Early indicators of a gilt's reproductive potential

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Abstract

The reproductive output of the sow breeding herd determines the productivity and subsequent profitability of any piggery. The average replacement rate for Australian sow herds is currently 55-58% (Australian Pig Industry Benchmarking Report 2019-2021), which is much higher than the acceptable level of 38% (Hughes and Varley, 2003). To reduce this replacement rate, the aim of this thesis was to determine if the reproductive potential of a replacement gilt could be identified prior to weaning, by determining a suite of markers that are indicative of *in utero* developmental programming of the reproductive axis. The markers of developmental programming and reproductive potential assessed in this thesis included birth weight, maternal parity, anti-müllerian hormone (AMH) concentration and gestated sex ratio of the litter. The overarching hypothesis was that low birth weight, gilt progeny would be reproductively inferior and less able to cope with suboptimal management, as proven by an imposed lactational feed restriction. The results of the three studies conducted suggest that selection of replacements from gilt litters is acceptable, providing they are not classified as low birth weight. While some significant differences were found in the ovarian development of offspring of male or female biased litters, the main commercial implication of the current study is that until further research is conducted selection from biased litters should be avoided. The results of this thesis conclude that the developmental programming of a gilt plays a significant role in her reproductive potential. To reduce replacement rates of the Australian sow herd the developmental programming needs to be considered when selecting potential replacement gilts.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

I give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

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Signed:

Lauren Staveley

Date: 10 December 2022

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Chapter 1: Literature Review

1.1 Introduction

The reproductive quality of a farm's breeding sow herd ultimately determines the productivity and subsequent profitability of any piggery. The average replacement rate for Australian sow herds currently sits between 55-58% (Australian Pig Industry Benchmarking Report 2019-2021), and while this seems representative of the global situation (Spinka and Illmann, 2015), it is much higher than the acceptable level of 38% required to improve productivity and profitability of the herd, as reported by Hughes and Varley (2003). The causes of the suboptimal performance of the Australian herd fall under two main categories; the first being poor management, and the second due to reproductive failure (Patterson and Foxcroft, 2019). This combination has led to high incidences of premature culling, resulting in early parity sows making up the vast majority of the current herd. This is disadvantageous to the productivity of the herd, as litter size tends to increase up to the fourth or fifth parity (Alsing *et al*. 1980), due to an increase in both ovulation rate and embryonic survival. Management practices should therefore be focused towards improving sow retention, as this will increase herd average litter size, individual sow lifetime piglet production, and ensure a positive financial return on gilt investment costs.

One of the fundamental performance indicators of sow productivity is the number of pigs produced per year. In Australia the average number of piglets weaned per sow per year is 23, with an average of 11.5 pigs born alive (Australian Pig Industry Benchmarking Report 2021). Many European countries are able to achieve greater numbers, with the Danish average sitting at 34 pigs per sow per year and an average born alive of 19.9; however, this increase is

also associated with an increase in piglets born dead and pre-weaning mortality (Australian Pig Annual, 2013), with the average total piglet mortality of Danish herds in 2021 sitting at 23.4% (Hansen, 2022). Therefore, further research is necessary to determine whether it is beneficial to produce higher quality pigs or simply produce more piglets.

This review will summarise the demands placed on the modern sow by production systems, pre and post-natal development of the reproductive axis, and provide an overview of the concept that lifetime reproductive performance is determined by the conditions experienced *in utero*.

1.2 Demands on modern sows and current requirements

Breeding sows are under immense pressure to perform on a yearly basis. Current, accepted reproductive targets for Australian breeding sows are 2.4 litters per year and >25 piglets weaned per sow each year, with a total born of >13 piglets per litter, and conception and farrowing rates in excess of 90% and 85%, respectively (Australian Pig Annual 2014). Reproductive failure is the single highest reason for sow removal, and is more prevalent in sows of parity 2 or less (D'Allaire and Drolet, 1999). Reproductive failure can be attributed to a number of reasons, including, but not limited to; failure to achieve puberty, anoestrus following puberty or weaning, irregular returns to service post-weaning and failure to farrow. Since it is gilts and primiparous sows that have the greatest rates of reproductive failure (D'Allaire and Drolet, 1999) it stands to reason that the greatest improvement in sow retention rates will be achieved by focusing on strategies to improve the reproductive potential, and output, of these younger animals.

1.3 Pre-pubertal development of the female reproductive system in pigs

1.3.1 Prenatal ovarian development

The development of the hypothalamic-pituitary-gonadal axis occurs largely prior to birth. Within the ovary, processes such as migration, proliferation, degeneration, meiosis of germ cells, and folliculogenesis determine the size of follicle population at birth and the number of follicles that can be recruited during postnatal life. The initial growth of the ovarian follicle pool in pigs occurs relatively rapidly with the first primordial follicles beginning to appear between day 60 and 70 post conception (Christenson *et al*. 1985; McCoard *et al*. 2003), and the first primary follicles appearing around day 70 post conception. After this rapid initial growth, development slows with the first secondary follicles appearing around the time of birth, at 115 days post conception (Oxender 1979; Christenson *et al*. 1985). Formation of the ovarian follicle pool is completed during the pre- and early peri-natal period, providing the female pig with a finite supply of oocytes that is drawn upon throughout the animal's reproductive life (Kezele *et al.* 2002).

1.3.2 Prenatal development of the reproductive endocrine axis

Prenatal development of the reproductive axis commences with the differentiation of the pituitary, occurring between days 50-110 post conception. This differentiation is associated with an increase in the number of gonadotrophic cells and a corresponding rise in concentrations of the gonadotrophic hormones, follicle stimulating hormone (FSH) and luteinising hormone (LH) (Christenson *et al*. 1985). It is during this period, specifically days 60 - 80, that pituitary release of LH comes under the control of the hypothalamus (Parvizi, 2000) and the pituitary acquires the ability to respond to exogenous GnRH stimulation with elevated LH secretion. This phase is associated with an increase in pituitary concentrations of FSH and LH, in addition to the growth of gonadotrophic cells (Christenson *et al*. 1985). Circulating

concentrations of LH and FSH remain low before day 80 post conception with pituitary LH being measurable from approximately day 60 of gestation (Elsaesser *et al*. 1988). Follicle stimulating hormone increases until day 105 (Thomas *et al*. 1993), with LH reaching maximum plasma levels around day 90, before declining towards the end of gestation (Parvizi, 2000). Early work completed by Colenbrander *et al.* (1982) indicated that maturation and development of the fetal ovary occurs independently of gonadotropic support, and this is supported by the fact that although oestradiol concentrations rise during the fetal period of development, LH release occurs independently of ovarian negative feedback control (Elsaesser *et al.* 1979; Wise *et al*. 1981; Colenbrander *et al.* 1982).

1.3.3 Pre-pubertal follicle growth

Pre-antral follicles first begin to appear in the ovary at birth, forming approximately 30% of the follicle pool by around 90 days of age (Oxender *et al*. 1979). Constant exposure to increasing levels of FSH during the post-natal period drives the first appearance and continued proliferation of the antral follicle pool (Hughes *et al*. 1990; Evans and O'Doherty, 2001), as FSH promotes the transition of follicles from pre-antral to antral stages of development (Mao *et al*. 2002). Antral follicles begin to develop on the ovary at around day 60-70 after birth (Oxender *et al*. 1979; Dyck and Swiersta, 1983; Guthrie *et al*. 1984), with the mean number of small antral follicles increasing from 2 to 306 per ovary between days 70 to 112 post-partum (Dyck and Swierstra, 1983). From day 112 until the onset of puberty, the number of small follicles gradually decreases as the number of large follicles begins to increase (Dyck and Swierstra, 1983). Bolamba *et al*. (1994) conducted laparoscopic examinations of gilts at 5 day intervals between 160-180 days of age. This study reported dynamic and often rapid changes in the number of small and large ovarian follicles, concluding that during the pre-pubertal period ovarian follicle growth occurs in waves, and is characterised by the gradual growth of a pool of follicles. In the absence of the pattern of LH release that is required to support follicle

growth through to ovulation, these waves of follicles become atretic and are succeeded by the next wave of follicles (Foxcroft, 1991; Bolamba *et al*. 1994).

1.3.4 Pre-pubertal patterns of gonadotrophin release

The pre-pubertal period is characterised by the constantly changing patterns of gonadotrophic and steroid release (Lutz *et al*. 1984; Diekman and Trout, 1983; Camous *et al*. 1985; Dyck, 1988; Evans and O'Doherty, 2001). Follicle stimulating hormone concentrations increase during the peri-natal (Colenbrander *et al*. 1982) and post-natal periods (Camous *et al*. 1985), with maximum levels observed in 70 - 75 day old gilts, before declining from 80 days onwards (Colenbrander *et al*. 1982; Guthrie *et al*. 1984; Camous *et al*. 1985; Christenson *et al*. 1985; Foxcroft *et al*. 1985).

As described by Camous *et al.* (1985) during the initial 40 days of post-natal life LH levels continue to decline, followed by an increase in the frequency and amplitude of LH pulsing. This increase results in a corresponding rise in basal LH concentrations between 40 and 125 days of age. Plasma LH concentrations, pulse frequency and amplitude once again begin to decline between days 125 and 192 post-partum. Camous *et al.* (1985) observed LH pulses in only 65% of gilts between days 125-192 post-partum in comparison to 85% of gilts between days 83-125 of age.

Circulating concentrations of oestrogen and progesterone remain low during the pre-pubertal period (Elseasser, 1982; Pressing *et al*. 1992; Bolamba *et al*. 1994). Slight rises in oestradiol concentrations from 150 and 210 days of age have been characterised as occurring in surges, coinciding with the appearance and proliferation of the antral follicle pool (Camous *et al*. 1985; Pressing *et al*. 1992).

1.3.5 Pre-pubertal development of the reproductive axis

The synthesis and pulsatile secretion of the gonadotropin hormones, LH and FSH, are essential for the initiation and maintenance of reproductive cycles (Kraeling *et al*. 1986; Armstrong & Britt, 1987; Ford *et al.* 2000). During the gilt's first 90 days of life, serum concentrations of LH and pulse frequency are high (Christenson *et al*. 1985). Antral follicles first begin to appear on the ovary between 60 and 90 days of age (Oxender *et al.* 1979), as the hypothalamus and anterior pituitary gland become sensitive to oestrogen negative feedback (Foxcroft *et al.* 1984; Christenson *et al.* 1985). This sensitivity to oestrogen results in a pattern of LH secretion characterised by high-amplitude, low frequency pulses and a reduction in serum concentrations that continue until 10 to 20 days prior to puberty attainment. At this time the pattern of LH secretion changes to high frequency, low amplitude pulses, that result in the maturation of the ovarian follicles (Diekman *et al.* 1983; Lutz *et al*. 1984; Camous *et al*. 1985). A reduction in sensitivity to oestrogen negative feedback is what drives these changes in LH pulsatility (Berardinelli *et al.* 1984; Barb *et al.* 2010). Oestrogen from pre-ovulatory follicles increases the serum concentration of oestrogen, exerting a positive effect at the hypothalamus to stimulate greater secretion of GnRH, inducing an ovulatory surge of LH and resulting in pubertal oestrus and ovulation.

1.3.6 Mechanisms of puberty attainment

Puberty attainment in the pig is determined by the maturation of the hypothalamic ovarian pituitary axis. Female pigs are able to reach puberty at a relatively early age of 150-220 days and are considered to be polyoestrous breeders (Pond *et al*. 1991; Evans and O'Doherty, 2001; Soede *et al*. 2011). Prior to puberty attainment the concentration of oestrogen in circulation begins to rise (Pressing *et al*. 1992), reflecting successive waves of ovarian follicle growth (Bolamba *et al*. 1994). It is this rise in oestrogen concentration that triggers a cascade of endocrine events eventually leading to the onset of oestrus and ovulation (Paterson, 1980;

Esbenshade *et al*. 1982; Deligeorgis *et al*. 1984). However, the factor limiting the follicles from further developing is the low activity of the gonadotropin releasing pulse generator, which causes levels of LH to be inadequate and therefore unable to stimulate the final stages of follicular growth, consequently preventing ovulation (Camous *et al*. 1985; Plant, 2002).

Boar exposure is a commonly used method to reduce age at puberty attainment, due to the combined actions of olfactory, tactile, auditory and visual cues produced by the boar (Paterson *et al.* 1989; Hughes *et al.* 1990; Paterson *et al.* 1992; Patterson *et al*. 2002). It is an effective method of stimulating early puberty due to the release of priming pheromones, which increase the activity of GnRH neurons in the hypothalamus of the female pig (Rissman, 1996; Rissman *et al*. 1997; Bakker *et al*. 2001). Exposure to boars allows for alteration in the pattern of LH secretion in the gilt (Hughes *et al*. 1990; Kingsbury and Rawlings, 1993), which causes an increase in ovarian follicle growth, leading to an increase in circulating oestrogen concentrations.

1.4 Developmental programming

Developmental programming is defined as the effect the pre- and peri-natal environment can play in the future development and lifetime performance of the progeny. The majority of studies focusing on prenatal programming in pigs have investigated the impact of the maternal environment on disease risk and are covered in an excellent review by McMillen and Robinson (2005). For the purposes of this review, we will focus on the concept that reproductive performance is determined by conditions *in utero*, and not just post-natal or pre-pubertal management of the female pig.

1.4.1 Sex-bias and reproductive development

The gestated sex ratio of both litter bearing and non-litter bearing species has been shown to have significant effects on the reproductive potential of females, due to the effect sex bias can have on the steroidal environment *in utero*. The reproductive potential of any animal is influenced *in utero* by numerous genetic and environmental factors, with one environmental factor being the exposure to gonadal steroids. Based on studies in which sheep were treated with testosterone prenatally it is apparent that increased levels of testosterone caused by male littermates could lead to the androgenisation of the female, resulting in structural alterations in the ovary (Steckler *et al.* 2005). This androgenisation is the culmination of exposure to androgens, which are produced by developing male fetuses and pass through fetal membranes to masculinise females in the litter. This androgenisation of female fetuses affects behaviour and reproduction. Exposure to some androgens during gestation is normal; however, when this is in excess it can be detrimental to normal development. This excess could be due to either the overall proportion of males in the litter or the proximity to males. Thanks to work conducted by Vom Saal (1981) we know that the probability of a female fetus being positioned *in utero* between two males is a function of the litter size and the proportion of the litter that is male. In an industry that relies on reproductive uniformity, masculinisation could have potentially detrimental implications, as it has been proven to create non-genetic variation, as well as diversity in reproductive behaviour and timing of oestrus (Vom Saal, 1989; Uller *et al.* 2005). Non-genetic behavioural variation can be seen in the future offspring of a female and is caused by the intrauterine position of a female fetus. Female mice that were positioned between two females *in utero* were more likely to have a litter of 60% female, in comparison to females that developed next to one or between two males, which were likely to have litters consisting of 50% or 40% females, respectively (Ryan and Vandenbergh, 2002; Rekiel *et al.* 2012). In some species, the measure of anogenital distance (the distance between the anus and genitals) can be reflective of reproductive potential. Female mice that were

exposed to excessive androgens *in utero* had a longer anogenital distance, shorter reproductive lifespan, and produced smaller and fewer litters, that were also more likely to have a higher proportion of males (Vom Saal *et al.* 1999; Bánszegi *et al.* 2012). In comparison, females born to female biased litters had shorter anogenital distances, were more likely to be mated and gave birth to more litters (Vom Saal andh Bronson, 1978). Similarly, in pigs, females from male biased litters had longer anogenital distances and were less likely to conceive at their first mating in comparison to females from female biased litters (Drickamer *et al.* 1997; Vom Saal *et al.* 1999). Similar evidence of increased reproductive potential has been found in sheep, with the presence of a female co-twin increasing oocyte quality (Kelly *et al*. 2017). Recent work conducted by Seyfang *et al.* (2018) found that female pigs born to a female biased litter had a longer anogenital distance, contrary to previous findings. Although the measure of anogenital distance did not agree with previous research, similar effects on reproductive outcomes were found. Females born to female biased litters were heavier, achieved puberty earlier, mated younger, were more likely to be mated and gave birth to larger litters, when compared to females born to non-biased or males biased litters. Some of these weight and growth differences may be due to postnatal factors as work conducted by Dunshea (2001) found all female or equally mixed litters grew more quickly in the postnatal period when compared to all male litters. However, anogenital distance was only an effective predictor of reproductive outcomes at week 16 of life and not at any other time point (Seyfang *et al*. 2018). There is evidence that sows originating from female biased litters have increased reproductive potential, as they consistently farrow and wean a higher number of piglets, with these litters also tending to contain a higher proportion of females (Edgerton and Cromwell, 1987; Rekiel *et al*. 2012). The observed increase in the number of piglets weaned is likely a direct result of an increase in teat number. Drickamer *et al.* (1999a) reported that the number of teats on a gilt can be influenced by both the number of teats on the mother and the proportion of males in the litter, with gilts from female biased litters having a higher number

of teats than those from male biased litters. This has been consistently found across other species such as rabbits, mice and horses (Ryan and Vandenbergh 2002; Hotchkiss *et al.* 2007; Bánszegi *et al.* 2010) and is due to the increased testosterone *in utero* suppressing mammary tissue development (Kratochwil, 1971). We can therefore conclude that the sex ratio or sex bias of a litter should be considered when selecting gilts into the breeding herd.

1.4.2 Maternal Age

It is common practice on commercial pig farms to focus selection of breeding animals on progeny from multiparous sows; however, as gilts make up approximately one quarter of the breeding herd (Koketsu, 2007), selection from these gilt litters is likely. When compared to progeny from multiparous sows, the progeny of gilts were lighter at birth and weaning (Hendrix *et al*. 1978; Craig *et al.* 2017a), had reduced lifetime growth rates (Rehfeldt and Kuhn, 2006) and were more susceptible to disease (Miller *et al*. 2012; Carney-Hinkle *et al.* 2013; Craig *et al*. 2017a). Earlier studies concluded that gilt progeny had lower total serum IgG concentrations pre-weaning, and it was presumed that this was due to lower colostrum intake by the lighter gilt-reared progeny (Klobasa *et al*. 1986; Burkey *et al.* 2008). However, recent studies profiling the milk composition of primiparous and multiparous sows have shown no differences exist in the concentration of IgG, total protein, total fat and net energy (Quesnel, 2011; Decaluwe *et al.* 2013; Declerck *et al.* 2015; Craig *et al*. 2019). Therefore, it is more likely that the underperformance of gilt progeny can be attributed to lower colostrum and milk production, and subsequently less available to piglets, rather than reduced quality (Speer & Cox, 1984; King, 2000; Beyer *et al*. 2007; Hansen *et al*. 2012; Theil *et al*. 2012; Wijesiriwardana *et al.* 2022). Interestingly, the adaptive immune response to a novel antigen was reduced in gilt-born compared with sow-born progeny; however, this did not result in a significant difference in post-weaning survival (Miller *et al*. 2012).

Previous studies comparing light with heavy birth weight litter mates have demonstrated that low birth weight offspring have reduced survivability, reduced muscle growth potential, poorer carcass and meat quality, and take longer to achieve market weight and puberty than their heavier counterparts (Quiniou *et al*. 2002; Kuhn *et al.* 2003; Bee, 2004; Gondret *et al.* 2006; Rehfeldt and Kuhn, 2006; Foxcroft *et al.* 2009; Hales *et al.* 2013). It is well documented that gilt progeny are lighter than sow progeny, with gilt progeny having lower growth performance, muscle accretion and gastrointestinal development (Miller *et al*. 2012a; Alvarenga *et al.* 2013; Carney-Hinkle *et al*. 2013; Craig *et al.* 2017a). This lower birth weight is likely due to the lower uterine capacity of gilts, which increases competition between fetuses for nutrients and oxygen and results in lighter piglets at farrowing. In addition, the number of piglets produced per litter usually increases from first to third parity (Hughes and Varley, 2003), in line with growing uterine capacity.

