litters. The specific objectives were to determine the impact of unilateral oviduct cauterization on postnatal ADG during lactation (ADG_{LACT}), nursery (ADG_{NUR}), finisher (ADG_{FIN}), and from birth to market age ($ADG_{LIFETIME}$), BF and LOIN at 70 kg and 110 kg body weight, and ACTH-stimulated cortisol response at W 12 and 20 of age.

4.2 Materials and Methods

The experiment was conducted in a 300-sow farrow to finish farm with the permission of the University of Saskatchewan's Committee for Animal Care and Supply (permit #2008005). Twenty-five PIC Line 42 (Camborough Plus) sows of parity 4-8 were selected for the experiment. Each had 10-17 total born and was in good health and body condition at their previous farrowing and weaning, respectively. The selected sows were randomly divided into CTRL and CAUT at weaning. GRP were balanced and blocked by weaning week and parity. After weaning, the selected sows were kept in individual pens and fed 2.5 to 3kg commercial gestation diet per day. Fourteen days post-weaning, a right oviduct cauterization surgery was performed on 13 CAUT sows. No surgery or placebo treatment was performed on the other 12 CRTL sows, which albeit a limitation of the study, was not performed in keeping with the "3R's" of animal care. Sows were anesthetized intravenously with sodium thiopental (6mg/kg) and anesthesia was maintained with a close circuit system of isoflurane. Three one cm incisions were made at the umbilicus, 15 cm caudal and 10cm lateral to umbilicus. With an atraumatic right angle grasping forcep, bipolar cautery instrument and endolaparoscopic scissors, the right oviduct was coagulated and separated. After surgery sows were transferred to comfort pens and recovery assessed daily for 5 days. For pain control flunixin meglumine (2ml/45kg IM, Banamine, Intervet-Schering Plough Animal Health Canada, Kirkland, QC) was administered when necessary. Both CAUT and CTRL sows were heat synchronized using altrenogest (20 mg/sow/day orally, Regu–Mate, Intervet-Schering Plough Animal Health, Kirkland, QC) for 14 days, and artificially inseminated using pooled PIC337 semen once daily for up to 3 consecutive days.

A laparotomy was performed on all sows to determine the ovulation rate and total embryo numbers (EMB_TOT) between gestation D 32 and D39. Anesthesia was similar to that used for cauterization surgery. A mid-ventral incision was made and corpora lutea (CL) counted on both ovaries to determine the ovulation rate. All embryos were counted in both uterine horns and viability determined using real time ultrasound (Shimatzu SDU 350 XL, Universal Medical Systems, Bedford, NY). Viable embryos were those in which a prominent heart beat was observed. Post-surgical procedures were similar to those described above. For pain control, two 100 mcg/hr fentanyl patches (RAN-Fentanyl Transdermal System, Ranbaxy, Mississauga, ON) were applied to the upper medial aspect of each hind leg. One week prior to farrowing, sows were vaccinated for *E. coli* (2 ml Litterguard, Pfizer Animal Health, Kirkland, QC) and moved to an all-in-all-out farrowing room.

4.2.1 Farrowing procedures

Sows were induced to farrow on gestation D115 using 87.5 ug/sow cloprostenol administered twice at 8 am and 2 pm (Planate, Intervet-Schering Plough Animal Health) if they had not farrowed naturally. Farrowing was observed closely and oxytocin (20 USP IM, Vetoquinol, Lavaltrie, QC) administered between piglet expulsions when necessary in order to prevent stillborn delivery. As soon after birth as possible, piglets were individually identified. Within 24 hours after birth, litter size, gender (GNDR), crown-rump length (CRL), heart girth circumference and the individual weight of all live piglets were taken. Body mass index (BMI_{D0}) was calculated by dividing birth weight (WT_{D0}; kg) by CRL squared (cm^2, CRL). When possible, some of the piglets from each litter were judiciously cross-fostered to a sow in the opposing group allowing the potential discrimination of gestational versus the lactational environmental effects in the statistical analysis. Some piglets were cross-fostered to non-trial sows and piglets from non-trial sows were fostered to some trial sows. This was done to ensure that CTRL and CAUT litters were roughly similar in number and size of piglets, which avoided as much as possible bias towards CAUT litters which were smaller in size. All fostering was performed within the same farrowing week and approximately 20% of the piglets were cross-

fostered between CAUT and CTRL groups. Creep feeding was provided to all litters one week prior to weaning as per standard barn protocol. Six CAUT and nine CTRL sows were rebred after weaning allowing piglet performance data on their subsequent litter to be captured up to and including the nursery stage. A categorized variable (REP) was used to distinguish performance data collected at the initial and subsequent farrowing. Space limitations in the barn prevented capturing performance data from the REP 2 litters during the finisher stage.

