THE UNIVERSITY
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AUSTRALIA

Management and Nutrition of the Replacement Gilt

A thesis submitted in total fulfillment of the requirements for the degree of
DOCTORATE of PHILOSOPHY

By

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B.Sc. (Honours)

in the

School of Agriculture Food and Wine

Discipline of Agricultural and Animal Science

The University of Adelaide, Australia

March 2008

Table of Contents

List of Figures

List of Tables

Abstract

Replacement gilts and early parity sows constitute a large, and increasing, proportion of modern breeding herds. Breeding herd profitability is therefore increasingly dependant on the efficiency of gilt management strategies as well as litter size at first farrowing; however, incidences of reproductive failures and small first litter sizes are common within cohorts of replacement gilts. Hence, this thesis had two primary aims which were addressed in four experiments; one, to identify whether the puberty stimulation and mating strategies developed for genotypes of 20 to 30 years ago are suitable for today's heavier yet leaner genotypes; and two, to better understand the influence of pre-pubertal growth rate and metabolic status on reproductive maturation, puberty attainment and potential litter size.

In experiment 1, 192 Large White/Landrace crossbred gilts were used to compare the effects on puberty attainment of commencing boar exposure at 161, 182 or 203 days of age, and the effect of first mating gilts at either the pubertal or second oestrus on ovulation rate and early embryo survival. Gilts were artificially inseminated at the allocated oestrus, with the reproductive tracts collected at 22.8 $\pm$ 0.4 days after first mating (mean $\pm$ S.E.M), and the numbers of corpora lutea and viable embryos recorded. Delaying first boar contact until 182 or 203 days of age significantly ($P < 0.01$) reduced days-to-puberty and increased the proportion of gilts attaining puberty within 10 days of start of boar exposure. Gilt age at mating had no effect on potential litter size; however, there was a tendency for gilts mated at their second oestrus to shed 0.6 more ova, and possess one more embryo at day 20 of pregnancy.

Experiment 2 determined the effects of long- (chronic) and short- (acute) term moderate dietary restriction on ovarian development and oocyte developmental competence in 161- and 175- day old, pre-pubertal gilts. Both chronic and acute periods of moderate feed restriction reduced the number of medium follicles present on the ovaries of 161- and 175-day old gilts, as well as the proportion of

oocytes reaching Metaphase II *in vitro.* However, feeding level during the 14 days prior to ovary collection had the greatest effect on follicular growth and oocyte quality. Experiments 3 and 4 investigated the effects of the same dietary treatments on the timing of puberty attainment in response to boar exposure and potential litter size following mating at the pubertal oestrus. Chronic dietary restriction during the pre-pubertal period delayed puberty attainment, but the timing of the pubertal response was unaffected by acute, moderate dietary restriction of previously well-fed gilts during the period immediately prior to, and coincident with, boar exposure. Acute dietary restriction or repletion stimulated an increase or decrease, respectively, in pubertal ovulation rates; however, neither the number of viable embryo present on day 22 of gestation nor embryo survival were affected by the nutritional treatments used in these studies.

Overall, these results demonstrate that the timing and synchrony of puberty attainment is significantly improved when gilts first receive boar exposure at 182 days of age (or older). It is, therefore, concluded that sexual maturity, as measured by responsiveness to boar contact, occurs later in modern genotypes. It is also evident that within the age range investigated, delaying first mating until the second oestrus does not significantly increase either ovulation rate or embryo number at day 20 post-mating. Further, the current data provide the first evidence that despite profoundly affecting the size and morphology of the antral follicle pool as well as pubertal ovulation rates, subtle alterations in dietary intake have no affect on the number or proportion of embryos surviving the pre-and peri-implantation period. It is evident the litter size of gilts mated at the pubertal oestrus is not determined by the number of ova shed, with the current data demonstrating that increasing ovulation rates results in increased embryo mortality.

Declaration

This thesis is a record of original work and contains no experimental material which has been accepted for the award of any other degree or diploma 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.

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William van Wettere

31st March, 2008

Acknowledgements

Chapter 1　Introduction and Literature review

1.1 Preamble

Currently, the annual sow replacement rate in Australia is 61% (Australian Pig Annual, 2005), approximately 1.6 times greater than the acceptable level of 38% reported by Hughes and Varley (2003). This high replacement rate means that unmated gilts constitute 13% of the breeding herd (Australian Pig Annual, 2005), and as a result their subsequent reproductive performance has a profound effect on the productivity of the breeding herd as a whole.