Two large scale retrospective investigations were conducted on Australian commercial sites and included records on approximately 24,000 sows (Craig *et al*. 2017b; Hewitt *et al*. 2017). Both of these studies reported gilt progeny selected into the breeding herd to be one day older at first breeding with few significant differences in performance indices following this. As such it is thought that the majority of selection should occur from multiparous sows, due to their ability to produce heavier birth weight piglets; however, more research is necessary to optimise maternal age for selection purposes.

1.4.3 Uterine Capacity

Pigs are a polytocous species and as such they are capable of ovulating from between 15-30 follicles at a time (Soede *et al.* 2011), and if managed correctly will generally have fertilisation rates in excess of 95% (Pope and First, 1985). In polytocous species there is often considerable variation in embryonic growth, resulting in asynchronous development, and a proportion of the fetuses failing to receive sufficient nutrients (Dziuk, 1987; Pope, 1988; Geisert and

Schmitt, 2001). As such, despite the high number of oocytes shed at ovulation, only about 50-70% of these will develop into live piglets at birth (Ferguson *et al*. 2007). This is predominantly due to early embryonic loss (day 10 to 30 post conception) and fetal death (day 31 to 70) (Pope and First, 1985; Geisert and Schmitt, 2002). The causes of early embryonic loss are extensive, complex and often interrelated (Geisert and Schmitt, 2002), but are most commonly due to fertilisation of oocytes from less developed follicles and poor timing of conception. Early studies (Webel and Dziuk, 1974) indicate that decreasing available uterine space by 50% did not affect embryo survival to day 30 but resulted in a lower proportion of embryos surviving after day 30. Since this initial trial a number of studies have focused on experimentally increasing the number of embryos up to day 30 of gestation; however, in each study the number of pigs farrowed was never different to the control (Ford *et al.* 2002). These findings demonstrate that it is not until after day 30 of gestation that uterine capacity becomes a limiting factor and is therefore not associated with embryonic mortality, but is a factor associated with fetal death. As such, a focus on selection for placental efficiency may be a more effective way to improve litter sizes (Vonnahme *et al.* 2002; Vonnahme and Ford, 2004; Foxcroft *et al.* 2006). The placental efficiency of an individual is influenced by utero-placental and umbilical blood flow; the means by which the necessary nutrients for fetal growth are delivered by circulation to, and from, the placenta (Reynolds and Redmer, 2001). Pigs with increased placental efficiency have larger litters than pigs with lower efficiency (Wilson *et al.* 1999).

When uterine capacity is at its maximum, as inevitably occurs when selecting for prolificacy, the sow is unable to meet the circulatory demand of each fetus and as such a number of these fetuses will be compromised, ultimately resulting in fetal death, low birth weight and low viability piglets (Campos *et al*. 2012). Although total uterine blood flow increases as the

number of fetuses increases (Reynolds *et al*. 1985; Pere and Etienne, 2000) uterine blood flow per fetus decreased.

Although an increase in litter sizes may be achieved by simply selecting for an increase in live born pigs, the above studies suggest that many of the adverse prenatal programming effects associated with inadvertent uterine crowding will not result in an increase in net revenue per sow. This poses a major limiting factor on the profitability of a breeding sow as an increase in litter size is currently correlated with an increase in lightweight and low viability piglets. Therefore, selection for placental efficiency, rather than litter size, may be the most economical way to improve the number of pigs weaned per sow (Vonnahme *et al.* 2002; Vonnahme and Ford, 2004; Foxcroft *et al*. 2006).

1.4.4 Sub optimal maternal nutrition

The majority of research into the long-term effects that sub optimal maternal nutrition can have on offspring has investigated the linkage to increased risk for adult-onset disease, including cardiovascular disease, obesity and diabetes (Gluckman and Hanson, 2004; McMillen and Robinson, 2005; de Boo and Harding, 2006). As such, it is well known that maternal nutrition influences the prenatal growth potential and physiology of the major organ systems (Robinson *et al*. 1999); however, there have been minimal studies conducted into the effects of maternal nutrition on the subsequent reproduction of their offspring. The reproductive potential of an animal depends on the development of the reproductive axis, with most of the structural and neuroendocrine development occurring prior to birth (McNatty *et al.* 1995). Studies conducted on animals and humans have shown that environmental factors, such as maternal nutrition, can have adverse effects on prenatal growth, subsequently influencing aspects of postnatal reproductive development that affect age at puberty (Da Silva *et al.* 2001) and age at menopause (Cresswell *et al.* 1997). Borwick *et*

al. (1997) conducted a comparison of twin bearing ewes that received either a high (150%) or low (50%) proportion of their energy requirements for maintenance during the first third of pregnancy, and found that germ cell degeneration in the fetal ovary was delayed in the low group at day 47 and 67 of gestation, occurring independently of changes in placental or fetal mass. In contrast, both Wallace *et al.* (1996, 1997) and Da Silva *et al.* (2002) found that over feeding of adolescent sheep throughout gestation resulted in rapid maternal growth rates at the expense of the nutrient requirements of the gravid uterus. This resulted in a significant change in expression of LHβ mRNA in the pituitary gland of fetuses of over-fed dams, and a reduction in placental growth and progeny birth weight when compared with moderately fed adolescent ewes. In cows, restricting feed intake to 60% of maintenance energy requirements prior to conception and through the first trimester resulted in a 60% reduction in antral follicle counts of the offspring when compared with control animals, despite no differences in birth weight (Mossa *et al.* 2009). These findings imply that maternal nutrition may play a significant role in development of the ovarian reserve, as antral follicle counts are positively correlated with the size of the ovarian reserve (Ireland *et al*. 2008).

In addition to the effect maternal nutrition can have on the reproductive potential of a gilt, it has been widely reported that under nutrition in gestation resulting in light weight piglets, is primarily associated with a reduced number of secondary muscle fibres (Handel and Stickland, 1987; Dwyer *et al*. 1994). This effect of maternal nutrition occurs between day 25 and 50 of gestation, which is the period immediately after secondary muscle fibre hyperplasia (Dwyer *et al.* 1994). Dwyer *et al.* (1994) also reported a positive correlation between total number of muscle fibres and growth potential, establishing that littermates with a higher number of fibres grew faster and more efficiently than littermates with lower muscle fibre numbers.

Previously, imposing feed restriction in late lactation in pigs reduced oocyte quality, subsequent litter size and embryonic survival while increasing weaning to oestrus interval (Zak *et al.* 1997*a*, 1997*b*; Foxcroft *et al*. 2005; Vinsky *et al*. 2006). However, later studies using similar experimental designs and achieving similar levels of sow tissue catabolism have reported a reduced effect on reproductive performance following weaning, with the only consistent effect being the decrease in embryo weight of the subsequent litter (Oliver *et al*. 2011; De Bettio *et al.* 2016). De Bettio *et al*. (2016) placed sows on a 50% feed restriction for 21 days of lactation, observing no reduction in reproductive output. These results suggest that due to the genetic selection for increased ovulation rate and litter sizes, the biology of the commercial sow may have been altered, with sows better able to adapt to the metabolic challenges associated with tissue mobilisation during lactation. It can therefore be concluded that maternal nutrition plays an important role in the health, reproductive development and feed efficiency of the progeny, making it an important factor to consider in breeder selection programs and for efficient pork production. However, further research into the biology of the modern sow, and how selection pressures may have altered the nutritional requirements in lactation is necessary, before any conclusions can be drawn.

1.5 Phenotypic markers of reproductive success

Selection of replacement gilts at birth often begins with selection of the larger female progeny of purebred lines. Each farm will have various other criteria, but size is usually the starting point of any protocol. While this has been reasonably successful the Australian industry still has a replacement rate of 56.1% (Australian Pork Limited, 2013; Australian Pig Industry Benchmarking Report 2019-2021). It is therefore necessary to find and introduce phenotypic markers of reproductive success to this selection protocol. A proven phenotypic marker has been reported by Foxcroft and Patterson (2010) that gilts that reach puberty more rapidly in

response to boar contact are likely to be retained in the breeding herd and have a longer reproductive lifetime when compared to their slower developing counterparts.

While phenotypic markers such as birth weight and plasma concentration of anti-müllerian hormone (AMH) may prove to be effective, the environment these animals are reared in must also be considered. Phenotypic plasticity is the ability of one genotype to produce more than one phenotype when exposed to different environments, therefore a variety of factors must be considered such as the size of the litter the progeny are reared in, both *in utero* and/or during lactation, as well as pre-weaning growth, mortality and morbidity.

1.5.1 Birth weight

Productivity of the breeding sow depends partly on the number of piglets born and weaned per litter, with more piglets born and weaned increasing profitability of the breeding herd (Stein *et al*. 1990; Tummaruk *et al*. 2001). Due to increased incidence of premature culling, and reduced age at culling, early parity sows make up a high percentage of the breeding herd, with profitability becoming increasingly dependent on litter sizes at early parities (Hughes and Varley, 2003). However, this dependence on high litter sizes could potentially have detrimental effects on herd genetics and piglet survival. Increases in gestational litter size are associated with more lightweight and potentially low viability piglets born in each litter (Campos *et al.* 2012). As the pig is a highly fertile species each conception yields more embryos than the female can support, resulting in high early embryonic loss and often a number of lightweight and low viability piglets at farrowing. It is these low birth weight and low viability piglets that increase pre-weaning mortalities and decrease sow efficiency. Work conducted in both indoor and outdoor farms across Europe has found that production records showed a detrimental influence of high average total born on piglet mortality and increases in birth weight variability (Prunier *et al*. 2014). These substantial increases in litter sizes can detrimentally affect pig welfare, particularly when the litter size is supernumerary to

functional teats, leading to intense teat competition and starvation (Rutherford *et al*. 2013). Low birthweights can affect following generations, with lightweight piglets giving birth to successive litters that are smaller in both number and weight (Corson *et al.* 2009). This is partly due to the birth weight of a piglet affecting ovarian mass and follicular development, with activation and growth of the primordial follicle pool impaired in low birthweight piglets (Corson *et al.* 2009). Birth weight was directly positively correlated to ovarian mass and the number of primary follicles at birth (Da Silva-Buttkus *et al*. 2003). It is therefore assumed that the intergenerational repercussions of low birth weight are due to alterations in the size of the ovarian follicle pool and, possibly, ovarian function. Da Silva *et al.* (2002) also observed that the morphological structure of the ovary collected from low birth weight piglets at farrowing resembled that of an ovary that had been collected from a piglet several weeks prior to birth. Regardless of the physiological issues these lightweight piglets face they are also at a physical disadvantage, with a lower capacity to compete with heavier littermates for colostrum and lower energy reserves to enable them to adapt to extra uterine life (Hayashi *et al.* 1987; Fraser *et al.* 1995). Therefore, it is important to include birth weight in any selection program, whether the breeding herd or commercial production, as a decrease in birth weights will have a detrimental effect on both the short and long term output of any production unit.

1.5.2 Anti-Müllerian hormone (AMH)

AMH, also known as Müllerian-inhibiting substance (MIS), is a dimeric glycoprotein, and member of the transforming growth factor-beta (TGFβ) superfamily of growth and differentiation factors. AMH is an important intra-ovarian regulator of follicle growth (Durlinger *et al.* 2002) and has been shown to play an inhibiting role in the cyclic process of follicular recruitment by determining FSH threshold levels (Almeida *et al.* 2018). This function acts to inhibit excessive recruitment of follicles from the primordial pool into primary follicles

and prevent premature exhaustion of the ovarian follicular reserve (di Clemente *et al.* 1994; Monniaux *et al.* 2012).

1.5.2.1 The role of AMH in regulation of ovarian reserve

Embryonically, AMH is the growth factor that induces degeneration of the paramesonephric Müllerian ducts during male fetal sex differentiation (Behringer *et al*. 1994). AMH along with its receptor, AMHR2, is expressed in granulosa cells of primary and growing follicles in the postnatal ovaries of gilts (Rajpert-De Meyts *et al*. 1999; Weenen *et al.* 2004; Baarends *et al.* 1995; Durlinger *et al.* 2002). In AMH-deficient mice, the absence of AMH results in early depletion of the primordial follicle pool in comparison to control mice (Durlinger *et al*. 2002). Between 4 and 13 months of age, AMH-deficient female mice have fewer primordial follicles, suggesting that AMH inhibits growth and recruitment of primordial follicles, with an absence of AMH leading to more rapid depletion of the primordial follicle pool. AMH reduces the sensitivity of follicles to FSH, with the absence of AMH potentially having a proliferative effect in the sexually mature ovary, therefore leading to greater recruitment of follicles and early depletion of ovarian reserve (Durlinger *et al.* 2001). AMH decreased the sensitivity and responsiveness of growing follicles to FSH in cultured granulosa cells collected from immature ovaries of rats and pigs (Di Clemente *et al*. 1994). These findings were supported by Durlinger *et al.* (2002) who conducted *in vitro* studies on the ovaries of two-day old postnatal mouse ovaries. When the ovaries were cultured in the presence of AMH they showed fewer growing follicles than when cultured in the absence of AMH. The results suggest that AMH is one of the factors determining sensitivity of ovarian follicles to FSH, and that AMH is a dominant regulator of early follicle growth. Behringer *et al.* (1990) found that transgenic mice that over-express AMH are infertile and have fewer germ cells at birth, and lose all germ cells in the span of two weeks post birth. Furthermore, AMH concentrations have been found to decrease

in line with the decline in follicle numbers as rodents age (Kevenaar *et al*. 2006) and women (Piltonen *et al*. 2005; van Rooij *et al*. 2005).

In many species, AMH is expressed solely by the granulosa cells of pre-antral and small healthy antral follicles, (Ibanez *et al*. 2002; Da Silva-Buttkus *et al*. 2002, 2003; Cushman *et al.* 2009; Ibanez and de Zegher, 2006; Hansen *et al*. 2011), as shown in cattle using immunohistochemistry. Studies of intrafollicular AMH levels in antral follicles of goats, sheep and cows found AMH concentrations to be highest in small antral follicles, decreasing markedly as follicles increased to their preovulatory size (Monniaux *et al*. 2012). Monniaux *et al.* (2012) reported a significant relationship between the number of small antral follicles and circulating AMH concentrations, concluding that changes in individual concentrations can be attributed to the numerical changes in the population of high AMH-secreting follicles. However, this does not appear to be true for porcine AMH. When intrafollicular AMH concentrations were measured in cycling gilts no significant difference was found in follicular fluid collected from antral follicles of varying sizes (Monniaux *et al*. 2012). In this particular study, follicular AMH concentrations were approximately 300 – 400 times lower in pigs when compared to goats, sheep and cows; however, this was concluded to be likely due to the differences in the affinity of the AMH antibodies in the AMH immunoassay. Recent findings, using porcine AMH specific ELISAs, found that contrary to other species, prepubertal AMH concentrations in pigs were not indicative of antral follicle counts in both immature and pubertal gilts (Steel *et al.* 2019). These findings can possibly be explained by work conducted by Almeida *et al.* (2018), demonstrating that unlike other species, expression of porcine AMH is not limited to the granulosa cells of growing follicles, but it is also expressed by the theca cells of preovulatory follicles, and the luteal cells post ovulation. It was hypothesised by Almeida *et al.* (2018) that the role of AMH expression in the luteal cells of the corpus luteum may be to reduce the action of FSH, thus preventing unnecessary follicle recruitment and early

exhaustion of the ovarian follicle pool. In the pig, the onset of increasing progesterone levels occurs 40-48 hours after the LH surge (Soede *et al*. 1994), unlike most species where this occurs around the same time as the LH surge (Noguchi *et al.* 2010). This delay results in the initial rise in FSH levels immediately after the LH surge (Noguchi *et al.* 2010), leading to increased follicular recruitment and wasting of follicular reserve.

1.5.2.2 The use of AMH as a marker of ovarian reserve in livestock species

In female ruminants, AMH is expressed solely in the granulosa cells of healthy, growing ovarian follicles (Monniaux *et al.* 2012), with expression occurring from the secondary follicle through to early antral follicle stages, later decreasing as follicle size increases (Campbell, *et al*. 2012). Importantly, atretic follicles do not express AMH, and AMH can, therefore, be used as a reliable marker of the number of healthy growing follicles present on the ovary (Campbell *et al*. 2012). As such AMH is currently used as a reliable endocrine marker of ovarian reserve of growing follicles in sheep (Lahoz *et al.* 2012), and in cattle as a predictor of lifetime fertility (Rico *et al*. 2009). Concentrations are highly variable between individual cows and goats (Ireland *et al.* 2008, Ireland *et al.* 2011; Rico *et al*. 2009; Monniaux *et al*. 2011) as this is closely related to the number of small antral follicles. This large inter-individual variability in follicular populations was also reported previously in cattle with the use of ovarian histology and ultrasonography (Durocher *et al.* 2006; Ireland *et al*. 2007). As expected, individual plasma AMH levels remain static throughout the oestrous cycle (Ireland *et al.* 2011), as the population of small antral follicles is considered to show little numerical change during this period. Similarly, in the goat, a seasonal breeder, plasma AMH concentrations showed little change with season (Monniaux *et al.* 2011). In a study involving cyclic and pregnant mares, no difference in AMH concentration was observed regardless of cycle stage, or month of gestation (Almeida *et al*. 2011). In cows, plasma AMH concentrations are an accurate predictor of an individual's response to the gonadotrophin hormones (Rico *et al.* 2009) used

to hyper-stimulate the ovary to maximise the number of fertilised and transferable embryos collected (Monniaux *et al.* 2010). However, the high degree of unpredictability in the ovarian response to these hormonal protocols affects both the profitability and efficiency of embryo transfer programs. Therefore, a single sample of plasma AMH can be used to accurately predict the ovulatory responses of individual animals prior to undergoing any kind of multiple ovulation process. As summarised in a recent review by Daly *et al.* (2020), it has been found that donor heifers and cows with high AMH enrolled in multiple ovulation embryo transfer or multiple *in vitro* embryo transfer programs, produce more total embryos (Sakaguchi *et al*. 2019), more transferrable embryos (Hirayama *et al*. 2012), and a higher percentage of embryos transferred resulting in viable offspring (Ghanem *et al*. 2016). There is the potential for similar results in donor lambs for juvenile *in vitro* embryo transfer programs, as animals with higher AMH concentration at 5 weeks of age had more antral follicles and a higher rate of blastocyst development following hormonal stimulation at 6-8 weeks of age (McGrice *et al*. 2020). This suggests that AMH could prove to be a successful selection tool for donor ewes. There is a lack of research able to describe the association between prepubertal AMH concentrations and lifetime fertility in the pig. However, recent work conducted by Am-in *et al*. (2020) has found a positive correlation between prepubertal serum AMH concentrations and age at puberty attainment. Am-in *et al.* (2020) demonstrated that gilts with higher levels of AMH reach spontaneous puberty earlier than gilts with lower levels, and anoestrous gilts have significantly lower levels of AMH than any animal that attained puberty. As such, AMH is well situated for the potential use as an early life indicator of increased ovarian reserve and quality in the pig, but further research is necessary.

1.5.3 Colostrum

Colostrum is the milk produced by the sow during and immediately post farrowing, forming during late pregnancy through the process of colostrogenesis (Langer, 2009). The composition

of colostrum is optimised to support the immunological, nutritional and developmental needs of the piglet (Farmer and Quesnel, 2009), with intake being a major determinant of neonatal survival and development (Farmer and Quesnel, 2009; Vallet *et al*. 2013). The lactocrine hypothesis is well reviewed by Bartol *et al*. (2017) and predicts that disruption of lactocrine signaling will affect the developmental trajectory of uterine tissues and compromise reproductive performance. Multiple studies have tested this hypothesis and found that low colostrum intake (as measured by serum iCrit values at day 0 of life) was associated with reduced lifetime fecundity (Bartol *et al*. 2013), reduced average pre-weaning growth rate, increased age at puberty and a reduction in number of piglets born alive (Vallet *et al*. 2015). As previously described low birth weights, especially when associated with high litter sizes, reduces the piglet's ability to consume colostrum due to already low energy reserves (Hayashi *et al.* 1987; Fraser *et al.* 1995) and potentially high competition for teat access (Rutherford *et al*. 2013). In addition, maternal parity may play a role in the availability of colostrum, as multiple studies have described the underperformance of gilt progeny (Speer & Cox, 1984; King, 2000; Beyer *et al*. 2007; Hansen *et al*. 2012; Theil *et al*. 2012). This was previously thought to be due to lower quality colostrum; however, recent studies profiling the milk composition of primiparous and multiparous sows have shown no differences exist in the concentration of IgG, total protein, total fat and net energy (Quesnel, 2011; Decaluwe *et al.*, 2013; Declerck *et al.*, 2015; Craig *et al*. 2019). Therefore, it is more likely that the underperformance of gilt progeny can be attributed to lower colostrum and milk production, and subsequently less available to piglets, rather than reduced quality. As such birth weight and maternal parity are both factors that should be considered as insufficient colostrum ingestion at birth may impair reproductive and uterine gland development as well as lactation performance of replacement gilts.