4.2.2 Body Weights

Piglets were weighed at D21 (WT_{D21}) of age and ADG_{LACT} was calculated. Each pig was re-weighed at weaning (WT_{WEAN}) approximately at W3.5, then sorted by weight and placed in 2.5m x 1.04m nursery pens. Each nursery pen contained 5 to 12 pigs, depending upon the number of pigs weaned each week. Average pen density in the nursery was calculated and used in the statistical analysis. Pigs were fed *ad-libitum*, re-weighed 4W post-weaning and ADG_{NUR} calculated. Shortly thereafter, pigs were moved into finisher pens. Three to six pigs of similar weight were housed in each 2.4m x 1.7m pen and average pen density calculated. Commercial *ad-libitum* diets were provided. Pigs were re-weighed at W11, W15 and W19, and ADG_{FIN} (W7 to W19) and $ADG_{LIFETIME}$ (birth to W19) calculated.

4.2.3 Backfat and Loin Eye Measurement

P2 backfat and loin eye depth were measured in all pigs when they weighed between 65-80 (BF_{70}, $LOIN_{70}$) and 105-115 kg (BF_{110}, $LOIN_{110}$) live weight. Measurements were taken ultrasonically over last rib, 5 cm off the midline on the right flank (Pie 2000, Universal Medical, NY).

4.2.4 Mortality

The ID, date and reason of piglet mortality were recorded. The reasons for pre-weaning deaths (MT_{PRE}) were assigned by the farrowing technicians based on clinical signs and history, whereas postmortem examinations were performed on all pigs dying post-weaning (MT_{POST}). Inguinal, mesenteric and bronchial lymph nodes, heart, liver, lung, spleen, and small intestine were collected, formalin fixed and processed routinely. The cause of MT_{POST} was determined based on presenting history and clinical signs, gross and histological lesions.

4.2.5 Adrenocorticotropin (ACTH) Stimulation

Fifty-two pigs were selected from 22 sows, by selecting one male and one female pig of average WT_{D0} from each biological sow. One additional pig was selected at random from available litters on each test week (n = 10) and served as a saline placebo-treated ACTH control. ACTH stimulation was performed twice at W12 and W20 of age. Three baseline saliva samples were collected before ACTH (Synacthen Depot, 5 IU/kg, Novartis Pharmaceuticals Canada Inc., Dorval, QC) administration at -60 minutes, -30 minutes and immediately before ACTH injection (time 0; T_0). ACTH was administered intramuscularly in the neck and saliva samples were collected at 60, 120, 180, 240, 300, 360, 420, 480, 600, 720, 840 and 960 minutes after administration. Saliva was collected by allowing each pig to individually chew a cotton salivette (Sarstedt Inc., Montreal, QC) tied to an aluminum rod with dental floss, for 2 minutes or until thoroughly wet. A few grains of citric acid (Citric Acid USP, Vetoquinol Canada Inc., Lavaltrie, QC) were added to the salivette to induce salivation when needed. Saliva samples were typically collected from outside the pen and when that wasn't possible, the pen was entered. To account for potential collection stress, behaviour at the time of collection was scored 1 to 5, whereas: "1" corresponded to a pig that willingly chewed the salivette when the sampler remained outside the pen; "2" corresponded to a pig that chewing the salivette with some hesitance while the sampler remained outside the pen; "3" corresponded to a pig that chewed the salivette willingly after sampler entered the pen; "4" corresponded to a pig that chewed the salivette hesitatantly after the sampler entered the pen; and "5" corresponded to a pig that required restraint (boarded against

the pen) in order to collect the sample. Following collection, the salivette was visually inspected and the presence of water (pigs that drank water during the collection), blood or feed was recorded. Salivates were centrifuged at 2800 rpm for 10 minutes, saliva transferred to a plastic vial and stored at -20^{O}C pending analysis.