Appropriate management of replacement gilts has been identified as a key determinant of sow lifetime productivity (Aumaitre et al., 2000). Combining appropriate prepubertal nutrition with effective stimulation of puberty and mating strategies facilitates gilt entry into the breeding herd and maximises subsequent reproductive performance (Evans and O'Doherty, 2001). However, reproductive disorders, including failure to reach puberty, unpredictable timing of first oestrus, low conception rates and low first litter sizes, remain a common problem associated with replacement gilts (Whittemore, 1996). Lucia et al. (2000) reported that anoestrus and pregnancy failure in parity 0 sows accounted for 12.4% of all early culls, with a further 2.2% attributed to low first litter size. In the more recent Australian study of Hughes and Smits (2002), approximately 27% of premature culling from the breeding herd took place at parity zero.

In order to address this problem, it is necessary to investigate whether current gilt management strategies, largely developed 20 – 30 years ago, are suitable for today's genotypes (Hughes and Varley, 2003). Compared to their counterparts of three decades ago, current genotypes typically have higher rates of lean growth, reduced body fat content, and greater mature weights, and are now heavier yet leaner when puberty stimulation and mating take place. More importantly, they are also likely to be at a lower proportion of their mature weight and therefore physiologically less mature. Consequently, a number of recent reviews have suggested that selection for improved production traits

may also be a selection against early puberty attainment (Whittemore, 1996; Edwards, 1998; Close and Cole, 2000; Evans and O'Doherty, 2001; Slevin and Wiseman, 2003).

In addition to genotype effects, sexual activity and reproductive processes in the female pig are controlled and coordinated by the combined actions of socio-sexual and nutritional signals (Cosgrove and Foxcroft, 1996; Prunier and Quesnel, 2000; Martin et al., 2004). Socio-sexual signals originating from mature boars act as a potent stimulant of ovulatory activity in female pigs, and repeated studies have shown that daily physical contact with a mature boar (the 'boar effect') is an effective method of stimulating precocious puberty attainment in replacement gilts (Hughes and Cole, 1976; Kirkwood and Hughes, 1979). However, reproductive activity in the female pig ultimately depends on nutrient availability (Cosgrove and Foxcroft, 1996). Ranked behind thermogenesis and growth with respect to nutrient prioritisation, reproductive activity is reduced or even postponed when nutrient availability is limited, or during periods of extreme growth (Cosgrove and Foxcroft, 1996). Considering the low fat content and strong inherent drive toward lean tissue accretion typical of current genotypes (Close and Cole, 2000; Hughes and Varley, 2003), it is likely that reproductive performance of today's gilts, namely timing of puberty attainment and first litter size, will be highly sensitive to changes in nutritional and environmental management during the pre- and peri-pubertal period. This literature review will discuss current knowledge pertaining to the attainment of puberty in replacement gilts and the determination of first litter size.

1.2 Reproductive development in the gilt

In the mature female pig, communication and feedback within the hypothalamic-pituitary-ovarian axis drive and coordinate reproductive function (**Figure 1.2.1**). Pituitary synthesis and secretion of the gonadotrophic hormones, luteinizing hormone (LH) and follicle stimulating hormone (FSH), are tightly controlled by gonadotrophin releasing hormone (GnRH) of hypothalamic origin. In turn, gonadotrophins play an integral role in the later stages of ovarian follicle growth and ovulation, while ovarian secreted steroids and peptides act at both the hypothalamic and pituitary level to regulate the release of GnRH, LH and FSH (Prunier and Quesnel, 2000; Evans and O'Doherty, 2001). Puberty in the gilt depends on the progressive integration of this reproductive axis, and this section will review the sequence of endocrine and ovarian events resulting in the first ovulation and onset of reproductive capability.