1.6 Conclusion

The productivity and subsequent profitability of the Australian pork industry is currently limited by high replacement rates of gilts and sows. Reproductive failure is the single largest cause of replacement within breeding herds, and therefore, more research is necessary to address this issue. Sow performance is highly variable, and it is this variability that lowers the overall performance of the Australian herd. As such the simplest way to decrease this variability would be to develop a suite of early phenotypic indicators of a sow's reproductive potential. Due to the extensive effects of prenatal programming, it is essential that the gestational environment and maternal characteristics be taken into consideration when selecting gilts. It is logical that the phenotypic markers of maternal age, gestated sex bias, birth weight and AMH concentrations prepubertally, should give a clearer prediction of an animal's ovarian reserve and reproductive potential, allowing for selection during the pre-weaning period and enabling greater manipulation of the subsequent environment that the animal is reared in. If these easily identifiable measures were incorporated into the current selection process it is possible that the replacement rate could be reduced relatively quickly, improving both the productivity and profitability of the Australian pork industry.

This thesis presents the results from three aspects of a single study investigating the role of developmental programming on the reproductive potential of gilts. The objectives of this thesis were:

- To determine if a suite of easily identifiable, neonatal indicators (birth weight, maternal parity and AMH concentrations) should be identified for use within commercial systems when selecting replacement gilts
- To determine if the gestated sex ratio of a litter should be used as a tool for selection or exclusion of gilts from the replacement herd

- To determine if developmental programming, as assessed by birth weight and maternal parity, plays a role in a sow's ability to cope with a lactational feed restriction.

The overarching hypothesis was that low birth weight, gilt progeny would prove to be reproductively inferior and less able to cope with suboptimal management, when compared to high birth weight, multiparous progeny, as proven by the lactational feed restriction.

Additionally, we hypothesised that

- gilts with higher circulating concentrations of AMH pre-pubertally would be indicative of gilts with a higher ovarian reserve and quality.
- gilts gestated in a male biased litters would show reduced fertility, when compared to gilts gestated in litters that were either female or unbiased.

Chapter 2: Methods

All animal procedures were conducted at the University of Adelaide's piggery, Roseworthy, South Australia with approval from the Animal Ethics Committee of The University of Adelaide, in accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council, 2013).

2.1 Experimental animals

Seven hundred and eighty four piglets were initially selected from 196 Large White x landrace litters (parities 0-7, average parity 1.6) over 20 replicates between March 2015 to May 2017 for inclusion in this study. Of the 784 piglets, 214 had reproductive tracts collected and 126 entered the Nutritional Challenge component of this study. On the day of farrowing (day 0) number of piglets born alive, number of stillborn piglets, piglet sex and the sex ratio of the litter were recorded. Piglets were individually weighed and the two heaviest (1.21 - 2.60 kg, average 1.79 kg) and lightest (0.80 - 2.02 kg, average 1.35 kg) female piglets from each litter were identified and tagged (focus piglets). Piglets weighing less than 800 g at birth were deemed to be low viability and were excluded from the study. Focus piglets remained with their litter, minimal fostering occurred, and litter sizes were not standardised, but were recorded, to minimise interference with production protocols. These focal animals were managed according to the farm's production protocols, with gilts either sent to a local abattoir at day 154 ($\pm$ 9) for collection of reproductive tracts (n = 214) or selected to be replacement animals (n = 126) and managed within the breeding herd. These focal piglets were allocated to observation groups dependent on their birth weight and maternal parity (H0; High birth weight born to a gilt (n = 69), L0; Low birth weight born to a gilt (n = 50), HM; high birth weight born to a multiparous sow (n = 133), and LM; Low birth weight born to a multiparous sow (n = 94)).

2.2 Piglet measures

At 24 hr post-farrowing, every piglet within each litter was weighed individually, and a 3 mL blood sample was collected from the focal piglets by venipuncture of the vena cava using a 23-gauge ¾" needle and syringe. Following collection, the blood sample was evenly divided between a 4 mL lithium heparin coated Vacutainer (Vacuette®, Griener Labortechnik, Austria) for plasma collection, and a 4 mL silica coated Vacutainer (Vacuette®, Griener Labortechnik, Austria) for serum collection. Samples for plasma collection were stored on ice before being centrifuged for 15 min at 3000 x rpm within 1 hr of collection, and plasma was removed from the blood tube and stored at -80°C. Samples for serum collection were stored for 24 hr at 4°C and centrifuged for 15 min at 3000 rpm the following day. Serum was removed and stored at -20°C. Focus piglets were individually weighed at weaning (20 ± 0.25 days), and day 154 of life. Blood samples were collected at weaning (4 mL) and day 154 (9 mL) into a lithium heparin coated Vacutainer (Vacuette®, Griener Labortechnik, Austria). Samples for plasma collection were centrifuged for 15 min at 3000 rpm within 1 hr of collection, and plasma was removed and stored at -80°C.

2.3 Reproductive tract collection

Two hundred and fourteen of the focus animals were sent to the abattoir at 154 days of age for collection of reproductive tracts. Tracts were recovered within 20 min post-mortem. Reproductive tracts of selected gilts were collected opportunistically, with collection from as many animals as possible whilst limiting interference both on farm and at the abattoir. The left ovary from 121 randomly selected gilts was used to provide oocytes for *in vitro* embryo production, while the right ovaries from 40 randomly selected gilts were used for histological analysis. Ovaries were removed from the tract and the left ovary was placed into 50 mL

phosphate buffered saline (PBS) at 33°C and 7.1 pH and stored in a warm foam box, while tubes containing the right ovary were placed on ice. The ovaries were transported to the laboratory within 1 hr of collection.

2.4 In vitro embryo production

All chemicals were obtained from Sigma Chemical Co. (St Louis, MO, USA) unless otherwise stated.

2.4.1 Collection and oocyte recovery

The left ovary of 121 gilts, randomly selected from the first 10 replicates, was used for *in-vitro* embryo production. In the laboratory, excess moisture was removed from each ovary with paper towel. The ovary was weighed, and the diameter of all visible surface follicles was measured using electronic Vernier calipers (Absolute Digimatic Caliper, Mitutoyo, Japan). Follicles measuring <4 mm (small follicles) were counted, follicles ≥4 mm (large follicles) and ≥6 mm (preovulatory follicles) were counted, diameter recorded, and follicular contents were collected by aspiration into a sterile 5 mL syringe, using an 18-gauge needle. Cumulus oocyte complexes (COCs) were collected into Hepes buffered TCM199 containing 4 mg mL^{-1} Bovine Serum Albumin (BSA) (Fraction V; Invitrogen Corp., Auckland, New Zealand), 100 μg mL^{-1} streptomycin sulphate (CSL Limited, Parkville, Victoria, Australia), 100 IU mL^{-1} penicillin G (CSL Limited), and 100 IU mL^{-1} heparin (Pharmacia and Upjohn, Bentley, Western Australia). Cumulus-oocyte complexes were recovered from the follicular fluid using a dissection microscope and placed into *in vitro* maturation medium (IVM).

2.4.2 *In vitro* oocyte maturation

Immediately following recovery COCs were washed three times in IVM medium (sodium bicarbonate-buffered TCM 199 containing 20% (v/v) porcine follicular fluid (pFF), 0.1 mM sodium pyruvate, 5 µg mL^{-1} FSH (Folltropin; Bioniche Life Sciences Inc, Belleville, ON, Canada), 5 µg mL^{-1} LH (Lutropin; Bioniche Life Sciences Inc), 1 µg mL^{-1} oestradiol, 10 µg mL^{-1} epidermal growth factor (EGF), 100 µM cysteamine, 100 µg mL^{-1} streptomycin sulphate and 100 IU mL^{-1} penicillin G). Cumulus-oocyte complexes were then matured in culture wells (Nunc Inc., Naperville, IL, USA) containing 600 µL of IVM under 300 µL of mineral oil for 42 hr in a humidified atmosphere of 5% CO_2 in air at 38.6°C (Weaver *et al*. 2013).

2.4.3 *In vitro* fertilisation

In vitro fertilisation (IVF) was conducted in a modified TRIS medium containing 4 mg mL^{-1} BSA, 2.5 mg mL^{-1} caffeine, 100 µg mL^{-1} streptomycin sulphate and 100 IU mL^{-1} penicillin G. Following maturation, COCs were transferred into a 0.1% hyaluronidase solution for 30 seconds in order to remove excess cumulus cells, whilst leaving the corona radiata cells intact. Oocytes were washed three times in fertilisation medium and transferred to a culture well containing 500 µL of IVF medium under 300 µL of mineral oil. Spermatozoa were added to the oocytes to give a final concentration of 0.5 x 10^6 spermatozoa mL^{-1} in each well. Spermatozoa were co-incubated with oocytes in a humidified atmosphere of 5% CO_2 in air at 38.6°C for 5 hr.

2.4.4 Sperm preparation

The day before IVF two tubes of freshly collected and extended mixed semen sample from terminal line sires were purchased from a commercial boar semen collection center (SABOR Pty. Ltd., Clare, South Australia). For IVF, 1 mL of semen from each tube was centrifuged for 5 min at 780 *g*. The supernatant was removed from each sample and spermatozoa were re-

suspended in 5 mL of sperm preparation medium (SPM; Hepes Synthetic Oviductal Fluid (SOF) supplemented with 5 mg mL^{-1} BSA, 50 mg mL^{-1} caffeine and 20 mg mL^{-1} heparin). Spermatozoa were re-centrifuged at 780 *g* for 5 min, and the supernatant was discarded. The spermatozoa were re-suspended in 5 mL SPM and incubated at 38.6°C for 45 min. Following incubation, the tube was gently inverted and centrifuged for 5 min at 780 *g*. One hundred µL of the spermatozoa pellet from each sample was re-suspended in 900 µL of SPM. Sperm concentration and viability were determined for each sample with the highest viability samples being used for IVF.

2.4.5 *In vitro* embryo culture

Following incubation, spermatozoa and any remaining cumulus cells were removed from the surface of the zona pellucida using a fine bore glass pipette. Presumptive zygotes were washed three times in *in vitro* culture (IVC) medium (Hepes SOF supplemented with 5 mg mL^{-1} BSA, 0.1 mM sodium pyruvate, amino acids at sheep oviduct fluid concentrations (Walker *et al*. 1996), and 5 mM hypotaurine) and incubated in a culture well containing 600 µL of IVC medium under 300 µL of mineral oil at an atmosphere of 5% CO_2, 5% O_2 and 90% N_2 at 38.6°C. Cleavage rate was recorded 48 hr post-fertilisation with embryo development assessed, and blastocysts counted, on day 6 post-fertilisation.

2.5 Histological analysis

The right ovary from a subset of 40 gilts was processed for histological analysis (H0; n = 10, HM; n = 10, L0; n = 10, LM; n = 10). The ovary was extracted from the PBS and excess moisture removed with paper towel before weighing and measurement of follicles. Using a scalpel, the ovary was dissected longitudinally, and each half was placed in a separate mega tissue embedding cassette (ProSciTech, Thuringowa, Queensland, Australia) and immersed in 3.5% paraformaldehyde. Ovaries were fixed in paraformaldehyde (Sigma Chemical Co. St Louis,

MO, USA) for 48 hr before washing with PBS four times over the succeeding 48 hr, and then stored in 70% ethanol until embedding.

2.5.1 Embedding, sectioning, and staining of ovaries

Ovaries were dehydrated in increasing concentrations of ethanol (70-100%). Xylene was used to remove excess alcohol and samples were then embedded in paraffin wax.

Histological examinations were conducted on 5 µm tissue sections cut on a Thermo Scientific Microm HM340E microtome (Microm International GmbH, Walldorf, Hessen, Germany) and floated onto a Starfrost® glass slide (ProSciTech, Thuringowa, Queensland, Australia).

Sections were stained with Lillie-Mayer's hematoxylin (Australian Biostain Pty Ltd, Traralgon, Victoria, Australia) and then counterstained with eosin yellow and mounted in dibutylphthalate polystyrene xylene medium (Ajax Finechem Pty Ltd, Ringwood, Victoria, Australia).

2.5.4 Analysis of follicle numbers

Every 20th section was analysed using 4x magnification on an Olympus WH B10X\20 microscope (Olympus, Tokyo, Japan) and a Colorview Soft Imaging System CX41 camera (2048 x 1536 pixel resolution; Soft Imaging System, Brook-Anco Corp, Rochester, New York, USA) with the aid of the imaging analysis program analysis Five (Olympus, Tokyo, Japan). Only follicles with a visible oocyte nucleus were counted. A total of 40 sections per ovary were analysed and follicles were classified according to their developmental stage as primary (single layer of cuboidal granulosa cells around the oocyte), secondary (more than one complete layer of cuboidal cells around oocyte) (Hulshof *et al*. 1994; Carámbula *et al.* 1999), antral (clearly defined antral space) (Torres-Rovira *et al.* 2014) or atretic (disorganised

granulosa cells and the presence of pyknotic nuclei) (Hay *et al.* 1976). The total number of follicles in each category (primary, secondary, antral and atretic) was estimated using the formula developed by Block (1952):

$Nt = (N_0 \times St \times ts)/(S_0 \times d_0)$

Nt = Estimated total number of follicles in each category.

N_0 = Number of follicles observed in the ovary.

St = Total number of cuts done in the ovary.

ts = cutting thickness.

S_0 = Total number of sections evaluated.

d_0 = Mean diameter of the follicle nucleus of each category.

2.6 Anti-Müllerian Hormone (AMH) assay

Plasma concentrations of AMH were determined using the CUSABIO Pig AMH ELISA Kit (CUSABIO Technology LLC, Houston, Texas, USA) as outlined in the manufacturer's instructions. The CUSABIO Pig AMH ELISA kit was validated, and research published previously (van Wettere *et al.* 2015; Meng *et al.* 2020; Zhuo *et al.* 2022). Eighty-four piglets were selected for testing based on availability of plasma samples from days 1, 20 (weaning) and 154, in addition to having lifetime weight records, ovarian weight and surface antral follicle count data (excluding histology). All incubation steps were performed at 33°C. Before assaying plasma samples were thawed, vortexed and centrifuged to remove any interfering cell fragments. Once all samples, standards, HRP-conjugate and antibody reached room temperature, 50 µL of standard or sample was added to each well, followed by 50 µL of HRP-conjugate and antibody in duplicate. Only the antibody was added to the blank well. Each well was mixed using a multichannel pipette before being sealed and incubated at 33°C for 1 hr. After incubation the plate was washed three times with wash buffer (15 mL CUSABIO wash

buffer concentrate diluted into 285 mL distilled water). Following this 50 µL of Substrates A and B were added to each well, mixed with a pipette, sealed and incubated for a further 15 min. After this final incubation, 50 µL of stop solution was added to each well and the plate was gently tapped to ensure mixing. Optical density was immediately determined for each well using a microplate spectrometer reader (Benchmark Plus, Bio-Rad Laboratories, Hercules, CA, USA) set to 450 nm with a wavelength correction of either 540 or 570 nm to allow for removal of irrelevant wavelength measures. Intra and inter-assay coefficients of variation were less than 10% and 13%, respectively. The detection range of this assay was 1.25 ng/mL – 50 ng/mL and the detection sensitivity was 1.25 ng/mL.

2.7 Immunoglobulin G

Radial-immuno diffusion was used to determine the concentration of Immunoglobulin G (IgG) in the serum from day old piglets. This was conducted by the Veterinary Diagnostics Laboratory, University of Adelaide, Australia. Agar plates were prepared using a 1% agarose solution (SeaKem agarose (Sigma Chemical Co. St Louis, MO, USA) in phosphate buffered saline), which was heated to dissolve the agarose. Once the solution had cooled to between 50-60°C, Biorad Purified Pig IgG (Gladesville, NSW, Australia) was added and the solution plates were allowed to set. Once solidified, wells were cut into the agar and serum samples were added to each well. As the antibody diffuses into the agar it forms a precipitin ring where the antibody reacts with the antigen in the agar. The petri dish was left in a humidified container at room temperature for 48 hr before the diameter of the precipitation rings was measured and IgG quantified using a method modified from Berne (1974).

2.8 First parity measures and the effects of restricted nutrition during late lactation

A late lactation nutritional challenge was imposed upon all gilts that were selected into the breeding herd (n = 126) over 12 replicates. These gilts were selected by having met the farm's selection protocols based on breed (Pure or F1), conformation and size at week 21 of life according to the APL best practice gilt management for fertility and longevity (Plush and Athorn, 2019). Animals selected were from the groups: H0; High birth weight born to a gilt , L0; Low birth weight born to a gilt, HM; high birth weight born to a multiparous sow, and LM; Low birth weight born to a multiparous sow (Table 2.1).

Selected gilts were housed in a dry sow shed and received 20 min of full contact boar exposure daily during heat detection from 28 weeks of age. At first oestrus, the selected gilts were inseminated with dead semen, followed by insemination with terminal line semen at second oestrus, around 32 weeks of age. Selected gilts were then housed in an eco-shelter until 5 days prior to the average due date. Farrowing data was recorded at day 0 (born alive, still born, sex ratio), with sow weight and individual piglet weights recorded at 24 hr post farrow. Litter size was standardised to 10-11 piglets.

Selected gilts were randomly allocated to either a 5 kg or 7 kg daily diet of pelleted feed (14.6 MJ DE/kg, 18.7% CP, 1% total lysine), which was equivalent to a 30% or 0% deficit, respectively. Feed restriction was imposed from day 13 $\pm$ 2 of lactation until weaning at 20 $\pm$ 2 days. Any residual feed was collected and weighed daily to calculate average feed wastage. On day 13 and weaning, total litter weight, and sow weight were recorded, and a blood sample was collected from the sow via venipuncture of the jugular vein. Transrectal ultrasound was performed with a 7.5 MHz linear-array transducer (Esaote, Genova, Italy) to assess ovarian follicular growth on days 13 and 20 postpartum. Ultrasound was performed in the farrowing

crate with no restraint necessary. Both ovaries were scanned and all follicles greater than 1 mm were measured. Once weaned all sows (n = 126) re-entered the commercial herd and were managed according to standard farm protocols, with subsequent mating data and farrowing data collected from the farm's Elite Herd (Genetic Solutions, Palmerston North, New Zealand) records.

Table 2.1 Visual representation of treatment allocation in the first parity nutritional challenge study

n	Birth weight grouping	Maternal age	Treatment allocation (kg)
8	High	Gilt	5
7	High	Gilt	7
5	Low	Gilt	5
3	Low	Gilt	7
28	High	Multiparous	5
34	High	Multiparous	7
23	Low	Multiparous	5
18	Low	Multiparous	7
126			

Birthweight grouping: High or Low weight at birth;

Maternal age: Born to a gilt or multiparous sow;

Treatment allocation: 5 or 7 kg of feed provided daily from day 13 of first lactation until weaning.

2.9 Statistical Analysis

2.9.1 Statistical analysis for the effect of maternal parity and birthweight on reproductive potential

All data analysis was conducted using the statistical package SPSS (IBM SPSS Version 21.0; IBM, Armonk, NY).