4.2.6 Cortisol ELISA

Salivary cortisol was measured in all samples by ELISA as per procedures described by [10] with some modifications. Briefly, 96 well, high-affinity binding ELISA plates (Costar, VWR, Batavia, IL) were coated with 260 µl of 1/40,000 rabbit polyclonal antibody (Cortisol-3, Cedarlane Diagnostics, Burlington, ON) except for two wells reserved for detecting non-specific binding. Coated plates were incubated overnight at 4^{O}C. Unbound antibody was washed twice using wash buffer and blocked with blocking buffer for 30 minutes at room temperature. All the buffers were prepared as described in table 4.3. The plates were washed twice with 260 µl of standard and label buffer. Fifty µl of standards, positive quality control and samples were dispensed in duplicate. One-hundred µl of 1/1000 Cortisol Horseradish Peroxidase Conjugate (HRP; YJ Bio-Products Inc., Rancho Cordova, CA) was added, and the plates were incubated overnight at 4^{O}C. Unbound antigen was washed twice with wash buffer and 100 µl of tetramethylbenzidine (TMB) mixture (Pierce soluble TMB Substrate Kit, Fisher Scientific, Ottawa, ON) was added to each well. After 10-20 minutes, once a dark blue colour developed, the reaction was stopped by using 50 µl of 2M sulphuric acid (Fisher Scientific, Ottawa, ON). Plates were read (Kinetic ELISA microplate reader, Molecular Devices, Sunnyvale, CA) at 450 nm wavelength.

4.2.7 Statistical analysis

Descriptive data was analyzed using STATA v10 (Statacorp LP, College Station, TX). A Student's *t*-test was used to determine significant differences in outcome variables between REP. No statistical differences were found and piglets from both REPs were included in the analysis.

Descriptive analysis to determine GRP differences was performed using a Mann-Whitney U test using litter as the experimental unit.. The effect of GRP and EMB_TOT (categorized by mean <= 12 or > 12) on MT_{PRE} and MT_{POST} was analyzed using a Fisher's Exact test.

Regression models were developed to determine the most significant factors affecting WT_{D0} and ADG. For these analyses, multilevel mixed-effects linear regression (XTMIXED) was used, where litter was included as a random intercept. These models used piglet as the experimental unit, and separate models were developed to evaluate the potential effects of GRP and EMB_TOT. Univariate analysis was first used to identify significant variables for inclusion in the full models. For the WT_{D0} model, GRP or EMB_TOT, parity of dam, REP, and GNDR were evaluated for potential inclusion. For all ADG models, GRP or EMB_TOT, GNDR, WT_{D0} and BMI_{D0} (a proxy measure of asymmetrical fetal growth), were evaluated for potential inclusion. Parity, post-foster litter size at D0 (LS_{POSFOS}) and whether or not the piglet was cross-fostered (yes, no; FOS_{YN}) were evaluated in ADG_{LACT} models. REP was evaluated in ADG_{LACT} and $ADG_{NURSERY}$, but not in ADG_{FIN} or $ADG_{LIFETIME}$ models since only REP 1 data was used for these latter models. Average pen density was included in ADG_{NUR} and ADG_{FIN} models. ADG_{LACT}, a proxy measure of milk production, lactational appetite, and post-natal catch-up growth, was evaluated in the ADG_{NUR}, ADG_{FIN} and $ADG_{LIFETIME}$ models. Since ADG_{LACT} was poorly correlated with WT_{D0} (Pearson's correlation coefficient, $r = 0.322$) both were included in models where biologically appropriate. Biologically relevant but non-intervening variables with $P < 0.2$ in the univariate analysis were selected for inclusion in the full model. Final models were developed using a stepwise backward selection and variables were retained in final model if $P < 0.05$. To determine the most significant factor influencing the postnatal ADG, inclusive models that contained GRP, EMB_TOT and WT_{D0} and other biologically relevant factors were also developed. Final mixed models were checked for assumptions of normality and homoscedasticity. Individual data points with residual values greater or less than three standard deviations from mean value were removed as outliers from the analysis and the final models re-run.

Multilevel mixed-effects linear regression was used in a similar manner to determine the most significant factors affecting BF_{70}, BF_{110}, $LOIN_{70}$ and $LOIN_{110}$. GRP or EMB_TOT (evaluated in

separate models), GNDR, WT_{D0} and BMI_{D0} and ADG from birth to the date of scanning were evaluated for potential inclusion in these models. The development and checking of final models were identical to that described above.

Multilevel mixed-effect logistic regression (XTMELOGIT) was used to determine the effect of GRP and EMB_TOT (evaluated in separate models), on MT_{PRE}. As above, individual piglets clustered by litter were used as experimental unit. WT_{D0}, $BMI_{D0,}$ and GNDR were regressed with MT_{PRE} and MT_{POST} in separate models to evaluate potential variables for the inclusion in the final model.