1.2.1 Pre-pubertal patterns of gonadotrophin and steroid release

The period from birth to puberty is characterised by specific patterns of gonadotrophin and steroid release (Lutz et al., 1984; Diekman et al., 1983; Camous et al., 1985; Evans and O'Doherty, 2001). Following an initial decline during the first 40 days of post-natal life, the frequency and amplitude of LH pulsing increases, resulting in a corresponding rise in basal LH concentrations between 40 and 125 days of age (**Figure 1.2.2).** FSH concentrations also increase during the peri- and post-natal periods, reaching maximal levels at approximately 50 to 70 days of age, with concentrations declining from 80 days of age onwards (**Figure 1.2.2;** Colenbrander et al. 1982; Camous et al., 1985; Christenson et al., 1985). Plasma LH levels as well as pulse frequency and amplitude also decline between days 125 and 192 post-partum (**Figure 1.2.2**), and during this period, LH pulses were observed in only 65% of gilts, compared to 85% between 83 and 125 days of age (Camous et al., 1985).
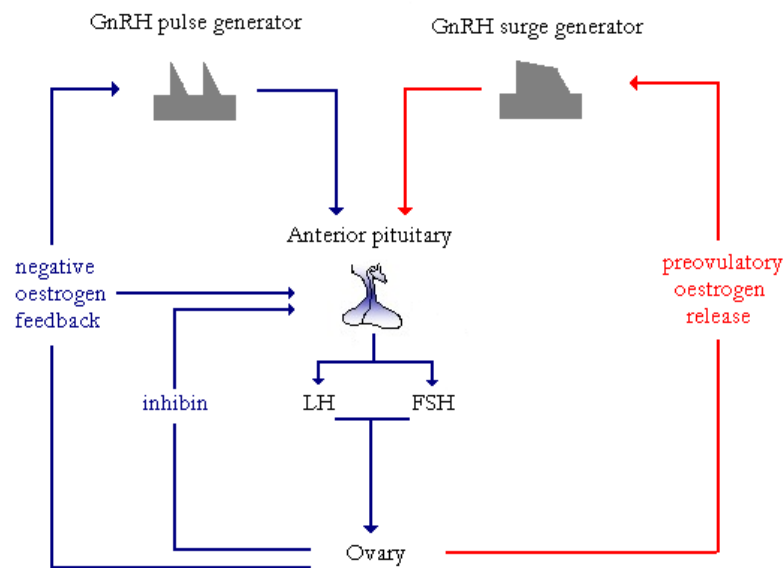
Figure 1.2.1 Main components of the porcine hypothalamic-pituitary-ovarian axis (Adapted from Prunier and Quesnel, 2000)

Circulating concentrations of oestrogen and progesterone remain low throughout the pre-pubertal period (Elseasser, 1982). Concentrations of progesterone remain below 0.3 ng/ml between 70 and 190 days of age, and oestradiol levels remain at 6 – 9 pg/ml from 70 to 150 days of age (Pressing et al., 1992), with Bolamba et al. (1994) reporting no real change in oestradiol concentrations between 160 and 180 days of age. However, additional studies indicate a 5 pg/ml (Pressing et al., 1992) rise in oestradiol concentrations between 150 and 210 days of age (Pressing et al., 1992). Based on the urinary oestrone excretion data of Camous et al. (1985) it appears that rises in oestrogen secretion occur in surges: the first two elevations coinciding with the appearance and proliferation of the antral follicle pool, with the third rise occurring concurrent with the increase in the number of larger follicles (**Figure 1.2.3**).

Figure 1.2.2 Circulating gonadotrophin levels in gilts between between 15 and 192 days of age: A) Plasma concentrations (ng/ml) of LH (■) and FSH (x); B) frequency (▲) and amplitude (○) of LH pulsing (adapted from Camous et al., 1985)

1.2.2 Pre-pubertal development of the ovarian follicle pool

Following the appearance of the first preantral follicles at birth, their number increases steadily forming approximately 30% of the ovarian follicle pool by the time the gilt is 90 days of age (Oxender et al., 1979). A facilitating role for FSH has been suggested during preantral follicle growth in rodents (McGee et al., 1997; Fortune, 2003) and pigs (Morbeck et al., 1992), and FSH is known to promote the preantral to antral transition in porcine follicles (Mao et al., 2002). Consequently, the first appearance of antral follicles in the gilt's ovary and subsequent proliferation of the antral follicle pool are stimulated by constant ovarian exposure to elevated FSH levels during the post-natal period, as well as the rise in LH secretion characteristic of this period (Hughes et al., 1990; Evans and O'Doherty, 2001).