Statistical analysis was conducted on the 214 focal piglets selected for collection of reproductive tracts. Focal piglets were allocated to observation groups dependent on their birth weight and maternal parity (H0; High birth weight born to a gilt (n = 54), L0; Low birth weight born to a gilt (n = 41), HM; high birth weight born to a multiparous sow (n = 67), and LM; Low birth weight born to a multiparous sow (n = 52). However, as selection of focal piglets in the litter was based on the two heaviest and two lightest piglets in the litter, the complete data set included a range of birthweights. Similarly sows with a range of parities were included in the data set. It was therefore decided that for analysis the data should be divided into 9 groups, organised by maternal parity (gilt, young or old sow) and BW (low, medium or high). BW was divided by quartiles with the lowest quartile of birthweights in the focal piglet population defined as low BW (0.90-1.40 kg), the highest as high BW (1.80-2.60 kg) and the middle two quartiles as medium BW (1.41-1.79 kg). BW was classed as low (≤1.40 kg), medium (1.41-1.79 kg) or high (≥1.80 kg) for each maternal age grouping of gilt (parity 0), young sow (parity 1-2) or old sow (parity ≥3). All variables were tested for normality and anything not normally distributed was transformed. Data were analysed using a linear mixed model, with dam as a random factor. Analysis of all data included the fixed factors of season farrowed (summer, autumn, winter, spring, in lieu of replicate), reared litter size (low $\leq$ 8: n = 25, medium 9-11: n = 143 and high $\geq$12: n = 46) and maternal parity (gilt vs sow). Pairwise comparisons were determined using a Bonferroni posthoc test.

Analysis of *in-vitro* embryo production measures included replicate in the model instead of season farrowed as there were 8 replicates conducted from August 2015 to January 2016.

Average daily gain (ADG) was calculated by subtracting the BW of an individual from the wean weight and dividing by the age (days) at weaning. Neonatal fractional growth rate (NFGR) was calculated as the ADG from birth to wean, divided by the individual's weight at birth.

2.9.2 Statistical analysis: Gestational sex bias

Of the 214 piglets previously discussed, data was available on the proportion of male and female piglets within the litter for 214 piglets from 166 litters. For these piglets' outcome measures were also analysed according to the sex bias of the gestated litter.

Gestated litter classification was determined by the upper and lower quartile of sex ratio as biased litters, with the middle two classified as unbiased. Litters were therefore classified as female biased if ≥60% of the litter was female, male biased if ≤40% of the litter was female, and no bias was any litter that fell between this at 41-59% female. All variables were tested for normality and any variables not normally distributed were log transformed, with both transformed and untransformed data presented where necessary. Data were analysed using a linear mixed model, with sex bias as the independent variable and dam as a random factor. Analysis of all data included the effects of season farrowed (in lieu of replicate) and reared litter size (low ≤ 8, medium 9-11 and high ≥12). Average daily gain (ADG) and neonatal fractional growth rate (NFGR) were calculated for the preweaning period as described in Section 2.9a.

Analysis of the effects of gestational sex ratio on *in-vitro* embryo production measures included replicate in the model instead of season farrowed as there were 8 replicates conducted from August 2015 to January 2016.

2.9.3 Statistical analysis: Subsequent reproduction and response to a nutritional challenge

Data were then analysed by dividing piglets into 4 groups, by birth weight and maternal parity. Birthweight was classed as low or high for the individual litter, with maternal parity classed as

either gilt (first litter) or multiparous (second litter onwards). Data were analysed by division into the original 4 groups for this analysis as there was not enough data to allow for a more extensive division of data. All variables were tested for normality and any data not normally distributed was transformed. Data were analysed using a linear mixed model, with dam as a random factor. Analysis of all data included the fixed factors of season farrowed (in lieu of replicate), litter size, and maternal parity group. Nutritional challenge treatment group was initially included in the analysis, but later removed as it did not significantly ($P > 0.05$) affect any outcomes measured in this trial.

Chapter 3: The effect of maternal parity and birth weight on the developmental programming and reproductive potential of a gilt.

3.1 Introduction

Targeted management of replacement gilts typically occurs during and in the post-selection period (16-21 weeks), with the majority of the development of reproductive tissue and endocrine pathways occurring prior to this rudimentary time point. Selection is then based on simplistic criteria (Plush and Athorn, 2019), which are important, but due to the unacceptably high replacement rate of Australian sow herds, are obviously not capturing enough data to allow for a thorough evaluation of a reproductive gilt. Approximately 27% of replacement gilts are culled prior to completion of their first parity, and 40% prior to parity three, with reproductive failure being the single largest cause for removal (Serenius *et al*. 2006; Hughes *et al.* 2010; Plush *et al.* 2016). This premature culling of sows has resulted in the average Australian herd parity profile sitting at 2.7 (Plush *et al.* 2016) and as a sow does not become profitable until parity three to six (Stadler *et al.* 2003), there is an obvious need for a more effective and efficient form of selection of sows into the breeding herd.

It is now understood that prenatal and early postnatal development can affect all aspects of an animal's lifetime growth and performance (Gatford *et al*. 2018). Known as developmental programming, the maternal *in-utero* environment and early postnatal period affects (programs) future development and performance of the progeny. Therefore, consideration of early life factors could provide a strategy to enable earlier selection of more suitable replacement sows for the breeding herd. In addition, with gilts making up approximately one quarter of the breeding herd (Koketsu, 2007), selection of replacement animals from gilt

litters is highly likely. Progeny of gilts have been found to be lighter at birth and weaning (Hendrix *et al*. 1978), have lower lifetime growth rates (Rehfeldt and Kuhn 2006), greater susceptibility to disease (Miller *et al*. 2012), and lower muscle accretion and duodenal mucosa height (Alvarenga *et al*. 2013). This is thought to be due to the lower uterine capacity of gilts (Hughes and Varley, 2003; Hughes, 1994b), resulting in a reduced ability to deliver the nutrients necessary for fetal growth. In addition, as the Australian industry continues to select for prolificacy, there will be an inevitable increase in the number of compromised and lightweight piglets which are born (Campos *et al.* 2012) and, therefore, a higher incidence of pre-weaning mortality. In addition, carcass value at slaughter and the efficiency of carcass production will likely decrease, as studies have demonstrated that low birth weight offspring have reduced muscle growth, poorer carcass and meat quality, take longer to achieve market weight and are older at puberty than their heavier birthweight (BW) litter mates (Quiniou *et al*. 2002; Kuhn *et al*. 2003; Bee, 2004; Gondret *et al*. 2006; Rehfeldt and Kuhn. 2006; Foxcroft *et al*. 2009). Delayed onset of puberty has been found to be an accurate marker of reduced reproductive potential (Foxcroft *et al.* 2009). As piglet birthweight is positively correlated to ovarian mass and the number of primary ovarian follicles present at birth (Da Silva-Buttkus *et al.* 2013), light birthweights have intergenerational repercussions, with these animals giving birth to successive smaller litters of lightweight piglets (Corson *et al*. 2009). Therefore, it stands to reason that the birth weight of a gilt should continue to be taken into consideration when selecting replacement breeding animals.

A second predictor of reproductive potential that is proving useful in humans and ruminant species is anti-müllerian hormone (AMH). Anti-müllerian hormone is a dimeric glycoprotein, and member of the transforming growth factor-beta (TGFβ) superfamily of growth and differentiation factors and is an important intra-ovarian regulator of follicle growth. Anti-müllerian hormone is expressed by the granulosa cells of preantral and small antral follicles

and inhibits recruitment from the primordial follicle pool to prevent premature exhaustion of the ovarian follicular reserve (di Clemente *et al*. 1994; Monniaux *et al*. 2012). In sheep, AMH is a reliable prepubertal endocrine marker of the size of the ovarian reserve and the number of growing follicles (Lahoz *et al*. 2012; Torres-Rovira *et al*. 2013), and in cattle it is an accurate predictor of lifetime fertility and response to superovulation (Rico *et al*. 2009; Monniaux *et al*. 2010; Guerreiro *et al*. 2014). However, the use of AMH as an accurate prepubertal predictor of ovarian reserve in the pig is yet to be determined, as currently there is limited data on the role of AMH in the regulation of follicle growth in the pig. Studies of intrafollicular AMH levels in cycling gilts, goats, sheep and cows found concentrations to be highest in small antral follicles in all species except the pig, where no differences were observed between follicles of different sizes (Monniaux *et al.* 2012).

Therefore, the primary aim of the current study was to investigate the effect of maternal age and birth weight on the developmental programming of post-natal growth and reproductive potential in gilts. We hypothesise that gilts with heavier birth weights and gestated and reared by multiparous sows will show increased markers of reproductive potential as measured in this study. As AMH is yet to be proven as a reliable marker of reproductive potential in the pig the secondary aim of this study was to determine whether prepubertal concentrations of plasma AMH correlate with surface antral follicle counts, histological follicle counts, and oocyte quality, as indicated by *in vitro* embryo production measures, which are the currently accepted markers of ovarian development. We hypothesised that prepubertal concentrations of AMH will be positively correlated with small follicle populations and oocyte quality.

3.2 Methods

All animal procedures were conducted at the University of Adelaide's piggery at Roseworthy, South Australia with approval from the Animal Ethics Committee of The University of Adelaide (S-2014-193a) in accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council, 2013).

This study was conducted over 20 time replicates from March 2015 to May 2017, with 214 piglets followed to the abattoir, of the initial 464 piglets identified from 116 Large White x Landrace purebred and crossbred sows. On the day of farrowing (day 0) litters were weighed to enable identification and tagging of the two heaviest and lightest female piglets from each litter. Low viability animals weighing <800 g were not tagged.

Selected gilts were weighed on day 1, weaning and day 154 of life, and a blood sample was collected at each time point for IgG (day 1 sample only) and AMH analysis (Chapter 2.6, n=214). Reproductive tracts were collected on day 154 of life for analysis of ovarian development, through surface antral follicle counts (Chapter 2.3, n=214), and histological analysis of follicle development (Chapter 2.5, n=40) and oocyte quality through *in vitro* embryo production measures (Chapter 2.4, n=121). *In vitro* embryo production was conducted from ovaries of gilts whose tracts were collected in the first 8 replicates only.

3.3 Statistical analysis

Data were then analysed by dividing piglets into 9 groups, by maternal parity and birth weight (Table 3.1). Birth weight was classed as low (≤1.40 kg), medium (1.41-1.79 kg) or high (≥1.80 kg) for each maternal age grouping of gilt (parity 0), young sow (parity 1-2) or old sow (parity ≥3). All variables were tested for normality and any data not normally distributed were transformed. Data were analysed using a linear mixed model, with dam as a random factor.

Analysis of all data included the effects of season farrowed (in lieu of replicate), litter size, and maternal parity as main factors. Pearson product-moment correlation analysis and step-wise multiple regression analysis were used to examine the relationship between measures of growth and development, with post-hoc analysis to determine which groups were different from one another.

Table 3.1 Visual representation of grouping of data for analysis by maternal age and birthweight.

Maternal Parity	Birth weight		
	Low	Medium	High
Gilt (P=0)	n=42	n=32	n=21
Young sow (P=1-2)	n=14	n=26	n=26
Old Sow (P=3+)	n=13	n=20	n=20

Analysis of *in-vitro* embryo production measures was performed for 8 of the 20 replicates. These analyses were performed between August 2015 and January 2016, therefore replicate was included in the model for analysis of IVP results, instead of season farrowed.

3.4 Results

3.4.1 Effect of maternal parity on litter size and born alive

The number of piglets born alive and reared litter size were higher in gilts compared with both young and old sows, with gilts giving birth to fewer stillborn piglets (Table 3.2). The number of piglets born alive, total born, litter size post fostering and stillborn numbers were similar between multiparous sows (Table 3.2).

Table 3.2. The main effects (mean ± SEM) of maternal parity (Gilt, Young Sow, Old Sow) on farrowing data.

Parity Group	n	Av. Parity	Born alive*	Litter size**	Total born	Still born
Gilt (P0)	95	0 00± 0.00	11.37 ± 0.25[a]	10.85 ± 0.16[a]	12.55 ± 0.37[a]	0.38 ± 0.07[a]
Young sow (P1-2)	66	1.44 ± 0.06	10.64 ± 0.28[b]	9.65 ± 0.21[b]	11.49 ± 0.38[b]	1.02 ± 0.16[b]
Old sow (P3+)	53	4.57 ± 0.17	10.77 ± 0.39[b]	9.77 ± 0.23[b]	10.97 ± 0.47[b]	1.30 ± 0.17[b]
Significance			0.001	0.001	0.010	0.012

[a-b] Within a column, means without a common superscript differ (P < 0.05).

* Born alive refers to the number of piglets live born to the sow prior to fostering.

**Litter size refers to the number of piglets reared by the sow until weaning.

3.4.2 Effect of maternal parity and birthweight on growth performance

Birthweight affected growth performance at all ages. Weight at slaughter (day 154) was lowest in the low BW group, followed by the medium BW group and the high BW group remained heaviest (Table 3.3). Average daily gain from birth to weaning (ADG) was positively correlated with BW (P<0.05) and was lowest in the low BW gilts followed by the medium BW gilts, and highest in high BW gilts (Table 3.3). ADG was positively correlated with BW (P<0.0001, r = 0.405, n = 214). Neonatal fractional growth rate (NFGR) and BW were negatively correlated (P<0.0001, r = -0.370, n = 214) and NFGR (P<0.05) decreased with increasing BW group, being highest in low BW gilts followed by medium BW gilts and lowest in the high BW gilts (Table 3.3).

Table 3.3 Postnatal growth performance (mean ± SEM) of female pigs when grouped by birthweight (BW, Low, Medium, High) and parity (Gilt, Young Sow, Old Sow). Average daily gain and neonatal fractional growth rate were measured in the preweaning period only.

Parity	BW Group	N	Birthweight (g)	Wean weight (kg)	Day 154 weight (kg)	Average daily gain (kg)	Neonatal fractional growth rate (%/day)
Birth weight	Low	69	1.19 ± 0.02^{a}	3.82 ± 0.08^{a}	86.3 ± 1.8^{a}	0.203 ± 0.01^{a}	17.0 ± 0.01^{a}
	Med	78	1.61 ± 0.01^{b}	4.54 ± 0.08^{b}	92.5 ± 1.6^{b}	0.224 ± 0.01^{b}	14.0 ± 0.01^{b}
	High	67	2.04 ± 0.02^{c}	5.20 ± 0.12^{c}	98.7 ± 1.7^{c}	0.249 ± 0.01^{c}	12.3 ± 0.01^{b}
Gilt (P0)	Low	42	1.19 ± 0.02^{a}	3.89 ± 0.13	84.5 ± 2.23	0.208 ± 0.01	17.7 ± 0.01
	Med	32	1.62 ± 0.02^{b}	4.48 ± 0.15	90.3 ± 2.27	0.222 ± 0.01	13.9 ± 0.01
	High	21	1.97 ± 0.03^{c}	5.46 ± 0.17	96.9 ± 2.68	0.268 ± 0.01	13.6 ± 0.01
	Average	95	1.50 ± 0.33	4.42 ± 0.09	89.4 ± 1.52	0.225 ± 0.01	15.6 ± 0.00
Young Sows (P1-2)	Low	14	1.15 ± 0.04^{a}	3.57 ± 0.22	91.4 ± 3.75	0.188 ± 0.02	16.9 ± 0.01
	Med	26	1.61 ± 0.03^{b}	4.52 ± 0.17	98.0 ± 3.08	0.217 ± 0.01	13.5 ± 0.01
	High	26	2.04 ± 0.03^{c}	4.92 ± 0.17	100.4 ± 2.97	0.239 ± 0.01	11.8 ± 0.01
	Average	66	1.68 ± 0.36	4.48 ± 0.12	97.6 ± 1.97	0.220 ± 0.01	13.6 ± 0.00
Old Sows (P≥3)	Low	13	1.23 ± 0.04^{a}	3.79 ± 0.24	86.6 ± 4.01	0.198 ± 0.02	16.4 ± 0.01
	Med	20	1.59 ± 0.03^{b}	4.66 ± 0.21	91.6 ± 3.29	0.235 ± 0.01	14.7 ± 0.01
	High	20	2.12 ± 0.03^{c}	5.24 ± 1.17	97.8 ± 3.21	0.240 ± 0.01	11.4 ± 0.01
	Average	53	1.70 ± 0.38	4.73 ± 0.18	92.9 ± 1.84	0.229 ± 0.01	13.6 ± 0.01
Significance	Parity group		Ns	ns	Ns	Ns	ns
	BW group		0.001	0.001	0.001	0.001	0.001
	P x BW		0.050	NS	Ns	Ns	NS

[a-b-c] Within a column and group, means without a common superscript differ ($P < 0.05$).

Immunoglobulin G concentration at 24 hours of life was positively correlated with BW (P = 0.042, *r* = 0.19, n=78) and unaffected by maternal parity (Table 3.4).

Table 3. 4 Immunoglobulin G serum concentration (mean ± SEM) at day one of life of female pigs when grouped by maternal parity (Gilt, Young Sow, Old Sow).

Parity	n	IgG (g/L)
Gilt (P0)	39	35.9 ± 2.15
Young Sow (P1-2)	17	41.3 ± 3.25
Old Sow (P≥3)	21	42.5 ± 2.86
Significance		0.146

Maternal parity did not affect weight or growth rates at any time points. However, when birthweight groupings were removed from the analysis maternal parity did affect birthweight, with gilt progeny weighing significantly less than old sow progeny (*P* = 0.007, gilt progeny: 1.52 ± 0.035, young sow progeny: 1.67 ± 0.045, old sow progeny: 1.72 ± 0.053), but was not significant at any other time points. There was a significant interaction between BW and maternal parity, with piglets classed as 'high BW' being born heavier if gestated by an older sow, of parity three and above (Table 3.3).

Weaning weight was positively correlated with BW, day 154 weight and ADG from birth to weaning (Table 3.5). NFGR was not related to weaning weight or day 154 weight (Table 3.5). ADG from birth to weaning was also positively correlated with weight at day 154 (P<0.001, *r* = 0.29, n = 214).

Table 3.5 Correlation coefficients between weaning weight and lifetime growth.

Variable	P Value	*R*
Birth weight	**<0.001**	**0.679**
154 day weight	**<0.001**	**0.340**
ADG	**<0.001**	**0.850**
NFGR	0.219	-0.058

Average daily gain (ADG), neonatal fractional growth rate (NFGR).

3.4.3 Effect of maternal parity and birthweight on ovarian development at day 154

Ovarian weight at day 154 was not affected by maternal parity (P>0.05) or BW (P>0.05) of the gilt. Ovarian weight correlated positively with weight at day 154 (P = 0.045, *r* = 0.38, n = 214), but was not significantly related to birthweight or any other growth performance measures. Ovarian weight was positively correlated with the number of small follicles on the ovary surface (Table 3.6), and negatively correlated with preovulatory surface follicle counts (≥6 mm) (Table 3.6). A positive correlation was observed between ovarian weight and the number of atretic follicles, while numbers of primary, secondary and antral follicles were not related to ovarian weight (Table 3.6).

Table 3.6 Correlation coefficients between ovarian weight (log transformed) and both surface and histological follicle counts.

Variable	n	P Value	*R*
Small follicles (<4 mm)	214	**<0.001**	**0.313**
Large follicles (≥4 mm)	214	0.147	-0.072
Pre-ovulatory follicles (≥6 mm)	214	**0.013**	**-0.151**
Histological primary follicles	39	0.398	-0.043
Histological secondary follicles	39	0.140	-0.177
Histological antral follicles	39	0.237	0.118
Histological atretic follicles	39	**0.043**	**0.278**

Serum IgG concentrations at day 1 of life did not correlate (all P>0.05) with any measures of ovarian development.

The number and size of surface antral follicles were not affected by birth weight group or maternal parity (Table 3.6). There were no effects of BW group on histological follicle counts (Table 3.6). Progeny from young sows had fewer primary follicles than progeny from gilts or

older sow litters (P < 0.05, Table 3.6). There was no effect of maternal parity on the numbers of secondary, antral or atretic follicles (Table 3.6).