ACTH stimulated cortisol data analyses were performed using SAS 9 (SAS Institute Inc.). Data were analyzed using the Proc Mixed procedure. Group differences in cortisol value over time were assessed. Analysis was performed on time zero (T_0)adjusted and non-adjusted cortisol levels at W12 and W20 of age (two analysis) was found while controlling for sow effects (parity categorized and sow ID), pig effect (GNDR), time point effect (behavior scores, sample contamination with feed, water, and blood), and temporal time effect (week of sows farrowed). The interaction of time and group was included. Similar analysis was done to determine the effect of EMB_TOT (EMB_TOT< 12 or >= 12) on cortisol values.

4.3 Results

4.3.1. Descriptive Statistics

The ovulation rate did not differ between GRP (CAUT = 23.9 ± 4.9, CTRL = 27.0 ± 4.1; P = 0.15). The number of CL's present in the ovary ipsilateral to the non-cauterized oviduct and therefore available for fertilization was significantly reduced in CAUT compared to CTRL sows (CAUT = 12.9 ± 3.5, CTRL = 27.0 ± 4.1; $P < 0.001$). Gestational and farrowing parameter measurements are reported in Chapter 3.

CAUT offspring were significantly heavier than CTRL at birth, D21 ($P < 0.01$), and trended to be higher at weaning (P =0.06), and W7 (P =0.07). There was no significant GRP difference in

body weight at W19. CAUT piglets were 31% heavier at birth, 14% heavier at D21, 9% heavier at weaning, and 6% heavier at W7. CAUT piglets had higher ADG_{LACT} by 12% ($P = 0.01$) and ADG_{WEAN} by 9% ($P = 0.04$)(Table 4.1). ADG_{FIN}, $ADG_{NUR,}$ and $ADG_{LIFETIME}$ did not differ by GRP.

Eighteen percent of pigs died from birth to W19. MT_{PRE} was 15.1% and MT_{POST} was 3.5%. MT_{PRE} was significantly higher in CTRL (CTRL = 39/155, CAUT = 13/127; Fisher Exact $P = 0.01$). Across both groups the predominant reasons for MT_{PRE} were: crushing by sow (56%), low viability (15%), congenital defects (5%), or death due to internal bleeding, anemia, sudden death or enteritis (3.3%). When comparing death reason by group, only low viability pigs were significantly higher in CTRL (CTRL = 9/155, CAUT = 0/127; Fisher's Exact $P = 0.005$). MT_{POST} did not differ by GRP, however MT_{POST} was significantly increased in litters in which the embryo count exceeded 12 at gestation D35 (EMB_TOT $\leq$12 = 2/85, EMB_TOT >12 = 8/80; Fisher Exact $P = 0.05$).

4.3.2 Factors affecting birth weight

WT_{D0} was unconditionally associated with GRP ($P < 0.001$), EMB_TOT ($P < 0.001$) and GNDR ($P = 0.05$), but not parity of dam or REP. When WT_{D0} was regressed with GRP and GNDR, only GRP was significant, and was higher in CAUT pigs by 310 gm ($P = 0.001$, $\beta = 0.31$, 95% CI = 0.17, 0.46). When EMB_TOT and GNDR were regressed with WT_{D0}; only EMB_TOT was significant. Each additional embryo at gestation D35 decreased WT_{D0} by 31 gm ($P < 0.001$, $\beta = -0.031$, 95%CI = -0.048,-0.014).

4.3.3 Factors affecting average daily gain during lactation (ADG_{LACT})

ADG_{LACT} was unconditionally associated with GRP ($P = 0.007$), EMB_TOT ($P = 0.003$), WT_{D0} ($P < 0.001$), BMI_{D0} ($P < 0.001$), $LS_{POSTFOS}$ ($P = 0.12$), FOS_{YN} ($P = 0.138$), and REP ($P = 0.09$), but not parity or piglet GNDR. ADG_{LACT} was 36gm/day higher in CAUT than CTRL pigs ($P = 0.007$, $\beta = 0.036$, 95% CI = 0.01, 0.061). Each additional embryo at gestation D35 decreased

ADG_{LACT} by 4gm/day ($P < 0.001$, $\beta = -0.0047$, 95%CI = -0.0065, -0.0028). When ADG_{LACT} was regressed with WT_{D0}, BMI_{D0}, $LS_{POSTFOS,}$ FOS_{YN}, REP, and either GRP or EMB_TOT (two different models), the only factors significantly affecting ADG_{LACT} were WT_{D0} and REP. For every 1 kg increase in WT_{D0}, ADG_{LACT} increased 67gm/day ($P < 0.001$, $\beta = 0.067$, 95%CI = 0.046, 0.088). ADG_{LACT} was 28gm/day higher in the second compared to the first REP ($P < 0.02$, $\beta = 0.028$, 95%CI = 0.003, 0.053).