Antral follicles first appear on the ovaries at around 60 to 70 days of age (Oxender et al., 1979; Dyck and Swierstra, 1983; Guthrie et al., 1984), and between 70 and 112 days of age the mean number of small (1 – 3 mm) surface antral follicles present on each animal's ovaries increases from 2 to 306. The number of small antral follicles then declines gradually until the onset of puberty (**Figure 1.2.3**; Dyck and Swierstra, 1983), concurrent with a gradual increase in the number of larger (> 3mm) follicles from 5.5 to 25 (**Figure 1.2.3**; Dyck and Swierstra, 1983). More recently, Bolamba et al. (1994) performed laparoscopic examinations at 5 day intervals between 160 and 180 days of age, reporting dynamic, and often rapid, changes in the number of small (1 – 3 mm) and large ($\geq$ 6 mm) ovarian follicles during this period. These authors concluded that during the pre-pubertal period ovarian follicle growth occurs in waves, characterised by gradual growth of a cohort of follicles which, in the absence of the pattern of LH release required to support follicle growth through to ovulation, become atretic and are succeeded by a subsequent wave of follicles (Bolamba et al., 1994; Foxcroft, 1991). Although antral follicle growth beyond 2 mm is certainly dependant on gonadotrophin support (Driancourt et al., 1995), it is noteworthy that these changes in the dynamics of the ovarian follicle population appear to occur in the absence of any change in circulating concentrations of either LH or ovarian secreted steroids (Bolamba et al., 1994). Consequently, the ability of follicles to grow and develop in response to the relatively constant pre-pubertal pattern of gonadotrophin secretion observed by Bolamba et al. (1994) is likely dependent on expression and activity of the various intrafollicular growth factors and steroids known to determine follicle sensitivity to gonadotrophins (Cardenas and Pope, 2002).

Figure 1.2.3 Mean number of surface antral follicles present on the ovaries of gilts from birth to puberty. Closed triangles (▲) represent follicles with a diameter of 1 – 3 mm, crosses (x) represent follicles > 3mm in diameter (adapted from Dyck and Swierstra, 1983)

1.2.3   Ovarian feedback control of GnRH and gonadotrophin secretion

Gonadotrophic hormone secretion is driven by the pulsatile, quantal discharge of stored GnRH from axonal terminals in response to the rhythmic and synchronous firing of entire populations of GnRH neurones (Austin and Short, 1982), and is controlled primarily by the inhibitory and stimulatory effects of ovarian steroids and peptides (Levine, 1997). GnRH is discharged from axons situated in the median eminence, diffuses into the capillaries of the pituitary portal vasculature, and is transported to the anterior pituitary. At the anterior pituitary GnRH binds with specific receptors on the surface of gonadotrophic cells, stimulating the synthesis and secretion of gonadotrophins, as well as increasing the expression of its own receptors (Nett et al., 2002). Oestrogen elicits a biphasic response in GnRH and LH secretion, initially inhibiting and then, once oestrogen concentrations surpass a "critical threshold", promoting LH release (Kesner et al., 1989; Britt et al., 1991). These negative and positive feedback actions of oestrogen appear to be mediated by independent neuronal pathways involving two separate populations of GnRH neurons (Herbison,

1998; Kraeling et al., 1998). The cell bodies of the GnRH neurones controlling tonic, pulsatile release of gonadotrophins are commonly referred to as the pulse generator and reside in the arcuate nucleus of the medial basal hypothalamus. The cell bodies of the neurones responsible for the generation of the pre-ovulatory surge of GnRH release are situated in the preoptic area and suprachiasmatic nuclei (Austin and Short, 1982; Nishihara et al., 1999).

Negative feedback effects of oestrogen control the frequency and amplitude of tonic, or episodic, LH release, and rising oestrogen concentrations completely suppress pulsatile LH release for a period of 54 – 60 hours prior to the preovulatory LH surge (Foxcroft and van de Weil, 1982; Britt et al., 1991). Although oestrogen appears to inhibit pituitary responsiveness to GnRH for the first 6–12 hours, oestrogen induced attenuation of LH release is primarily due to the elimination of GnRH discharge from the median eminence (Kesner et al., 1989; Britt et al., 1991; Elsaesser et al., 1998). Oestrogen inhibition of LH pulsing appears to involve the promotion of gamma aminobutyric acid (GABA) release, which acts directly on GnRH neurones to inhibit GnRH release (Herbison, 1998; Smith and Jennes, 2001; Malyala et al., 2005), as well as alterations in the activity of the noradrenergic and adrenergic neural system (Elsaesser et al., 1998; Smith and Jennes, 2001). Although, in vitro studies indicate that oestradiol suppresses FSH production by reducing pituitary cell production of activin, a peptide known to stimulate FSH synthesis (Nett et al., 2002), Hasegawa et al. (1988) reported an inverse relationship between FSH and ovarian secreted inhibin, but not oestradiol, and it is generally accepted that inhibin selectively suppresses FSH release from the pituitary (Elsaesser et al., 1988; Knight, 1996).