Table 3.7 The main effects (mean ± SEM) for maternal parity (Gilt, Young Sow, Old Sow) and birthweight (BW, Low, Medium, High) on histological follicle counts, post slaughter at day 154 of age.

	N	Primary follicles	Secondary follicles	Antral Follicles	Atretic follicles
Gilt	20	26321 ± 15159[b]	9082 ± 5800	1541 ± 1036	14738 ± 18531
Young sows (P1-2)	10	14786 ± 9223[a]	7278 ± 4059	1821 ± 1057	11626 ± 6066
Old sows (P≥3)	10	30153 ± 13376[b]	11994 ± 7434	2438 ± 1457	15614 ± 9106
Low BW (0.90-1.40kg)	16	24713 ± 3985	9186 ± 1520	1504 ± 245	10139 ± 1349
Medium BW (1.41-1.79kg)	13	23005 ± 3985	9942 ± 2102	1949 ± 297	15131 ± 2392
High BW (1.80-2.60kg)	10	26655 ± 4117	9087 ± 1194	2219 ± 496	19659 ± 7991
Significance	P	0.008	Ns	Ns	Ns
	BW	Ns	Ns	Ns	Ns

[a-b] Within a column and group, means without a common superscript differ (P < 0.05).

The number of preovulatory follicles (> 6 mm) on the surface of the ovary correlated positively with BW, and the number of small surface follicles was negatively related to BW (Table 3.8). The number of large surface follicles was not related to BW (Table 3.8).

The number of antral follicles, as measured by histological analysis, correlated positively with BW (Table 3.8). Numbers of primary, secondary and atretic follicles were not related to BW (Table 3.8).

Weight at weaning was positively correlated with histological antral and atretic follicle counts (Table 3.8). Neonatal fractional growth rate was positively correlated with small surface follicle counts (P = 0.027, *r* = 0.14, n=214), but not correlated to any other measure of ovarian development. Average daily gain from birth to weaning was not correlated with any measures of ovarian development.

Table 3.8 Correlation coefficients between ovarian development, birth weight and weaning weight.

		Birth weight		**Weaning weight**	
Variable	**N**	**P Value**	***R***	**P Value**	***R***
Ovarian weight	214	0.138	0.075	0.218	0.058
Small follicles (<4 mm)	214	**0.047**	**-0.115**	0.474	0.005
Large follicles (≥4 mm)	214	0.159	0.069	0.406	0.018
Pre-ovulatory follicles (≥6 mm)	214	**0.046**	**0.115**	0.450	-0.009
Histological primary follicles	39	0.390	-0.046	0.220	0.139
Histological secondary follicles	39	0.454	-0.019	0.369	-0.061
Histological antral follicles	39	**0.034**	**0.295**	**0.049**	**0.294**
Histological atretic follicles	39	0.116	0.196	0.032	0.326

Adult weight at slaughter correlated positively with average follicle diameter (P = 0.045, *r* = 0.13, n=214) and preovulatory surface follicle number (P = 0.021, *r* = 0.15, n=214), but was not correlated with any other measures of follicle development.

3.4.4 *In vitro* embryo production measures

BW did not directly affect any measures of *in vitro* embryo production. Maternal parity affected the percentage of oocytes that cleaved following *in vitro* fertilisation, with a higher percentage of oocytes from gilt and old sow progeny fertilised and cleaved when compared with oocytes from progeny of young sows (P< 0.05, Table 3.9). However, maternal parity had

no effect on blastocyst development rates, when expressed as a proportion of total oocytes collected or oocytes that cleaved (Table 3.9).

Table 3.9 In-vitro embryo production data (mean ± SEM) when grouped by maternal parity (Gilt, Young Sow, Old Sow).

	n of ovaries	n of oocytes	% cleaved	% blastocysts / cleaved	% blastocysts / oocytes
Gilt	54	463	52.5 ± 5.1 [a]	14.6 ± 3.5	12.2 ± 3.2
Young sows (P1-2)	34	380	35.9 ± 5.8 [b]	6.3 ± 2.3	7.6 ± 3.4
Old sows (P≥3)	32	339	53.3 ± 5.3 [a]	17.3 ± 3.7	13.6 ± 3.0
Significance	P		0.048	Ns	Ns

[a-b] Within a column and group, means without a common superscript differ (P < 0.05).

3.4.5 Seasonal effects

Gilts that were born in spring and slaughtered in autumn were significantly (P < 0.05) heavier at slaughter than gilts farrowed in autumn and slaughtered in spring (106.5 ± 4.1 kg, 88.0 ± 1.4 kg, respectively). Other than slaughter weight there were no seasonal effects on BW, weaning weight or preweaning growth. There were no differences in ovarian weight or surface antral follicle counts between seasons. The number of primary follicles was affected by season, with decreased numbers of primary follicles in gilts farrowed in summer and slaughtered in winter (P < 0.05), compared to gilts farrowed in autumn, slaughtered in spring and farrowed in winter, slaughtered in summer (summer farrowed; 13980 ± 1776, autumn farrowed; 26802 ± 3048, winter farrowed; 30799 ± 5330). No other histological follicle counts were affected by seasonal effects. Measures of *in vitro* embryo production were not affected by season.

3.4.6 Anti-müllerian hormone

AMH concentrations at day 1 and weaning were not affected by BW, preweaning growth, maternal parity, or season. AMH at day 154 was affected by BW with no difference between low and high BW animals, but lower plasma AMH concentrations in medium BW gilts in comparison to low and high BW animals (low BW: 6.74 ± 0.22 ng/mL; high BW: 6.71 ± 0.20 ng/mL; medium BW: 6.05 ± 0.27 ng/mL, P = 0.034).

AMH measured at weaning was negatively correlated with ovarian weight (P = 0.023, *r* = -0.242, n = 69 Fig 1, A) and histological antral follicle count (P = 0.047, *r* = -0.432, n = 69 Fig 1, B) at day 154. AMH concentrations at day 154 were negatively correlated with ovarian weight (P = 0.049, *r* = -0.182, n = 83 Fig 2, A), surface counts of small follicles (P = 0.014, *r* = -0.242, n = 83 Fig 2, B), histological antral follicle counts (P = 0.037, *r* = -0.408, n = 20 Fig 2, C) and atretic follicle counts (P = 0.034, *r* = -0.416, n = 20 Fig 2, D)

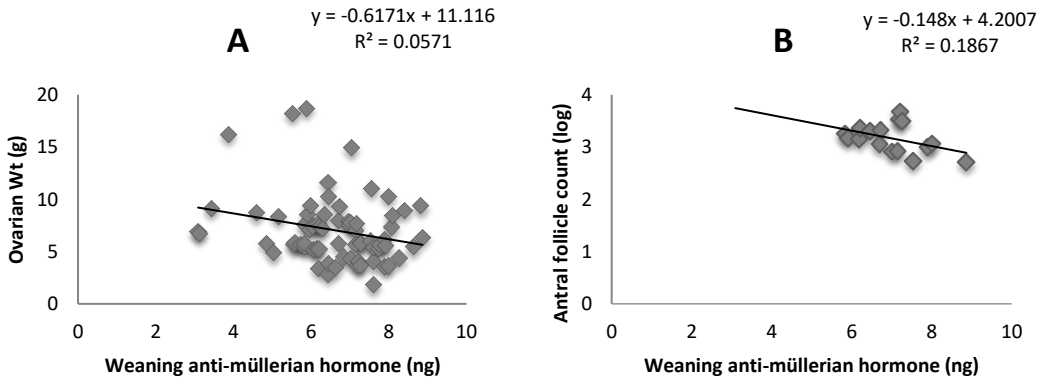

Figure 1 Correlations between plasma anti-müllerian hormone at weaning and ovarian weight day 154 (A) and plasma anti-müllerian hormone at weaning and antral follicle count (log) at day 154 of life (B).

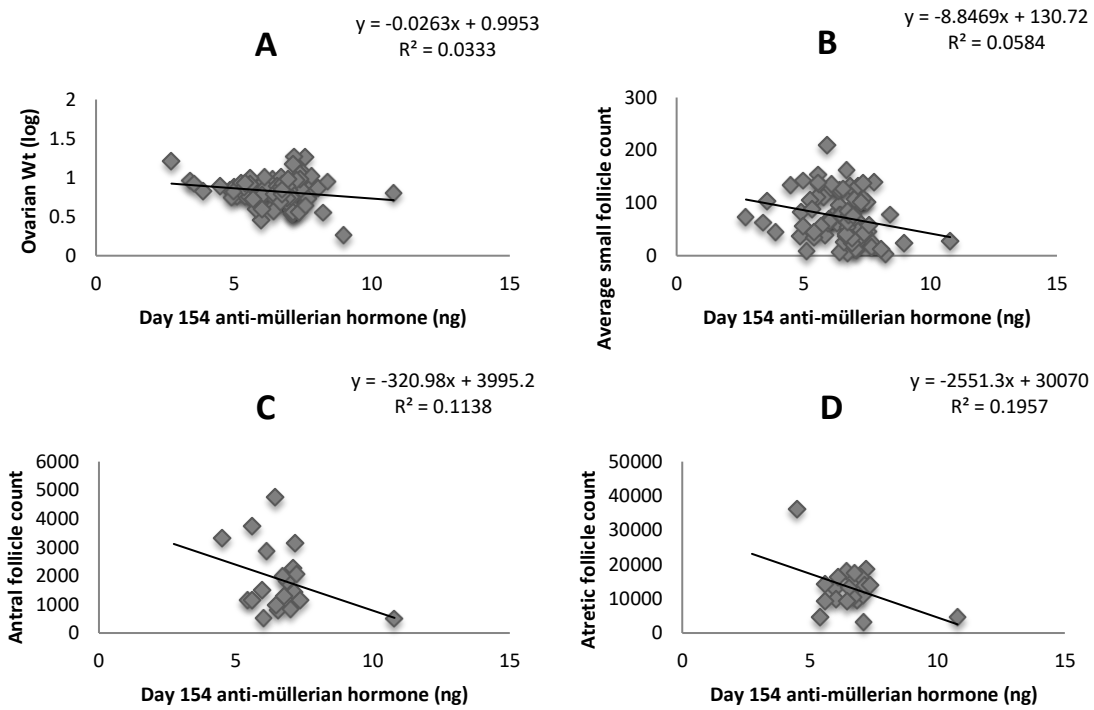

Figure 2 Correlations between plasma anti-müllerian hormone at day 154 of life and ovarian weight (log) (A), average small follicle count (B), antral follicle count (C) and atretic follicle count (D).

3.5 Discussion

The birth weight, but not maternal parity, of a gilt significantly affected all measures of growth performance throughout the animal's life. While low BW piglets had a higher fractional growth rate as neonates, they were unable to catch-up in terms of body weight and therefore remained lighter than their heavier littermates at 154 days of age. Assessment of ovarian measures at day 154 showed no observed effects of birth weight or maternal parity on the surface antral follicle population. However, histological assessment indicated that progeny from young sows had fewer primary follicles. Similarly, a lower percentage of oocytes from progeny of young sows cleaved following *in vitro* fertilisation, when compared with oocytes from the progeny of gilts or older sows. The hypothesis that AMH could be used as a reliable marker of ovarian reserve in a similar manner to other livestock species was not supported by this study. AMH concentrations at weaning and slaughter were negatively correlated with

ovarian weight and some measures of follicle size and development. It has recently been discovered that AMH may play a markedly different role in the porcine ovary when compared with many other livestock species (Almeida *et al.* 2018). Across species, AMH expression is limited to the granulosa cells of growing follicles and acts to reduce the sensitivity of primordial follicles to FSH. However, in the pig it is also expressed by the theca cells of preovulatory follicles and the luteal cells following ovulation (Almeida *et al.* 2018). As such the use of AMH as a direct marker of ovarian follicle populations in pigs is proving more complicated than other species; however, due to its effect on FSH sensitivity it may be a potential marker of ovarian longevity.

Similar to previous studies (Gondret *et al.* 2006; Beaulieu *et al*. 2010; Alvarenga *et al.* 2013; Almeida *et al.* 2015) low BW gilts had a lower ADG and remained lighter at weaning and slaughter than their high BW counterparts. However, NFGR increased as BW decreased, indicating higher growth rates relative to initial size, reflecting a greater partitioning of nutrients toward growth and potentially away from reproductive development. This form of accelerated growth within the neonatal period is termed catch-up growth (De Blasio *et al.* 2006) and in humans can predict detrimental growth and future health issues (Prada and Tsang, 1998; Karlberg *et al.* 1997; Hokken-Koelega *et al*. 1995). The hypothesis that nutrients are being partitioned away from reproductive development in the low birth weight animals is supported by the positive correlations between weaning weight and antral follicle counts, slaughter weight and ovarian weight and slaughter weight and average follicle diameter. NFGR was positively correlated with small surface follicles (<4 mm diameter), which in combination with the negative correlations between NFGR and BW suggest these animals were less likely to have reached puberty by day 154 of life. These increased rates of NFGR suggest that the low birth weight animals may have experienced placental restriction during early to mid-gestation. In rats and sheep, experimental placental restriction late in gestation, as opposed

to early, generally does not result in any form of catch-up growth, despite the lower birth weight of these animals (Cock *et al*. 2001; Simmons *et al*. 2001; Alexander, 2003). Most studies investigating the role of catch-up growth have focused on intra-uterine growth restricted animals and as any severely restricted animals (<800 g at birth) were excluded from this study it is interesting that even mild IUGR animals are showing similar results. As such we can conclude that the measures of birth weight and preweaning growth of a gilt are important factors to consider in breeder selection.

The relationship between birth weight and ovarian reserve has been well documented in many species (Ibanez *et al.* 2002; Da Silva-Buttkus *et al*. 2002, 2003; Ibanez and de Zegher, 2006; Cushman *et al*. 2009; Hansen *et al*. 2011), and there is strong evidence that females that are born small for gestational age possess smaller ovaries, fewer antral follicles and show a decreased ovulation rate. Most of these studies compared the effects of birthweight on ovarian mass and antral follicle counts in fetal or neonatal pigs, and in comparison, to growth restricted piglets (runts). This current study found no significant relationship between birth weight and ovarian mass at day 154. This may be due to the exclusion of very low BW runts (<800 g BW) from this study due to these animals being unlikely to be considered as replacement breeding sows. However, weight at slaughter was positively correlated to ovarian mass, which was correlated to the size of the pool of small growing follicles. Ovarian weight was negatively correlated with the number of large preovulatory follicles and positively correlated with atretic follicles, which could be explained by the prepubertal patterns of follicle growth and regression due to the lack of luteinising hormone necessary for further growth and ovulation. Further investigations should consider quantification of LH profiles in relation to birthweight.

The ovaries of gilts born to young sows contained fewer primary follicles and produced oocytes with inferior developmental competence compared with those born to gilts and older sows. It is well known that first lactation sows are unable to produce the same quantity or quality of milk and colostrum as multiparous sows (Klobasa *et al*. 1986; Burkey *et al*. 2008). As such it is unlikely that the impaired ovarian function observed in this study was a reflection of insufficient intake or nutrient supply, as it would be expected that these results would be reflected in weaning weights and found in gilt, not multiparous litters. The decrease in follicle numbers and developmental potential is possibly due to oocyte and embryo quality having been affected in young sows due to high body reserve mobilisation during their first and second lactation, which may impact on fetal development, including development of the ovary, in their offspring. These outcomes are in line with results described by Flowers (2008) where it was reported that first litter sows are especially vulnerable to the drain of lactation on body reserves, as the subsequent litter size was inversely related to first litter weaning weight. This finding was supported by work conducted by Hewitt *et al.* (2009) in which a reduction in first litter size, and thus use of body reserves for lactation, increased parity 2 litter size. The current study did not assess lactation measures; however, further research is necessary into the effects of first lactation on the reproductive potential of the subsequent litter.

Maternal parity did not affect serum concentrations of immunoglobulins in this study, which is to be expected as previous literature has found little difference in quality of colostrum produced by gilts or sows (Craig *et al.* 2019). Birthweight was positively correlated with serum concentrations of immunoglobulins in this study. Absorption of colostral IgG is essential within the first 24 hours of life, as piglets are born with almost no circulating immunoglobulins, due to no placental transfer of antibodies *in utero* (Brambell, 1958; Nguyen *et al.* 2013). Therefore, colostrum ingestion and establishment of passive immunity is one of the most important

factors contributing to piglet survival (Devillers *et al*. 2011). This positive correlation between BW and circulating IgG suggests that larger piglets have both the necessary energy reserves and physical strength to find and hold udder space. In addition, work conducted by Miller *et al.* (2008) and King *et al.* (1997) concluded that heavier piglets are able to drink more milk through to weaning due to increased suckling stimulus, causing increased mammary gland growth. The influence of BW on colostrum intake, while not directly found to affect ovarian development in this study, has the potential to affect preweaning growth, which as mentioned previously does play a vital role in all measures of gilt development.

Currently the most accurate measure of ovarian reserve is the population of small growing follicles (Scheffer *et al.* 2003), the determination of which requires either physical collection of the ovary for surface/histological analysis or ultrasonography. While ultrasonography does not involve the removal of an ovary, it is time consuming, often inaccurate, physically difficult in young gilts, and generally does not allow for measurement of small follicles, so is not an efficient measurement of ovarian reserve. A serum marker, such as AMH, which in many species is reflective of the size of the growing follicle pool, would be a beneficial addition to current breeder selection protocols. In many species AMH is expressed solely by the granulosa cells of preantral and healthy small antral follicles, (Ibanez *et al.* 2002; Da Silva-Buttkus *et al*. 2002, 2003; Cushman *et al*. 2009; Ibanez and de Zegher, 2006; Hansen *et al*. 2011) and has been shown to play an inhibiting role in the cyclic process of follicular recruitment by determining FSH threshold levels (Almeida *et al.* 2018). Durlinger *et al*. (2001) conducted a series of *in vitro* and *in vivo* experiments in mice to determine the relationship between AMH and FSH on early follicle recruitment, concluding that AMH inhibits FSH stimulated follicle growth, suggesting that AMH is one of the factors determining sensitivity of ovarian follicles to FSH, and that AMH is a dominant regulator of early follicle growth. As such, studies of intrafollicular AMH levels in antral follicles of goats, sheep and cows found AMH

concentrations to be highest in small antral follicles, with a marked decrease as follicles increased to their preovulatory size. However, when intrafollicular AMH concentrations were measured in cycling gilts no significant differences were found in follicular fluid from antral follicles of varying size (Monniaux *et al*. 2012). In the current study, plasma AMH at weaning and at day 154 was negatively correlated with follicle counts and ovarian mass at day 154. As described earlier, these measures are considered reliable indicators of ovarian reserve. In contrast, a recent study has found that contrary to other species, prepubertal AMH concentrations in pigs were not indicative of antral follicle counts in both immature and pubertal gilts (Steel *et al*. 2019). This can potentially be explained by work conducted by Almeida *et al.* (2018), demonstrating that in the pig AMH expression is not restricted to the granulosa cells of growing follicles, but is also expressed by the theca cells of preovulatory follicles and the luteal cells following ovulation. In the pig, the onset of increasing progesterone levels occurs 40-48 hours after the LH surge (Soede *et al*. 1994), unlike most species where this occurs around the time of the LH surge. This delay in increasing progesterone concentrations results in the initial rise in FSH levels immediately after the LH surge (Noguchi *et al*. 2010). As the role of FSH is to stimulate the growth of preantral follicles this would result in increased follicular recruitment. However, as AMH is known to reduce sensitivity to FSH, it was hypothesised by Almeida *et al.* (2018) that the role of AMH expression in the luteal cells of the corpus luteum in the pig may be to reduce the action of FSH, thus preventing unnecessary follicle recruitment and early exhaustion of the ovarian follicle pool. As such the negative correlations found in this study between AMH concentrations and follicle counts may be explained by reduced AMH expression resulting in increased sensitivity to FSH and therefore resulting in higher numbers of recruited follicles. This increased sensitivity to FSH associated with low levels of AMH may be a potential indicator of animals that will have shorter reproductive lifespans. However, further research is necessary into the lifetime performance of animals with differing AMH levels to investigate whether this 48-hour period

of follicular recruitment following each ovulation is enough to impact the reproductive lifespan of a sow. Steel *et al.* (2018) found no association between AMH concentrations at day 80 of life and mating, litter, or culling outcomes in pigs. However, as this data was collected until parity two and the average herd age in Australia is 2.7 it would be beneficial to analyse data on animals through to removal from the breeding herd.