4.3.4 Factors affecting average daily gain during nursery (ADG_{NUR})

ADG_{NUR} was unconditionally associated with GRP ($P = 0.174$), WT_{D0} ($P < 0.001$), BMI_{D0} ($P < 0.001$), ADG_{LACT} ($P = 0.007$), and REP ($P = 0.12$). ADG_{NUR} was not associated with EMB_TOT, average nursery pen density or GNDR. In the inclusive model, ADG_{NUR} was regressed with GRP, WT_{D0}, BMI_{D0}, $ADG_{LACT,}$ and REP. Only WT_{D0} and REP were significantly associated with ADG_{NUR}. For each 1 kg increase in WT_{D0}, ADG_{NUR} increased by 125 gm/day ($P < 0.001$, $\beta = 0.125$, 95%CI = 0.09, 0.157). ADG_{NUR} was 49 gm/day higher in REP 2 ($P = 0.01$, $\beta = 0.049$, 95%CI = 0.011, 0.087).

4.3.5 Factors affecting average daily gain during grow-finish (ADG_{FIN})

ADG_{FIN} was unconditionally associated with WT_{D0} ($P = 0.001$), BMI_{D0} ($P = 0.049$), GNDR ($P < 0.001$), and ADG_{LACT} ($P = 0.013$), but not average finisher pen density or body weight at nursery dispatch. Moreover, neither GRP nor EMB_TOT had any effect on ADG_{FIN}. In the inclusive model, ADG_{FIN} was regressed with WT_{D0}, BMI_{D0}, GNDR and $ADG_{LACT.}$ OnlyWT_{D0} and GNDR significantly affected ADG_{FIN}. With each 1 kg increase in WT_{D0}, ADG_{FIN} increase 87gm/day. ADG_{FIN} was 64gm/day lower in females compared to barrows.

4.3.6 Factors affecting ADG from birth to market age ($ADG_{LIFETIME}$)

$ADG_{LIFETIME}$ was unconditionally associated with GRP ($P = 0.158$), EMB_TOT ($P = 0.001$),

WT_{DO} ($P < .001$), BMI_{D0} ($P = 0.001$), GNDR ($P = 0.001$), ADG_{LACT} ($P < 0.001$). When $ADG_{LIFETIME}$ was regressed with GRP or EMB_TOT (in separate models), BMI_{D0}, WT_{D0}, GNDR, and ADG_{LACT}, only the latter three variables were significantly related to $ADG_{LIFETIME}$. $ADG_{LIFETIME}$ was 39gm/day lower in females compared to barrows ($P < 0.001$, $\beta = -0.039$, 95%CI = -0.056,-0.022) and for each 1 kg increase in WT_{D0}, $ADG_{LIFETIME}$ increased by 71gm/day ($P < 0.001$, $\beta = 0.071$, 95%CI = 0.037, 0.105). For each 1 kg increase in ADG_{LACT}, $ADG_{LIFETIME}$ increased 329gm/day ($P < 0.001$, $\beta = 0.329$, 95%CI = 0.152, 0.507). It is noteworthy that in these inclusive models, neither GRP nor EMB_TOT significantly affected $ADG_{LIFETIME}$.

4.3.7 Factors affecting backfat and loin depth at 70 (65-80) kg and 110 (105-115) kg body weight

In the descriptive analysis, BF_{110} was lower in CTRL compared to CAUT pigs ($P = 0.02$; Table 4.2) but not BF_{70}. $LOIN_{70}$ and $LOIN_{110}$ did not differ between GRP. In regression analysis, BF_{110} was unconditionally associated with GRP ($P = 0.01$) and GNDR ($P = 0.094$), but not parity, EMB_TOT, WT_{D0}, WT_{D21}, BMI_{D0}, ADG_{LACT}, body weight at scanning, or $ADG_{BIRTHSCANNING}$. When regressed with GRP and GNDR, BF_{110} was significantly related only to GRP, and was only 0.3 mm greater in CAUT than CTRL pigs ($P < 0.001$, $\beta = 0.2914$, 95%CI = 0.138, 0.444). GRP, EMB_TOT, GNDR, WT_{D0}, BMI_{D0} and ADG_{LACT} were not associated with $LOIN_{70}$, $LOIN_{110}$, or BF_{70}.

4.3.8. Factors Affecting Pre- and Post-Weaning Mortality

GRP ($P = 0.01$), EMB_TOT ($P = 0.05$), WT_{D0} ($P < 0.01$) and BMI_{D0} ($P < 0.01$) were unconditionally associated with MT_{PRE}. When MT_{PRE} was regressed with GRP, WT_{D0} and BMI_{D0}, only WT_{D0} was significant and it was highly related to MT_{PRE}. With each one kg decrease in WT_{D0}, the odds of piglet death prior to weaning increased 20.5 times ($P < 0.001$, OR = 20.5, 95%CI = 7.37, 57.14). By contrast, EMB_TOT had minimal impact on MT_{PRE}. With each

additional embryo present at gestation D35 the odds of MT_{PRE} increased by only 1.1 times ($P < 0.05$, OR = 1.09, 95%CI = 0.99, 1.19). There were insufficient post-weaning deaths to enable regression analysis.

4.3.9. ACTH induced Cortisol Response

Salivary cortisol concentration was analyzed before and after adjusting for baseline (T0) levels. There was no GRP difference in either baseline adjusted or non-adjusted salivary cortisol concentration following ACTH administration at W12 or W20. Results were consistent when analyzed using EMB_TOT as a categorized variable (<12 or ≥12). Saline placebo treated ACTH control pigs did not show any cortisol response after saline injection (data not shown).

4.4. Discussion

The overall objective of this study was to determine if early gestational litter size and intra-uterine crowding affects postnatal performance in pigs, specifically growth, backfat and loin muscle development, mortality and ACTH induced cortisol response. A less crowded uterus was created in CAUT sows using unilateral cauterization surgery. Our experiment expectedly demonstrated that piglets with higher birth weight grew faster during lactation, nursery, and the grow-finish stages, and from birth until market age at W19. Birth weight in fact, was the most significant factor affecting ADG. Moreover, we conclude that the detrimental effects of intrauterine crowding on postnatal growth are mediated through decreased birth weight, and that IUC *per se* results in no additive effect.

Our central hypothesis was that uterine crowding in gestation causes inferior post-natal performance. While a reduction in birth weight occurred as anticipated in CTRL litters, we were interested in determining if IUC exerted effects over and above that of birth weight.

Offspring from CAUT sows were heavier at birth, D21and trended to be heavier until the end of

the nursery stage. The ADG of CAUT piglets were higher during lactation, but not during nursery, finisher stage and lifetime. . These results contradict the study of Christenson [9] in which no significant difference in the weight of pigs of UHO and normal sows was found up to D14 of age. Furthermore higher catch up growth was noted in pigs of UHO gilts that had gestated in a more crowded uterus, since they had similar ovulation rate (UHO = 11.9, control = 12.1) at gestation D40 [9]. It must be noted that in the Christenson study, piglets from UHO and control sows were not cross-fostered between treatment groups, and as a consequence, lactational litter sizes differed markedly by group (UHO = 4.6 ± 0.4, Control = 7.6 ± 0.3 for live born litter size). This decrease in litter size would have no doubt led to the UHO pigs having a competitve advantage over the control piglets, resulting in greater weight gains during lacation. To avoid this, cross-fostering to balance lacational litter size was undertaken judiciously in the present study which may explain why catch-up growth during lactaion was not observed in CTRL pigs. Because cross fostering may by itself positively or negatively affect lactational growth, depending on the circumstance, it was always considered in our regresion analysis for average daily gain.

CAUT pigs were about 400 grams heavier than CTRL at birth, and were about 5 kg heavier at W19. In this study, CAUT and CTRL pigs were kept in identical nursery and finisher pens that contained the same number of animals, but varied by production week. Average pen density was considered in the regression analysis due to its potential effect on ADG.

The positive impact of birth weight on postnatal growth is well established. Past research has also demonstrated that heavier birth weight pigs have higher numbers of skeletal muscle fibres that are associated with higher lactational and post-weaning growth rates compared to low birth weight pigs [11, 12]. In pigs, primary and secondary muscle fibres are formed during fetal development, with the number largely fixed before gestation D90 [13]. The number of primary myofibre is unaltered by uterine conditions while intra-uterine crowding causes a reduction in the number and hyperplasia of secondary myofibres [4, 13, 14]. Thus, any reduction in myofibre number or hyperplasia may result in the slower growth of lighter birth weight pigs compared to those of higher birth weight [6]. Anticipated changes in small intestinal morphology also associated with IUGR, characteristically a longer and thinner small intestines with reduced

villous length, may further reduce postnatal growth rate by impairing nutrient absorption [15].