In summary, the lifetime reproductive potential of a gilt is influenced *in-utero* by several important and easily identifiable environmental factors. The current selection of breeding animals is focused on the progeny of multiparous sows; however, the results of this study seem to indicate that the exclusion of gilt progeny is not necessarily required. There were no quantifiable differences between the growth and development of gilt progeny and multiparous progeny, provided they were not categorised as low BW. However, the negative reproductive outcomes in progeny of young parity sows demonstrates that further research into the impacts of metabolic state prior to and during mating on the reproductive potential of their progeny is required. Birthweight has the potential to be a useful tool for selection as it is indirectly correlated with ovarian reserve and should be coupled with any selection for increased litter sizes to ensure selection is based around improved uterine capacity and not just fertilisation rate. Low BW animals should be avoided as in many species these animals have been shown to experience catch up growth, as such these gilts may reach an appropriate weight by selection; however, they may either show delayed onset of puberty or a reduced reproductive lifespan due to the early partitioning of nutrients away from reproductive development during the neonatal period. The result of this study, in combination with other recent work, leads us to hypothesise that AMH has the potential to be used as a reliable marker of reproductive longevity in the pig. However, further research into the association between AMH concentrations and lifetime reproductive performance is necessary to determine the exact role of porcine AMH within the ovary.

Chapter 4: The effect of the gestated sex bias of the litter on the future reproductive development of a gilt.

4.1 Introduction

Optimising sow longevity and lifetime productivity is integral to the creation of a reproductively efficient herd and lowering costs of production. However, variation in sow performance within the breeding herd continues to hinder productivity and efficiency. The pig industry relies heavily on reproductive uniformity and, therefore, a reliable early-life indicator of reproductive potential could markedly improve the reproductive efficiency of the breeding herd. It is possible that the sex distribution, or sex bias, of the gestated litter could be used as an early indicator of a gilt's reproductive development and, therefore, lifetime reproductive performance.

In a number of species (rats, mice, sheep, cattle and pigs) the masculinisation of females *in utero* occurs when the fetus is exposed to excessive amounts of androgens, and this can result in non-genetic variation in behaviour, reproduction and phenotype (Raeside and Sigman, 1975; Vom Saal, 1989; Hughes, 2001; Uller *et al.* 2005; Navara and Nelson, 2009; Seyfang *et al.* 2018). Androgens are predominantly produced by male fetuses and are able to pass through fetal membranes (Clemens *et al*. 1978), with exposure of female fetuses to androgens increasing with the overall proportion of males in the litter or proximity to male fetuses. The probability of a female fetus being positioned between two males *in-utero* is a function of the litter size and proportion of the litter that is male (Vom Saal, 1981). Sex bias of the litter is, therefore, an easily identifiable trait, with the chance of a female pig being exposed to high androgen levels increased in litters containing more than 60% male fetuses (Drickamer *et al.* 1997).

Studies in fetal sheep gestated by testosterone treated ewes indicated that a high level of testosterone exposure *in utero* results in quantitative and structural changes in the ovary at day 139-141 of gestation (full term is 147 d) (Steckler *et al*. 2005). In addition, female mice from male biased litters produced more male biased litters, fewer and smaller litters and had a shorter reproductive lifetime (Vom Saal *et al*. 1999; Bánszegi *et al*. 2012). Exogenous treatment of pregnant sows with testosterone increased testosterone concentrations in maternal and fetal circulation and amniotic fluid, with the progeny showing deficits in LH secretion at puberty (Elsaesser and Parvizi, 1979; Petric *et al*. 2004). However, this model of maternal testosterone treatment is potentially too extreme to emulate the testosterone exposure associated with a male biased litter, as fetal testosterone concentrations in serum or fetal fluids were similar in gilts that developed between either two males or two females *in utero* (Framstad *et al*. 1990; Wise and Christenson, 1992).

In some species, female anogenital distance is reflective of reproductive potential and is altered by the gestated sex bias of a litter. Porcine and rodent studies have demonstrated that females gestated in male biased litters had longer anogenital distances and were less likely to conceive at their first mating (Vom Saal and Bronson 1978; Vom Saal *et al.* 1999; Bánszegi *et al.* 2012). Seyfang *et al.* (2018) reported similar effects of litter sex bias on reproductive outcomes of the offspring in pigs. However, in contrast to earlier studies they observed a longer anogenital distance in females from female biased litters rather than male biased litters. Interestingly, gestated litter bias can also affect a sow's capacity to rear her litter, as *in utero* exposure to testosterone can suppress the development of mammary tissue (Kratochwil 1971), and gilts originating in a female biased litter have been found to have a higher number of teats than those from male biased litters (Drickamer *et al.* 1999a).

The use of an easily identifiable marker of reproductive success, such as sex bias of the litter, will allow insight into the environment the gilt has been exposed to during gestation, and the lifetime effects this may have on development. The aim of this study was to determine the effect of gestated sex bias on ovarian development at 154 days of age, with the hypothesis that gilts gestated in a male biased litter would have a reduction in the quality of ovarian follicles, when compared to gilts from female biased litters.

4.2 Methods

This study was conducted using the previously described 214 female piglets from 166 litters. Gestated litter was classified as female biased if ≥60% of the litter was female (n = 56), male biased if ≤40% of the litter was female (n = 53), and no bias was any litter that fell between this at 41-59% female (n = 105). Piglets were weighed at birth, weaning and day 154 of life before collection of ovaries. Surface antral follicle counts, measures of *in vitro* embryo production (see Chapter 2.4) and histological follicle counts were undertaken to determine ovarian development and function (see Chapter 2.5).

4.2.1 Statistical analysis

All variables were tested for normality and any variables not normally distributed were log transformed. Data were analysed using a linear mixed model, with dam as a random factor. Analysis of all data included the effects of season farrowed (in lieu of replicate) and reared litter size (low ≤ 8, medium 9-11 and high ≥12). Average daily gain (ADG) and neonatal fractional growth rate (NFGR) were calculated for the preweaning period. ADG was calculated by subtracting the birth weight from the weaning weight and dividing by age (days) at weaning. NFGR was calculated as the ADG divided by the individual's weight at birth.

Analysis of *in-vitro* embryo production measures was performed for 8 of the 20 replicates. These analyses were performed between August 2015 and January 2016, therefore replicate was included in the model for analysis of IVP results, instead of season farrowed.

4.3 Results

4.3.1 Postnatal growth

The gestated sex bias of a litter was not affected by maternal parity and neither had any effect on pre-weaning growth rates or weight of female offspring at any time point of the study (Table 4.1).

Table 4.1 The main effects of gestated sex bias of a litter on postnatal growth performance of female pigs

	n	Day 1 wt	ADG	NFGR %/day	Day 14 wt	Week 18 wt	Week 21 wt
Female bias	56	1.66 ± 0.05	0.233 ± 0.01	14.4 ± 0.01	4.66 ± 0.17	76.9 ± 2.05	89.5 ± 2.32
No bias	105	1.60 ± 0.04	0.218 ± 0.00	14.5 ± 0.01	4.45 ± 0.09	76.0 ± 1.16	95.0 ± 1.43
Male bias	53	1.60 ± 0.04	0.229 ± 0.01	14.7 ± 0.01	4.52 ± 0.13	73.5 ± 2.15	90.6 ± 1.88
Significance		ns	ns	ns	Ns	ns	ns

Female bias (≥60% of the gestated litter was female); No bias (41-59% of gestated litter was female); Male bias (≤40% of the gestated litter was female).

Neonatal fractional growth rate (NFGR)

4.3.2 Ovarian development at day 154

There was a significant interaction between maternal parity and sex bias of the litter on ovarian weight, with sow progeny from a female biased litter possessing lighter ovaries than gilt progeny from female biased litters. Litters with no gestated sex bias showed no difference in ovarian weight due to parity. Male biased litters were affected by maternal parity, with gilt

progeny possessing the lightest ovaries, followed by the progeny of older sows, with parity 1-2 sow progeny having the heaviest ovaries out of all groupings (Table 4.2).

Follicle size and count was not affected by maternal parity. Gilts gestated in female biased litters had fewer preovulatory follicles (≥6 mm), and a smaller average follicle diameter of the surface antral follicles (≥4 mm) at day 154 in comparison to gilts gestated in a litter with no bias (Table 4.2). Gestated sex bias did not affect the number of surface antral follicles <6 mm.

Table 4.2 The main effects of gestated litter sex bias on measures of ovarian development.

Sex bias		n	Ovarian weight (g)	Follicle count <4 mm	Follicle count 4-5.99 mm	Follicle count ≥6 mm	Av follicle diameter (mm)
Female bias		56	3.77 ± 0.2	85.6 ± 6.1	9.6 ± 0.7	0.8 ± 0.2[a]	4.6 ± 0.1[a]
No bias		105	4.24 ± 0.2	84.7 ± 4.4	8.9 ± 0.5	2.1 ± 0.3[b]	5.1 ± 0.1[b]
Male bias		53	4.00 ± 0.3	79.3 ± 7.1	9.9 ± 0.7	1.6 ± 0.3[ab]	4.9 ± 0.1[ab]
Female bias	**Gilt**	28	4.14 ± 0.2 [b]	93.4 ± 9.3	8.13 ± 0.9	0.4 ± 0.1	4.4 ± 0.2
	Young sow	15	3.34 ± 0.2 [a]	84.1 ± 12.1	11.1 ± 1.8	1.0 ± 0.4	4.7 ± 0.2
	Old sow	13	3.47 ± 0.3 [a]	70.3 ± 9.8	10.9 ± 1.3	1.5 ± 0.4	5.0 ± 0.1
No bias	**Gilt**	35	4.14 ± 0.3 [b]	86.4 ± 8.1	9.9 ± 0.8	1.9 ± 0.5	5.1 ± 0.1
	Young sow	39	4.23 ± 0.2 [b]	81.9 ± 7.5	8.7 ± 0.8	2.9 ± 0.6	5.2 ± 0.2
	Old sow	31	4.38 ± 0.4 [b]	86.2 ± 7.2	8.1 ± 0.8	1.5 ± 0.4	4.9 ± 0.2
Male bias	**Gilt**	32	3.66 ± 0.4 [a]	80.0 ± 9.5	9.6 ± 0.9	1.4 ± 0.3	4.8 ± 0.1
	Young sow	12	4.90 ± 0.7 [c]	81.3 ± 14.9	9.8 ± 1.5	1.8 ± 0.6	5.0 ± 0.1
	Old sow	9	4.25 ± 0.4 [b]	73.9 ± 16.4	11.3 ± 2.5	2.1 ± 0.9	5.0 ± 0.2
Significance	**Sex bias**		Ns	ns	Ns	0.017	0.024
	Parity		Ns	ns	Ns	ns	ns
	SB x P		0.050	ns	Ns	ns	ns

[a-c] Within a column and group, means without a common superscript differ (P < 0.01).

4.3.3 Effect of gestated sex bias of the litter on histological ovarian follicle counts

Gilts gestated in female biased litters had more primary follicles, when compared with gilts from litters with no sex bias (Table 4.3). Primary follicle numbers in gilts from male biased litters were not different from those from female bias or no bias litters (Table 4.3). Numbers of secondary and antral follicles were not affected by the sex bias of the litter (Table 4.3). Gilts gestated in male biased litters had a lower number of atretic follicles, compared with no bias and female biased litter progeny (Table 4.3).

Table 4.3 The main effects of gestated sex bias of the litter on histological measures of ovarian development.

	n	Primary	Secondary	Antral	Atretic
Female bias	12	29,932 ± 4,658[a]	10,081 ± 2,038	2,064 ± 409	19,732 ± 6,642[a]
No bias	21	20,555 ± 2,052[b]	8,531 ± 873	1,847 ± 255	12,416 ± 1,640[a]
Male bias	6	28,365 ± 9,106[ab]	11,162 ± 3,955	1,342 ± 266	9,667 ± 2,156[b]
Significance		0.042	0.93	0.22	<0.001

[a-b] Within a column, means without a common superscript differ ($P < 0.05$).

4.3.4 Effect of gestated sex bias of the litter on *in vitro* embryo production

Higher numbers of blastocysts developed when oocytes from gilts gestated in male biased litters were used for *in vitro* embryo production, in comparison with oocytes from gilts from female biased or no sex bias litters (Table 4.4). The percentage of oocytes that cleaved and the percentage of cleaved embryos that developed to blastocysts were also higher for offspring from male biased litters, compared with female or non-biased litters (Table 4.4).

Table 4.4 The main effects of gestated sex bias of a litter on in-vitro measures of oocyte quality.

	n ovaries	n COCs **	# Blastocysts	# Expanded Blastocysts	% Cleaved	% Blastocysts / cleaved	% Blastocysts / oocytes
Female bias	41	355	13[a]	6	45.2 ± 5.7[a]	7.7 ± 3.1[a]	5.9 ± 2.7
No bias	59	504	16[a]	16	44.2 ± 4.3[a]	12.1 ± 2.5[a]	11.0 ± 2.6
Male bias	20	323	22[b]	8	64.9 ± 7.7[b]	26.1 ± 6.4[b]	22.8 ± 6.1
Significance	Sex bias		0.030	ns	0.005	0.005	ns

* % blastocysts/cleaved and % blastocysts/oocytes were calculated by combining all stages of blastocyst development

** Cumulus oocyte complexes (COCs)

4.4 Discussion

These results suggest that the gestated sex ratio of a gilt's birth litter significantly affects her reproductive development, while showing no effect on birth weight or growth performance. Gilts from female biased litters had fewer large surface antral follicles, a smaller average follicle diameter, and a higher number of primary follicles when compared with gilts from unbiased litters. Ovaries of gilts from male biased litters had fewer atretic follicles, but other follicle populations did not differ from gilts gestated in unbiased or female biased litters. Differences in follicle dynamics, and the distribution of follicles between size categories, can reflect sexual maturation (Knox, 2019). Based on the distribution of surface antral follicles, it would appear that the gilts gestated in unbiased litters were showing signs of advance sexual maturation compared with gilts from female biased litters. In support of this, ovaries of gilts from female biased litters contained more primary follicles, indicative of reduced recruitment of follicles into the later stages of development.

As no differences were found in antral follicle populations between gilts gestated in a male or female biased litter, the increased oocyte quality of the male biased gilts may be due to

variability in steroid content and gonadotrophin-binding ability of the preovulatory follicles. Ding and Foxcroft (1992) found that follicle size is not a definitive measure of follicle maturity as preovulatory follicles can differ in size by up to 2 mm. As such, further research is necessary to determine physiological differences in preovulatory follicle development between gilts gestated in female and male biased litters. The apparent increase in number of mature follicles in male biased gilts, found in the current study, may be reflective of advanced sexual maturation. Lamberson *et al.* (1988) reported a younger age at first oestrus as the number of males in a litter increased, and gilts that developed *in utero* between two males reached puberty earlier than those gestated between two females (Parfet *et al*. 1990). In a study conducted by Veiga-Lopez *et al*. 2009, prenatal testosterone treatment of ewe lambs resulted in the early onset of puberty; however, the LH surge was delayed, and the amplitude decreased, indicating that the androgen exposure *in utero* may have compromised the development of the hypothalamic-pituitary-gonadal axis. Seyfang *et al.* (2017) found follicles from male biased gilts to be more responsive to a single dose of PG600, which was supported by the reduced rate of puberty attainment and number of corpora lutea observed in female biased gilts. However, it is difficult to define if this was a true pubertal response to PG600, as animals were terminated at the end of the trial so it is unclear whether they would have continued to cycle without ongoing treatment. In a later study, Seyfang *et al*. (2018) found there was no significant difference in age at puberty attainment for female versus male biased gilts, therefore, it is possible that the earlier study demonstrated the increased sensitivity of the follicles of male biased gilts to exogenous gonadotrophin treatment. However, whether this is due to alteration in development of the hypothalamic-pituitary-gonadal axis or the physiological differences in follicle development is yet to be determined and should be the focus of future research. As corpora lutea were not counted in this study it is difficult to determine if the male biased gilts were displaying advanced sexual maturation or physiological differences in follicle development.

In sheep, maternal exogenous treatment with testosterone has been found to androgenise the female fetus, resulting in a reduced ovarian reserve and increased numbers of developmentally advanced ovarian follicles containing larger oocytes, due to enhanced follicular recruitment (Steckler *et al*. 2005). Results from the current study indicate that oocytes collected from male biased gilts were more developmentally competent, as evidenced by ability to develop to the blastocyst stage *in vitro*. These animals produced more blastocysts when their oocytes were used for *in vitro* embryo production. This is most likely due to masculinised gilts showing advanced onset of puberty (Lamberson *et al*. 1988; Rohde Parfet *et al.* 1990), as blastocyst development is increased in oocytes from post pubertal pigs in comparison to prepubertal, due to increases in progesterone concentration in the follicular fluid (Bagg *et al*. 2007). Although the current data shows little effect of being gestated in a male biased litter on follicle distribution, oocyte development competence appears to be enhanced. However, there is growing evidence that despite this gilts from male biased litters may not be ideal for selection into the breeding herd. A number of studies have reported that gilts from male biased litters are less likely to conceive at first mating, are more sensitive to gonadotrophins, more aggressive and possess fewer functional teats (Drickamer *et al*. 1997; Drickamer *et al*. 1999; Seyfang *et al*. 2017, 2018). It was recently demonstrated by Seyfang *et al*. (2018) that gilts gestated in male biased litters showed inhibition of the LH surge, with no effect on tonic LH secretions. Their data showed no differences in puberty attainment in response to boar exposure between gilts from litters with varying sex bias; however, gilts from male biased litters were found to have a delayed LH surge with decreased duration and total secretion. Disruption of the LH surge has the potential to impact the onset of oestrus and timing of insemination relative to ovulation and may impair follicular luteinisation, ultimately affecting the chances of conception and ability to maintain pregnancy (Kirkwood and Aherne, 1985). The mechanisms by which the LH surge is disrupted are yet to be determined and are

in need of further investigation, as unlike rodents and sheep there is little information surrounding the regions of the brain controlling surge and tonic secretion of LH in the pig. There is evidence that specific aspects of a gilt's reproductive axis can be affected at different times throughout gestation, as studies involving maternal testosterone treatment at day 30, but not day 40 of gestation, had the ability to masculinise the external female genitalia (Petric *et al*. 2004). In addition, it was found that the LH surge of a gilt can be affected by testosterone treatment within the critical window of 35-39 days of gestation, but treatment between 30-36 and 40-46 of gestation did not affect tonic LH release (Petric *et al.* 2004). As the sex bias of a litter appears to be affecting the LH surge and follicle development, but not tonic LH, it is possible that the androgen exposure is having an effect at the neuroendocrine level, specifically on the population of neurons responsive to positive, but not negative, effects of steroids on GnRH release. The increased cleavage and blastocyst development rates when oocytes from gilts gestated in male biased litters were used for *in vitro* embryo production suggest that these oocytes may have been of higher quality than those collected from female or unbiased gestated gilts. However, further investigation is necessary into the effects of sex bias on the ovarian reserve and longevity in the breeding herd, due to previous research finding differences in sensitivity to gonadotrophins and LH surge disruption (Seyfang *et al.* 2017, 2018). Therefore, the results of the current study, in combination with previous research, suggest that the gestated sex bias of a replacement gilt is an important factor to consider in breeder selection.