The important relationship between lactational growth rate and $ADG_{LIFETIME}$ must be emphasized. The results of this study indicate that lactation may be the optimum period for a light birth weight pig to express catch-up growth. For example, ADG_{LACT} in addition to WT_{D0}, was positively associated with $ADG_{LIFETIME}$ indicating that by providing the best suckling environment possible, producers would help offset the disadvantages associated with low birth weight. Similar results have been reported by others. In a study using rats, pups that were growth restricted *in utero* exhibited higher catch-up growth and were heavier later in the life compared to the normal rats [16]. In this present study ADG_{LACT} however, was not positively related to ADG during the nursery or finisher stages suggesting that enhanced growth or feed intake during lactation did not positively program appetite later in life.

The results of this study fail to provide substantial evidence that IUGR affects backfat or loin deposition as measured by *in vivo* ultrasound. Although BF_{110} was statistically higher in CAUT, the difference compared to CTRL pigs was small in terms of biological importance. Moreover, the trend was in the opposite direction than we hypothesized in that BF_{110} was higher in CAUT not CTRL pigs. Our results partially concur with those of Wolter and Elis in which no difference in fat and loin eye depth was found at market weight in low versus heavy pigs at weaning [17]. Our results are also consistent with the observations of Gondret *et al.*, that backfat thickness was equal in low and high birth weight pigs at slaughter [7]. Our results, however, are contrary to the results of Poore *et al.* [2] who reported that P1 and P2 backfat was higher in low compared to high birth weight pigs at 12 months of age. Therefore, if IUC or IUGR causes age-dependent changes in fat deposition, it may not express itself until after sexual maturity.

Pre-weaning mortality was unexpectedly high in this study, and largely attributable to exceptionally high mortality levels in CTRL litters (20% versus 10%). This was not unexpected given the lower birth weight in CTRL litters, and that cross-fostering was more restricted during the study than normally undertaken at the research farm. “Low viability” pigs, defined as piglets of low birth weight and poor post-natal vigor, were statistically higher in CTRL litters, supportive of our hypothesis. A similar negative relationship between birth weight and pre-

weaning mortality has been reported by others [5]. It is noteworthy that in this present study, pre-weaning deaths due to crushes or trauma were numerically but not statistically higher in CTRL litters. It is generally accepted that there are underlying reasons explaining why many crushing or traumatic deaths occur during the early lactational period. These underlying reasons may include low birth weight in combination with chilling, hypoglycemia or under-nutrition.

In this study we found that birth weight had a positive influence on postnatal growth in every stage of production. We therefore propose that selection of sows with increased uterine capacity will enhance postnatal growth performance. In genetic selection programs pork producers should select on the basis of birth weight along with litter size to help minimize the potential effects of IUGR on postnatal growth performance. Moreover, producers should emphasize proper care during early postnatal life, as high lactational growth will partially compensate for low birth weight. In conclusion, selection of sows delivering offspring of higher birth weight, and proper management during early postnatal life will have additive effects leading to more efficient pork production.

4.5. References

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Table 4.1. Growth performance (mean ± SD) in offspring born of right oviductal cauterized (CAUT) and control (CTRL) parity 4+ sows.

Variables	N	Group		P-value
		CAUT	CTRL	
Body weight (kg)				
Day 0	38	1.7 ± 0.3	1.3 ± 0.2	<0.001
Day 21	37	8.1 ± 0.9	7.1 ± 1.0	0.002
Wean	38	9.4 ± 1.0	8.7 ± 1.2	0.06
W 7	35	23.0 ± 3.0	21.6 ± 2.4	0.07
W19*	21	98.05 ± 4.8	93.16 ± 9.08	ns
ADG during growth phases (kg/day)				
ADG_{LACT} (D0-D21)	38	0.300 ± 0.037	0.268 ± 0.040	0.01
ADG_{WEAN} (W1-3)	38	0.297 ± 0.031	0.273 ± 0.039	0.04
ADG_{NUR} (W3-7)	35	0.498 ± 0.092	0.472 ± 0.063	ns
ADG_{FIN} (W7-19)	21	0.955 ± 0.043	0.932 ± 0.034	ns
$ADG_{LIFETIME}$ (W0-19)	21	0.732 ± 0.030	0.696 ± 0.065	ns

*includes data from only REP 1 farrowings (n = 25), whereas data up to and including nursery included REP 1 and REP 2 (n =15) farrowings

Table 4.2. Backfat and loin eye depth (mean ± SD) in pigs from CAUT and CTRL sows measured at 70kg (range 65-80) and 110kg (range 105-115) body weight

Variables	N	GRP		P-value
		CAUT	CTRL	
Weight at 70 kg	20	69.94 ± 3.2	70.2 ± 1.87	ns
Weight at 110 kg	21	109.6 ± 1.7	109.5 ± 1.0	ns
Backfat depth at 70 kg (mm)	20	10.23 ± 0.4	9.9 ± 0.3	ns
Backfat depth at 110 kg (mm)	21	10.3 ± 0.3	10.0 ± 0.2	0.02
Loin depth at 70 kg (mm)	20	53.7 ± 4.7	54.8 ± 4.8	ns
Loin depth 110 kg (mm)	21	70.0 ± 4.6	69.3 ± 2.9	ns

Table 4.3. Buffers used for the cortisol ELISA on saliva samples

Standard and Label Buffer (pH - 8)[S & L Buffer]	
Ingredients	Volume (1L)
Tris 50mMol (2.1)	1L
BSA 2	10g
NaCl 3	8.76g
Tween 4	500µl
Blocking Buffer (pH-8)	
Tris 50mMol (2.1)	1L
BSA [2]	10g
NaCl [7]	8.76g
Wash Buffer (pH-8)	
Tris 50mMol (2.1)	1L
NaCl [7]	8.76g
Tween [4]	500µl
Coating Buffer 50mMol	
DDH_2O	1L
Sodium Carbonate (Na_2CO_3) [5]	5.3g
Sodium Bicarbonate ($NaH\ CO_3$) [6]	4.2g
Sodium Chloride (NaCl) [7]	2.9g

CHAPTER 5

CONCLUSIONS, CONSTRAINTS, AND FUTURE DIRECTION

This thesis describes the consequences of uterine crowding on embryonic and fetal growth and survival at gestation D35 and at farrowing. We also studied effects on postnatal piglet performance during lactation and the post-weaning periods, ACTH induced adrenal cortisol response, and backfat and loin depth of the offspring. In this concluding chapter, the key findings of the research are presented along with the strengths, limitations and the future direction for the further research.

To address the objectives of the thesis, unilateral oviduct cauterization was performed on 12 out of 25 multiparous sows, where the remaining 13 were kept as control. The data were collected from all the sows and their offspring. Eight cauterized and nine control sows were rebred and subsequently farrowed, and these offspring were tracked until 7 weeks of age. This experimental design allowed us to compare the differences between offspring that gestated in crowded and non-crowded uterine environments. As in previous studies, we observed that embryo survival decreased with increased ovulation rate. Birth weight and lactational average daily gain was also increased in litters from less crowded uteri. Despite cross fostering between sows of two groups to provide similar suckling environments, total pig weight at weaning did not differ between treatment groups, implying that a higher litter size does not necessarily result in higher total litter weight at weaning. This may be important insights for pork producers in that there are adverse consequences of selecting larger litters, such as decreased fetal survivability, birth weight, and weaning weight. Results from Chapter 4 demonstrate that uterine crowding not only affects embryonic and fetal survival, but also performance of offspring in later life, as higher average daily gain was noted in pigs from cauterized sows. More importantly it showed that birth weight is the most influential factor affecting postnatal growth and performance. Another important finding of this study is that growth during the lactational period, in addition to during gestation, is very important for better postnatal performance.

From this research we conclude that selection of pigs not only on the basis of litter size but also

for higher birth weight will be advantageous for the pig producers. In addition, better lactational management is important as it helps to compensate for low birth weight.

The main constraint of our research was the sample size. A sample size of 25 may not be enough to generalize the differences noted in pre- and postnatal performance. By increasing the sample size we might have strengthened our results but this would have required more financial, human and animal resources that were available for the research. Secondly, the ovulation rate of the multiparous sows used in the research was not as high as we had anticipated. Using breed of sows with even higher ovulation rates and selecting two or more breeds of sows, might have provided more interesting and comparative results. We only kept the pigs of the subsequent farrowing (REP 2) up to the end of nursery. Future direction for the research would be to extend the study to subsequent farrowings (i.e. additional parities). The data from consecutive farrowings may provide a better understanding of the long term consequences of selecting sows for higher litter size.

Further research can be directed in looking the relationship between intra-uterine growth retardation and immune function. From previous studies it has been found that development of immune system and the induction of immune tolerance occur in early life. Challenge studies against disease like swine influenza, and immune parameters post injection can be measured. The antibody response to vaccination against the different diseases in the pigs from cauterized and non cauterized pigs can be measured. Additionally lymphocyte proliferation test to count total lymphocytes could also show if there is any effect of intrauterine crowding on long term immune function.