In sheep and rats, prenatal maternal treatment with testosterone resulted in intrauterine growth restriction (IUGR) and low BW offspring (Steckler *et al.* 2005; Manikkam *et al.* 2004; Bremner and Cumming, 1978; DeHann *et al.* 1987; Recabarren *et al.* 2005; Wolf *et al.* 2002, 2004). Intrauterine growth restriction and low BW are precursors of postnatal catch up growth, commonly viewed as a risk factor for adult onset of disease (Ong and Dunger, 2002).

However, these studies involved exogenous testosterone administration to the dam, thus are likely to have exposed the fetus to higher testosterone levels, in comparison to the current study, which relied on naturally occurring levels from surrounding male litter mates. Although post-natal growth rate can affect ovarian development (van Wettere *et al*. 2012), there were no differences in body weight at any time point between the three observational groups. This finding supports previous evidence that pre-pubertal growth is similar for gilts from male and female biased litters (Seyfang *et al.* 2019). Therefore, the observed effects of sex-bias of the gestated litter on ovarian development were not due to differences in gilt weight or growth rate.

The differences observed between the fertility of gilts from male and female biased litters, in this and previous studies, may be a reflection of the differences in the LH surge but not ovarian function, as there were no differences found in follicle distributions. In an industry that relies heavily on reproductive uniformity, the use of gilts from biased litters has the potential to be detrimental due to the probable alterations in puberty attainment, conception rates and gonadotrophin sensitivity.

Chapter 5: The effect of birth weight and maternal parity on the subsequent reproduction of a gilt.

5.1 Introduction

Factors such as maternal age and birthweight are well known to affect the future performance of a gilt. Gilt progeny have consistently been found to be lighter at birth and weaning (Hendrix *et al.* 1978), have poorer lifetime growth performance (Rehfeldt and Kuhn, 2006), lower muscle accretion (Alvarenga *et al.* 2013) and greater susceptibility to disease (Miller *et al.* 2013). In addition, low birthweight animals have reduced muscle growth, poorer carcass and meat quality, and take longer to achieve market weight and puberty (Quiniou *et al.* 2002; Kuhn *et al.* 2003; Bee, 2004; Gondret *et al.* 2006; Rehfeldt and Kuhn. 2006; Foxcroft *et al.* 2009). Birthweight has also been directly correlated with ovarian mass and the number of primary follicles present at birth (Da Silva-Buttkus *et al.* 2003), with low birthweight gilts giving birth to smaller litters of lightweight piglets (Corson *et al.* 2009), indicating that prenatal development of the piglet may have intergenerational repercussions on reproductive potential. It is, therefore, crucial that the relationship between maternal age, birthweight and reproductive potential in the pig be explored further.

The causes of the suboptimal performance of the Australian herd fall under two main categories; the first being poor management, and the second due to reproductive failure (Patterson and Foxcroft, 2019). As such it is necessary to investigate how the potential suboptimal management of nutrition may affect a sows reproductive capabilities. In sows, feed restriction during late lactation reduces oocyte quality, subsequent litter size and embryonic survival, and increases the weaning to oestrus interval (WOI) (Zak *et al.* 1997a, 1997b; Vinsky *et al.* 2006; Foxcroft *et al.* 2005; Oliver *et al.* 2011). The WOI is affected by

lactation length, season and management, with manipulation of nutrition and litter size during lactation having significant effects on subsequent reproductive performance, particularly in first parity sows (Clowes *et al*. 1994). However, there appears to be an evolving relationship between lactational catabolism and reproductive performance of sows post weaning, and in a review of the literature, Foxcroft *et al.* (2005) concluded that the resilience of modern sows to experimental feed restriction appears to be increasing, due to the relatively minor impact of sow tissue catabolism on WOI, with often no effects observed on ovulation rate. In support of this, lactational feed restriction of first parity sows, in both a controlled research and commercial environment, did not affect wean to oestrus interval with greater than 85% of sows returning to oestrus within 3-5 days post weaning (Patterson *et al*. 2010 and 2011). Historically, experimental restriction during late lactation has resulted in extended wean to first service intervals (Foxcroft *et al*. 1995). Patterson *et al.* (2011) proposed the existence of a subpopulation of sows that respond differently to nutritional challenges during lactation, as the amount of lactational catabolism necessary to support litter growth to weaning, and the effect this had on the embryonic development of the subsequent litter, varied greatly between individuals. This difference could be explained by differences in developmental programming, which is the effect that the prenatal maternal *in-utero* environment and early postnatal period can have on future development and performance of the progeny. The offspring's metabolism and capacity to respond to metabolic challenge can therefore be programmed by both the maternal nutrition during gestation and postnatally. Maternal malnutrition, undernutrition or overnutrition during the neonatal period has been shown to have transgenerational impacts and reduce growth performance and feed efficiency in offspring throughout their life, often resulting in asymmetrical growth retardation and metabolic alterations (Ovilo *et al*. 2013; Gonzalez-Bulnes *et al.* 2014; Chen *et al*. 2017; Ji *et al.* 2017). If this subpopulation of sows is created by differences in developmental programming, the opportunity exists to identify and select for sows that are better equipped to cope with

suboptimal nutrition. As such the aim of this study was to determine whether differences in the maternal environment, as indicated by gilt weight at birth and maternal parity, contributes to differences in response to feed restriction during the last week of their first lactation. We hypothesised that the reproductive performance of light birth weight, gilt progeny would be greatly affected by a feed restriction in their first lactation, resulting in fewer large antral ovarian follicles present at weaning, extended WOI intervals, and smaller and lighter subsequent litters. The majority of previous studies have concluded with the slaughter of the animals that underwent nutritional restriction and subsequent litter characteristics being determined at days 30-50 post conception. However, in the current study animals were allowed to continue in the breeding herd, with subsequent farrowing and litter data collected, allowing for a more commercially relevant assessment of their ability to overcome a nutritional insult during their first lactation.

5.2 Methods

Gilts were originally identified and tagged at birth according to high and low birthweight, within litter, and maternal parity (gilt vs sow) as described in Chapter 2.2. Animals selected into the breeding herd were managed as per standard farm protocol. Initial selection occurred at day 154 of life, with assessment based on conformation, locomotion, teat number and weight. Gilts selected into the breeding herd will be referred to as 'selected progeny' from here onwards. Selected gilts were then provided with daily boar exposure from days 175 to 196 days of age, with any animals failing to display heat being removed from the gilt pool. Selected gilts were mated in their second heat, at which point they joined the breeding herd.

Selected gilts were identified prior to entering the farrowing house and allocated to their original observational groups of high or low BW born to a gilt or sow, then randomly allocated to receive either a 5 kg or 7 kg daily diet of pelleted feed from day 13 $\pm$ 2 of lactation until

weaning at 20 $\pm$ 2 days. Data was recorded for farrowing, litter characteristics, growth and reproductive performance as described in Chapter 2.8. Once weaned, all sows re-entered the commercial herd and were managed according to standard farm protocols, with all subsequent mating and farrowing data collected from the farm's Elite Herd records.

5.3 Statistical analysis

The two highest and two lowest birth weight (BW) female piglets were selected from litters of gilt and multiparous sows. A subset of these selected gilts then entered the breeding herd and underwent a nutritional treatment in their first lactation. Data was initially analysed by dividing gilts into the 8 groups (Table 5.1), with birthweight, maternal age and nutritional treatment as fixed effects; however, due to no significant effect of nutritional treatment this effect was removed from the analysis presented in this study (Supplementary data Table 1). Data were then analysed by dividing piglets into 4 groups, by birth weight and maternal parity. Birthweight was classed as low or high for the individual litter, with maternal parity classed as either gilt (first litter) or multiparous (second litter onwards). All variables were tested for normality and homogeneity of variance by histograms, gplots and Shapiro-Wilk test of normality and any data not normally distributed was transformed. Data were analysed using a linear mixed model, with dam as a random factor. Analysis of all data included the effects of season farrowed (in lieu of replicate), litter size, birth weight group and maternal parity as main factors. The tertiles of the distributions (33.3% and 66.6%) were calculated for amount of body weight lost by the sow during lactation. Sows were grouped as losing a low (+27 to -7 kg, n = 46) medium (-8 to -13 kg, n = 45) or high (-14 to -27 kg, n = 44) amount of weight during lactation.

Table 5.1 Visual representation of distribution of animals per treatment group when divided by birth weight and maternal parity

n	Birth weight grouping	Maternal age	Treatment allocation (kg)
8	High	Gilt	5
7	High	Gilt	7
5	Low	Gilt	5
3	Low	Gilt	7
28	High	Multiparous	5
34	High	Multiparous	7
23	Low	Multiparous	5
18	Low	Multiparous	7
126			

Birthweight grouping: High or Low weight at birth;

Maternal age: Born to a gilt or multiparous sow;

Treatment allocation: 5 or 7 kg of feed provided daily from day 13 of first lactation until weaning.

5.4 Results

Nutritional treatment group was initially included in the analysis; however, it was later removed as it did not significantly affect any outcomes measured in this trial (Table 5.2 and Supplementary data Table 1). As expected, sows allocated to the feed restricted group recorded significantly less feed refusal when compared to the control (Table 5.2). All further results will focus on birthweight and parity effects.

Table 5.2 Average weight and growth of the sows and their litter (mean ± SEM) when groups by nutritional treatments only.

	Nutritional treatment				P > 0.05
	7 kg	SEM	5 kg	SEM	Treatment
Day 1 litter weight (kg)	17.4	0.56	16.5	0.52	ns
Day 1 post farrow sow weight (kg)	196.6	2.96	199.5	2.91	ns
Day 13 sow weight (kg)	193.6	2.94	196.6	2.89	ns
Day 13 litter weight (kg)	37.9	1.11	39.3	1.16	ns
Day 20 sow weight (kg)	189.2	2.83	187.2	2.75	ns
Day 20 litter weight (kg)	56.4	1.44	55.0	1.37	ns
Sow weight loss	-7.4	1.0	-12.0	0.7	0.001
Average feed waste (kg)	0.87	0.11	0.44	0.10	0.001

5.4.1 Effect of maternal parity and birthweight on growth performance of selected gilts

The effect of parity of the selected gilts dam on body weight remained significant at all time points except weaning, with selected gilt progeny weighing more than selected sow progeny despite no difference in ADG prior to weaning (Table 5.3). NFGR was increased in selected sow progeny (Table 5.3). Selected low BW gilts remained lighter than selected high BW gilts at day 1 of life and weaning (Table 5.3). ADG to weaning did not differ between low and high BW sows, but NFGR to weaning was higher in low BW animals (Table 5.3).

The effect of maternal parity on body weight remained significant, with selected gilt progeny weighing more than selected sow progeny throughout lactation (Table 5.3). Selected low BW gilts remained lighter than selected high BW gilts at day 13 of their first lactation (Table 5.3). At day 1 and 20 of lactation there was no difference in weight between selected low or high BW sows (Table 5.3).

Table 5.3. Average weight (kg) and growth of selected sows when grouped by maternal parity (gilt vs sow) and birthweight (low vs high). Measurements (mean ± SEM) are reported between day 1 and weaning, with average weight at day 1 of life (D1), average weight at weaning, and average daily gain (kg.day)(ADG) and neonatal fractional growth rate (%/day) (NFGR) between birth and weaning. Measurements (mean ± SEM) during the sow's first gestation are reported as average weight at day 1 post farrow (D1PF), day 13 of lactation (D13), and day 20 of lactation (D20) and average weight loss throughout lactation (Wt loss).

		Measures as a piglet				**Measures during first gestation/lactation**			
	n	**D1**	**ADG**	**NFGR**	**Wean**	**D1PF**	**D13**	**D20**	**Wt loss**
Low	49	1.50 ± 0.0	0.199 ± 8	13.7 ± 0.1	3.8 ± 0.1	191.8 ± 3.0	188.1 ± 3.0	182.3 ± 3.0	-8.3 ±1.3
High	77	1.82 ± 0.0	0.208 ± 6	11.5 ± 0.0	5.3 ± 0.1	197.3 ± 2.1	194.3 ± 2.1	187.6 ± 1.9	-10.4 ± 0.7
Gilt	23	1.90 ± 0.1	0.186 ± 9	10.2 ± 0.0	4.50 ± 0.2	208.9 ± 5.0	204.5 ± 4.6	195.7 ± 4.7	-11.2 ± 1.7
Sow	103	1.66 ± 0.0	0.208 ± 6	12.8 ± 0.0	4.59 ± 0.1	192.7 ± 1.7	189.7 ± 1.7	183.7 ± 1.6	-9.4 ± 0.7
BW		0.000	ns	0.004	0.011	ns	0.022	ns	ns
Parity		0.003	ns	0.010	ns	0.000	0.001	0.006	ns

All data are presented in kilograms, except for ADG which is kg/day, and NFGR, which is reported as percentage of birthweight

5.4.2 Effect of maternal parity and birthweight of a sow on first parity outcomes

There were no interaction effects between a gilt's birth weight and maternal parity on first parity outcomes. There was no effect of parity of the selected gilts dam on the progeny's litter weight at any time point (Table 5.4). Selected gilt progeny wasted more feed between days 13 and 20 of lactation when compared with selected sow progeny (0.79 ± 0.2 vs 0.49 ± 0.1 kg/day, respectively). There was no effect of BW on the number of piglets born alive, still born or weight of the gilt's first litter at any time point (Table 5.4).

Table 5.4. Effect of birthweight and maternal parity on first pregnancy outcomes. Average number of piglets born alive, still born and the reared litter size, which was standardized at fostering following day 1 weights (kg), and average litter weight (Mean ±SEM) at day 1 post farrow, day 13 and day 20 of lactation in the first lactation when sows were grouped by maternal parity (gilt vs sow) and birthweight (low vs high).

	N	Born alive	Still Born	Litter Size	D1 litter wt	D13 litter wt	D20 litter wt
Low BW	49	11.2 ± 0.3	0.34 ± .09	10.7 ± 0.1	16.5 ± 0.7	39.5 ± 1.2	56.8 ± 1.5
High BW	77	10.9 ± 0.3	0.63 ± .11	10.7 ± 0.1	16.6 ± 0.5	38.4 ± 0.8	55.7 ± 1.0
Gilt Progeny	23	11.2 ± 0.4	0.52 ± .16	10.7 ± 0.1	17.1 ± 0.8	39.4 ± 1.8	55.8 ± 2.1
Sow Progeny	103	10.9 ± 0.2	0.50 ± .09	10.7 ± 0.1	16.3 ± 0.5	38.7 ± 0.7	56.2 ± 0.9
BW		ns	ns	ns	ns	ns	ns
Parity		ns	ns	ns	ns	ns	ns

5.4.3 Effects of maternal parity and birthweight on ovarian development at day 13 and 20 of lactation

Parity of the selected gilt's dam had no significant effects on follicle numbers between day 13-20 of the selected gilt's first lactation, as assessed by transrectal ultrasound. Sow BW had no significant effect on follicle growth and development during lactation (Table 5.5). However, there was a tendency ($P < 0.1$) for higher numbers of large follicles at both day 13 and 20 of lactation in the high BW group (Table 5.5)

Table 5.5 Ovarian follicle populations during first lactation in gilts classified as low or high BW for their litter, born to a gilt or a sow. Ovarian data was collected via transrectal ultrasound of both ovaries at day 13 ± 2 and day 20 ± 2 of lactation, with follicles classified as either small (1-3.99 mm diameter) or large (≥4 mm diameter) and average diameter, calculated from both ovaries.

		Day 13			Day 20		
	n	**Small follicle count**	**Large follicle count**	**Av follicle diameter (mm)**	**Small follicle count**	**Large follicle count**	**Av follicle diameter (mm)**
Low BW	44	30.5 ± 2.7	2.0 ± 0.5	2.3 ± 0.2	34.3 ± 3.8	3.8 ± 0.8	2.7 ± 0.2
High BW	80	24.9 ± 1.6	2.8 ± 0.7	2.2 ± 0.1	34.1 ± 2.1	6.3 ± 0.9	3.3 ± 0.3
Gilt progeny	21	24.1 ± 2.4	1.0 ± 0.0	2.0 ± 0.2	36.0 ± 3.7	5.3 ± 1.2	2.8 ± 0.1
Sow progeny	103	28.1 ± 1.6	2.7 ± 0.6	2.3 ± 0.1	34.8 ± 2.1	5.4 ± 0.8	3.2 ± 0.2
BW		0.076	0.063	ns	ns	0.095	0.062
Parity		ns	ns	ns	ns	ns	ns

5.4.4 Effect of sow weight loss during lactation on litter weights

Sow weight loss during lactation was not affected by birthweight, maternal parity or reared litter size at any time point. Nutritional treatment group did affect sow weight loss with sows in the restrict fed group losing more weight throughout lactation than the control (P = <0.001, -12.0 ± 0.7 kg vs -7.4 ± 1.0 kg, respectfully). Sow weight loss during lactation affected litter weight at day 13 and day 20 of lactation, with sows that lost the least amount of weight having significantly lighter litters (Table 5.6).

Table 5.6 Average litter weight (kg) on day 13 and day 20 of lactation when sows are grouped according to weight loss (kg) (low, medium, high) from day 1 post farrow until weaning.

	N	Average sow wt loss	D13 litter wt	D20 litter wt
Low	46	-1.6 ± 5.7	35.2 ± 1.0	51.3 ± 1.3
Med	45	-10.1 ± 1.5	39.1 ± 1.0	56.9 ± 1.2
High	44	-17.6 ± 2.5	42.4 ± 1.0	59.3 ± 1.5
Sig.			<0.001	<0.001

5.4.5 Effects of birthweight, maternal parity and nutritional restriction on second parity outcomes

A sow's BW, maternal parity or allocated treatment group in the nutritional challenge did not have a significant impact on her litter's weight at days 1, 13 and 20 of lactation (Table 5.2). However, litter weight at the subsequent farrowing (parity 2) was heavier for low BW in comparison to high BW sows (Table 5.7). Age of the sow at second farrowing, but not first farrowing, was affected by BW (Table 5.6), with high BW animals farrowing at a younger age, despite having a significantly longer weaning to oestrus interval (WOI) in comparison to low BW sows (Table 5.7). However, when sows with a WOI of >10 days (negative pregnancy test at 35 days post conception) were removed from the analysis there was no significant differences between groups (Supplementary Table 1). The subsequent gestation length was affected by a significant interaction between maternal parity and BW, with average gestation

length being significantly shorter for low BW gilt progeny when compared to high BW gilt progeny (114.5 ± 1.9 vs 116.1 ± 1.6 days, respectively). Gestation length of low or high BW sow progeny was not different (Supplementary Table 1).

Table 5.7 Reproduction outcomes in the second pregnancy of gilts classified as low or high BW for their litter. As measured by second litter; total born (TB), born alive (BA), still born (SB), litter weight (litter wt, kg), age of sow at farrowing (days), weaning to oestrus interval (WOI) (days), weaning to oestrus interval excluding negative pregnancy checks (WOI <10 days) and length of second gestation (days).

	n	TB	BA	SB	Litter wt	Age of sow	WOI	WOI <10 days	Gestation length
Low	40	11.1 ± 0.5	10.8 ± 0.5	1.9 ± 0.6	16.8 ± 1.2	500.9 ± 10.8	6.4 ± 0.9	5.1 ± 0.2	115 ± 0.2
High	74	11.1 ± 0.4	10.7 ± 0.4	1.7 ± 0.4	14.6 ± 0.9	488.4 ± 2.1	12.2 ± 1.8	5.0 ± 0.1	116 ± 0.2
Sig.		ns	ns	ns	0.010	0.035	0.004	ns	ns

5.5 Discussion

Previous studies have reported that imposing feed restriction in late lactation reduced oocyte quality, subsequent litter size and embryonic survival and increased weaning to oestrus interval in sows (Zak *et al.* 1997*a*, 1997*b*; Oliver *et al*. 2011; Vinsky *et al*. 2006; Foxcroft *et al*. 2005). However later studies, using similar experimental designs and achieving similar levels of sow tissue catabolism, have reported a reduced effect on reproductive performance following weaning, with the only consistent effect being a decrease in embryo weight of the subsequent litter (Oliver *et al*. 2011). These results suggest that due to the genetic selection for increased ovulation rate and litter sizes, the biology of the commercial sow has been altered, with sows better able to adapt to the metabolic challenges associated with tissue mobilisation during lactation. In the current study, there were no significant differences in reproductive outcomes between sows that were restrict fed during lactation and control sows. Similarly, De Bettio *et al.* (2016) recently observed no reduction in reproductive output

in sows that were placed on a 50% feed restriction for 21 days. It is, therefore, suggested the lack of an effect of feed restriction on sow reproduction observed in this study may reflect improvements in herd genetics, rather than the feed restriction not being severe enough to elicit a detrimental response. However, further research involving a more severe feed restriction would be necessary to determine whether it is genetic improvement or nutritional requirements responsible for the lack of reproductive outcomes observed in this experiment.

In contrast to Chapter 3, which found gilt progeny to be 180 grams lighter at birth than sow progeny, the selected gilt progeny used in this study were heavier than the selected sow progeny at birth. This can potentially be explained by the piggery's management practices, as the initial experimental design involved all selected piglets either having their reproductive tract collected or remaining in the herd for this first parity study. However, the study was also influenced by the farm's selection criteria, which could not be altered. Not all animals selected as focal pigs at birth reached ideal weight or were of sound structure at day ~154 of life. Therefore, this contradictory finding may be explained firstly by the overall low numbers of gilt progeny making it to selection and secondly, by light weight gilt progeny, as found in Chapter 3, remaining lighter until selection age, resulting in a greater number of high BW gilt progeny being selected into the breeding herd. Foxcroft *et al.* (2009) reported that low birth weight animals reach puberty later than their heavier counterparts and as a result show reduced reproductive potential. As the main selection tool at this piggery is to retain the heaviest animals at day ~154 of age, in combination with low birthweight gilt having higher removal rates due to anoestrus before first mating (Magnabosco *et al*. 2016), this potentially resulted in a greater number of the lightweight gilt progeny either remaining anestrous or not being selected into the breeding herd due to size.

However, as this study aims to focus on the within litter variation, and low birthweight (for their litter) animals were still selected into the breeding herd we can still draw some meaningful conclusions from the data. There was no difference in ADG during the pre-weaning period or weaning weight in offspring of sows or gilts in this study; however, sow progeny showed an increase in NFGR due to the 'catch-up growth' often experienced by low birth weight animals. Selected gilt progeny were heavier than selected sow progeny at first farrowing and remained heavier throughout lactation, as would be expected due to the higher birth weight. Gilt progeny had a significantly higher rate of average feed wastage during late lactation in comparison to sow progeny, whilst remaining heavier, suggesting these animals either had higher body reserves to rely on, or may have been more efficient in comparison to the lighter selected sow progeny.

The original birthweight of a sow significantly affected the weight of that animal throughout life , with high birth weight gilts remaining heavier in comparison to their lighter littermates. However, at the end of first lactation there was no significant difference found between the body weight of high or low birth weight animals. Total weight loss throughout lactation significantly affected litter weight at days 13 and 21, with sows that lost a higher percentage of weight producing heavier litters at both time points. Although not significant, high birth weight sows tended to lose more weight throughout their first lactation, which could explain the lack of significant differences in sow body weight at weaning. High birthweight sows had a longer WOI, and a lighter litter weight at the subsequent farrowing. The increased WOI and lighter subsequent litter weight experience by the high birthweight sows can likely be explained by the increased level of energy mobilisation seen in the first parity, as suggested by heavier first litter weights and the tendency for increased weight loss throughout lactation. Excessive energy mobilisation during lactation commonly results in subsequent reproductive failure, decline in ovarian function, milk protein concentration and piglet growth (Clowes *et*

al. 2003; Vinsky *et al*. 2006). In the current study we are unable to define the level of energy mobilisation experienced, however, we can suggest that the high birthweight gilts experienced increased levels of mobilization when compared to light birthweight gilts.

In an industry that continues to focus on breeder selection in the weeks surrounding puberty, resulting in an average replacement rate of 55-58% (Australian Pig Annual, 2013; Australian Pig Industry Benchmarking Report 2019-2021), the successful adoption of early indicators of reproductive potential would modernise the management of the replacement herd. It is well known that gilt progeny contribute significant performance variation, as they have a greater susceptibility to disease (Miller *et al*. 2013), are lighter (Hendrix *et al*. 1978), and consequently have reduced lifetime growth rates (Rehfeldt and Kuhn, 2006). However, due to high replacement rates, gilts tend to make up approximately 25% of the breeding herd (Koketsu, 2007), making selection from these litters inevitable. The results from this study suggest that the birth weight of a gilt may play a greater role in subsequent production and ability to cope with suboptimal management practices, than the maternal parity. However, further work is necessary to draw any solid conclusions about the effect of maternal parity, due to the uneven parity profile in this study. Low birth weight gilts showed reduced WOI and increased second litter weight with all other reproductive parameters displaying no significant differences, when compared to high birth weight gilts. It should, however, be noted that the average weight of the low birth weight category for this trial was 1.5 kg, as animals were selected as low birthweight for their litter, and as discussed above, numbers of low birthweight gilt progeny included in this study was low due to on farm selection processes. In addition, in the current study, there were no significant differences in reproductive outcomes between sows that were restrict fed during lactation and control sows. These results suggest that due to the genetic selection for increased ovulation rate and litter sizes, the biology of the commercial sow may have been altered, with primiparous sows better able to adapt to the metabolic challenges associated with tissue mobilisation during lactation. It is, therefore, suggested the

lack of an effect of feed restriction on sow reproduction observed in this study may reflect improvements in herd genetics, rather than the feed restriction not being severe enough to elicit a detrimental response. However, further research involving a more severe feed restriction would be necessary to determine whether it is genetic improvement or nutritional requirements responsible for the lack of reproductive outcomes observed in this experiment.

There were no differences found in reproductive output between selected sow and gilt progeny, suggesting that birthweight potentially plays a larger role in reproductive development than maternal parity, as previously described in Chapter 3. However, due to the potential bias of lightweight gilt progeny being mainly excluded, due to commercial farm practices that could not be altered, we are unable to draw any tangible conclusions about maternal parity at this time.

Supplementary Data Chapter 5

Table 1. Reproduction data for sows fed either 5 or 7 kg of feed daily from day 13-20 of first lactation.

All days refer to the day of lactation post farrowing.

	Nutritional treatment				P > 0.05
	7 kg	St. Error	5 kg	St. Error	Treatment
Age at farrow (days)	341.82	7.11	347.48	7.11	ns
1st litter # born alive	11.44	.35	10.87	.33	ns
1st litter # born dead	.476	.14	.433	.13	ns
1st litter # total born	11.90	.39	11.33	.37	ns
Day 13 small follicles	27.04	2.48	26.52	2.31	ns
Day 13 large follicles	1.86	.91	1.98	1.08	ns
Day 20 small follicles	34.42	3.39	36.40	3.30	ns
Day 20 large follicles	5.55	1.14	4.89	1.12	ns
2nd gestation length (days)	115.46	.27	115.49	.27	ns
3rd gestation length (days)	115.16	.31	115.49	.31	ns
Age at 2nd farrowing (days)	493.42	8.13	498.29	8.05	ns
Age at 3rd farrowing (days)	641.09	9.38	645.09	9.51	ns
1st WOI (days)	11.84	2.10	8.54	2.05	ns
2nd litter total born	11.54	.55	11.44	.52	ns
2nd litter born alive	10.99	.52	10.89	.50	ns
2nd litter weight (kg)	15.03	1.34	15.55	1.38	ns
2nd litter still born	1.95	.43	1.56	.57	ns

Chapter 6: General Discussion

6.1 Summary

This thesis presents the results from three studies investigating the role of developmental programming on the reproductive potential of gilts. The objectives of this thesis were:

- To determine if a suite of easily identifiable, neonatal indicators (birth weight, maternal parity and AMH concentrations) should be identified for use within commercial systems when selecting replacement gilts
- To determine if the gestated sex ratio of a litter should be used as a tool for selection or exclusion of gilts from the replacement herd
- To determine if developmental programming, as assessed by birth weight and maternal parity, plays a role in a sow's ability to cope with a lactational feed restriction

The overarching hypothesis was that low birth weight, gilt progeny would prove to be reproductively inferior and less able to cope with suboptimal management, with these gilts suffering reduced reproductive performance when subjected to a lactational feed restriction.

The results of the three studies suggest that selection from gilt litters is acceptable, providing the individual gilt is not classified as low birth weight. However, the litters of first and second parity sows should be avoided, as their progeny showed reduced measures of reproductive potential. While some significant differences were found in the ovarian development of male and female biased litters, the main commercial implication of the current study is that until further research is conducted selection from biased litters should be avoided where possible, in order to increase herd reproductive uniformity.

6.2 Chapter 3: The effect of maternal parity and birth weight on the developmental programming and reproductive potential of a gilt.

Chapter 3 explored the influence of developmental programming on the reproductive potential of a gilt, specifically the effect of maternal age and weight at birth. This thesis hypothesised that the progeny of gilt litters and light birthweight piglets would show reduced reproductive potential, as indicated by ovarian antral follicle counts and assessment of oocyte developmental competence, using *in-vitro* embryo production measures. It is currently common practice to avoid selection of breeding animals from gilt litters; however, in this study there were no detrimental effects of being born to a gilt on ovarian development, when compared to progeny of multiparous sows, provided the gilt offspring were not classed as low birth weight. However, embryos from the progeny of first and second parity sows were found to have significantly reduced cleavage rates, potentially due to the impacts of the metabolic state of the sow prior to and during the mating period following their first litter. Therefore, the hypothesis was partly rejected, as gilt progeny were not found to be of lower reproductive value. A gilt's birth weight was found to be a useful selection tool, as it is indirectly correlated with ovarian reserve, similar to previous findings (Corson *et al.* 2009; Da Silva-Buttkus *et al.* 2003). The use of plasma AMH concentration as an early indicator of reproductive longevity in the pig was potentially supported by this study; however, given that recent work in the field has revealed that porcine AMH is produced by the theca cells of preovulatory follicles and luteal cells following ovulation (Almeida *et al.* 2018), more research is necessary to determine if AMH is a worthwhile marker of lifetime reproductive performance in the pig.

Maternal parity and birthweight are both easily adoptable markers in commercial piggeries. As gilts are predicted to make up approximately one quarter of any breeding herd (Koketsu, 2007) the ability to select from these litters would be of great financial benefit. Many commercial farms have the ability to weigh piglets at birth; however, a skilled stockperson

would easily be able to determine the approximate weight of a piglet, making this a simple and effective addition to any selection program. The use of AMH commercially is currently not viable, due to it being a costly test, the skill and labour required for blood collection from young piglets, and the lack of conclusive evidence. As explained in Chapter 3, in many species AMH is already used as a definitive measure of ovarian reserve, however, this is currently not true for the pig. While this study has added to the current understanding of the relationship between plasma AMH and ovarian reserve in the pig, further work is necessary to enable a thorough understanding of the role of AMH in the porcine ovary.

6.3 Chapter 4: The effect of the gestated sex bias of the litter on the future reproductive development of a gilt.

Chapter 4 focused on one particular aspect of developmental programming in utero: the effect of gestated sex bias on the reproductive development of a gilt. The pig industry relies heavily on reproductive uniformity, and the sex bias of a litter has previously been found to alter the timing of ovulation (Veiga-Lopez *et al*. 2009; Seyfang *et al*. 2018). As such the use of gestated sex ratio of the litter in the selection criteria of replacement breeding stock has the potential to improve efficiency and productivity. My thesis hypothesised that gilts gestated in a male biased litter would encounter negative impacts on ovarian development when compared to gilts from gilt biased litters, with a reduction in the oocyte quality. However, the data from this trial suggested that progeny of unbiased litters may be more developmentally advanced compared to female biased gilts, with gilts from male biased litters falling somewhere in between. No differences were found in ovarian follicle populations; however, the ovarian development of progeny of male biased litters appeared to be more advanced as indicated by improved oocyte quality. These findings may be due to advanced sexual maturation and earlier puberty attainment in the gilts from male biased litters, as similar results were found previously by Lamberson *et al.* (1988) when monitoring ages at oestrus.

Despite these seemingly straight forward results, these animals may not be ideal for selection, as a number of studies have reported that gilts from male biased litters are less likely to conceive at first mating, are more sensitive to gonadotrophins, more aggressive and possess fewer functional teats (Drickamer *et al*. 1997; Drickamer *et al*. 1999; Seyfang *et al*. 2017, 2018). While some significant differences were found in the ovarian development of gilts from male or female biased litters, the main commercial implication of the current study is that until further research is conducted selection from biased litters should be avoided, where possible. It is clear that over exposure to androgens can disrupt the normal development of the reproductive tract, culminating in differences in timing of the LH surge (Seyfang *et al.* 2017) and consequential alterations in the reproductive uniformity of the breeding herd, contributing further to the notion that selection should not occur from biased litters.

6.4 Chapter 5: The effect of birth weight and maternal parity on the subsequent reproduction of a gilt.

Chapter 5 investigated the effects of the maternal environment on a sow's reproductive ability to cope with a late lactation feed restriction following their first pregnancy. Birthweight and maternal age were the two main indicators used to distinguish the differing pre-natal environments each gilt was exposed to. Studies conducted in the 1990's and 2000's demonstrated that restricting the feed intake of sows during late lactation reduced oocyte quality, subsequent litter size and embryonic survival, whilst also increasing weaning to oestrus interval (Zak *et al*. 1997a and b; Vinsky *et al*. 2006). However, the relationship between lactation catabolism and reproductive performance post weaning appears to be evolving, with both Foxcroft *et al.* (2005) and Patterson *et al.* (2010, 2011) concluding that reproductive traits are becoming less affected by catabolism during lactation. As described by Patterson *et al*. (2011) there appears to be a subpopulation of sows that respond differently to the challenge of a nutritional restriction in lactation, in terms of their ability to support litter growth to

weaning versus embryonic development of the subsequent litter. In an effort to determine if maternal parity or birthweight played a role in the developmental programming of ability to cope with a late lactation feed restriction, we hypothesised that light birth weight, gilt progeny, when exposed to feed restriction during their first lactation, would show measures of reduced reproductive output such as fewer large ovarian follicles around weaning, longer weaning to oestrus intervals, and produce subsequent smaller and lighter litters in the next pregnancy. This hypothesis was rejected as feed restriction during lactation had no effect on any reproductive outcomes. We believe this may be due to advancements in the genetics of modern sows, as work conducted over the past 20 years into the effects of feed restriction appears to be having declining effects on reproductive outcomes.

6.5 Future research

Chapter 3 assessed the effects of birthweight and maternal parity on reproductive measures at puberty, which provided valuable information on reproductive potential. However, an intergenerational observational study of lifetime performance would provide definitive evidence of the reproductive output, retention in the herd and the effects on the progeny. As the progeny of young parity sows showed reduced reproductive potential, it is necessary to investigate further the effects of a negative versus positive energy balance around lactation and mating to determine the effect this has on the subsequent litter's reproductive development.

The negative effects of sow body weight loss during lactation, namely the first lactation, have been well characterised, as sows will typically mobilise body tissues during lactation to offset deficiencies in energy and nutrient intake, to meet the demands of the litter (Clowes *et al*. 2003; Vinsky *et al*. 2006). Excessive mobilisation during lactation commonly results in subsequent reproductive failure, decline in ovarian function, milk protein concentration and

piglet growth (Clowes *et al*. 2003; Vinsky *et al*. 2006). These impacts seem to be more prevalent in parity 1 sows, as a negative impact on wean to service intervals are seen when these sows experience as little as 5% weight loss in comparison to the 10% losses necessary to see impacts in multiparous sows (Thaker and Bilkei, 2005). These adverse impacts on reproduction are thought to be due to changes in metabolic hormone concentrations when sows are in a state of negative energy balance. Catabolism during peak milk yield in late lactation negatively affects levels of insulin and insulin-like growth factor 1 (IGF-1) (Zak *et al*. 1997), and this influences follicular development via changed patterns of LH and FSH secretion (Pettigrew *et al.* 1993). Sows that mobilise high levels of protein during lactation have been found to have suppressed ovarian function, with fewer medium sized follicles and less follicular fluid (Clowes *et al.* 2003). In addition, the number of live embryos is reduced in pregnant sows that did not receive adequate energy levels in the previous lactation, with embryo survival rather than ovulation rate being affected (Vinsky *et al*. 2006). The current study did not see any difference in piglet growth rates or subsequent reproduction, however, it was found that the progeny of these young parity sows showed decreased oocyte quality and ovarian reserve when compared to progeny from gilt or multiparous sow litters. This is likely due to the effect of negative energy balances in late lactation on the oocyte and embryo quality prior to mating, as a negative energy balance may potentially influence embryonic and fetal development, with consequences for subsequent ovarian development. As such, the outcomes of this thesis warrant further investigation into the intergenerational effects that a negative energy balance in late lactation plays in the future reproductive development of the offspring.

The intense selection pressures that modern sows have been subjected to have resulted in biological changes in their metabolism. As such, the modern genotype of highly prolific sows has evolved to better cope with suboptimal nutrition, meaning that the current nutritional

requirements of the modern-day sow may need to be completely reevaluated. As this study found no differences in any reproductive outcomes from a 30% feed restriction for the last 7 days of lactation, it would make sense to investigate a further restriction for a prolonged period. As this study was limited by low numbers of gilts remaining in the system it would also be beneficial to include greater numbers in future experiments. As such, a lifetime study assessing the reproductive performance and retention rate of sows subjected to multiple lactational feed restrictions would allow for a deeper understanding of the role birthweight and maternal parity play in the developmental programming of a sow to better cope with suboptimal nutrition, allowing selection of the most reproductively resilient sows. It would be necessary to investigate the effect of the recurring feed restriction on the reproductive development of the progeny, and the extent of intergenerational repercussions this may cause. In Chapter 3 we described the reduction in ovarian reserve (as measured by primary follicle counts) and cleavage rates during *in-vitro* fertilisation from second parity sows, hypothesising that this may be due to the catabolic state of the sow during her first lactation. If this is true then multiple feed restrictions would have an intergenerational detrimental effect on the herd's reproductive performance.

Despite the fact that the role of AMH in the porcine ovary may differ from other livestock species there is still the potential for it to become an important part of the selection of replacement gilts. Studies to analyse fluctuations in AMH concentration throughout the oestrous cycle of a sow are required. In many livestock species AMH is produced by small growing follicles, the population of which is thought to remain stable, and as such the concentration of AMH has not been found to fluctuate greatly throughout the oestrous cycle. However, in the sow there is the potential for a peak in production as unlike other species AMH is also produced by the corpus luteum. In sows, the stage of the oestrous cycle may be an important factor to take into consideration when testing AMH concentrations. Almeida *et*

al. (2018) have hypothesised that AMH production by the corpus luteum in the sow may be to prevent new follicular growth and early depletion of the ovarian reserve, in addition to AMH reducing sensitivity of follicles to FSH. It would therefore be of value to determine if high AMH concentration is linked to an increased reproductive lifespan in the sow.

Chapter 4 in combination with recent work conducted by Seyfang *et al*. (2017 and 2018) found that the gestated sex bias of a gilt did not alter timing of puberty attainment but did cause alterations in the steroid content and gonadotrophin-binding ability of the pre-ovulatory follicles. Further work is required to increase understanding of the physiological differences occurring due to the effect of the gestated sex bias, specifically the mechanisms by which the LH surge is disrupted and what effect this is having on retention rates of the breeding herd.

In summary, the developmental programming of a gilt is an important consideration when selecting replacement stock, one which for the most part is currently overlooked. The inclusion of maternal parity, birthweight and gestated sex bias into replacement programs, would save the industry time and money currently spent on the rearing of either suboptimal gilts, or animals which will continue to cause variability within the breeding herd. As such, the results of this thesis suggest that it is worthwhile including two main selection criteria into future breeding programs: the use of a minimum birthweight and exclusion of animals from sex biased litters.

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