The release of the pre-ovulatory surge of LH secretion in response to the abrupt, surge-like discharge of GnRH from the median eminence is dependant on the accumulation of a readily releasable pool of LH during the period of strong negative feedback (Kesner et al., 1989; Britt et al., 1991), and the decline

in LH surge release precedes that of the GnRH surge, suggesting that the magnitude and duration of the pre-ovulatory LH surge reflects the size of the LH pool (Nett et al., 2002). Furthermore, studies involving sheep indicate that LH secreting granules migrate to the periphery of gonadotrophic cells prior to the pre-ovulatory release of GnRH. Pituitary sensitivity to GnRH is promoted by an oestrogen induced increase in GnRH receptor expression (Nett et al., 2002; Clarke et al., 2005), whilst inhibin also augments GnRH induced LH release (Knight, 1996). Studies involving ovariectomised gilts (Kraeling et al., 1998) indicate oestradiol concentrations in excess of 20 pg/ml must be maintained for approximately 24 – 48 hours of exposure in order to activate the specific neural pathways which temporarily override the normal inhibitory feedback effects of oestrogen and initiate the "surge specific pattern of GnRH secretion that superimposes itself upon the normal pulsatile pattern of GnRH release" responsible for the pre-ovulatory surge of LH release from the pituitary (reviewed by Karsch et al., 1997; Levine, 1997; Herbison, 1998).

The activity of the GnRH pulse generator appears to decline concurrent with the pre-ovulatory surge of GnRH (Nishihara et al., 1999), and of the 30% of GnRH neurones activated during the GnRH surge, the majority are situated predominantly in the preoptic area (Levine, 1997). Oestradiol reduces GABAergic neurone activity during positive feedback, and GABA levels in the preoptic area, but not the medial basal hypothalamus, decline concomitantly with a rise in LH secretion in oestradiol treated ovariectomised rats (Jennes and Smith, 2001; Mizuno and Teresawa, 2005). Activation of glutamergic inputs increases GnRH secretion, and a stimulatory effect of N-Methyl-D,L-Aspartate, a potent glutamate agonist, on GnRH secretion from the median eminence has been established in pigs (Estienne and Barb, 2005). Glutamate release in the preoptic area increases immediately before, or concurrent with, the steroid induced LH surge in rats (Smith and Jennes, 2001; Mizuno and Terasawa, 2005; Ojeda et al., 2005). An increase in the activity of adrenergic neurones also coincides with the initiation of the oestrogen induced LH surge, with inhibition

of adrenaline synthesis preventing the steroid induced LH surge (Smith and Jennes, 2001). In addition to augmenting gonadotrophic sensitivity to GnRH, it is clear that neuropeptide Y plays an important role in the control of GnRH release (Herbison, 1998). Neuropeptide Y innervates GnRH neurones in the preoptic area, and steroid induced LH surges are suppressed following administration of neuropeptide Y antiserum. Furthermore, neuropeptide Y appears to facilitate the positive feedback effect of oestrogen on the activity of GnRH neurones, and in addition to increasing the expression of neuropeptide Y mRNA, oestrogen enhances the stimulatory effect of neuropeptide Y on GnRH release from the median eminence (Herbison, 1998; Smith and Jennes, 2001; Estienne and Barb, 2005).

1.2.4 Maturation of ovarian feedback mechanisms

Studies involving exogenous hormone treatments (Elsaesser and Foxcroft, 1978; Dial et al., 1984; Pressing et al., 1992) clearly show that the individual components of the pre-pubertal gilts hypothalamic-pituitary-ovarian axis are operational before the initiation of first ovulation. Between 70 and 100 days of age, coincident with the development of antral follicles, gilts acquire the capacity to initiate follicle growth and ovulate in response to hourly infusions of GnRH (Pressing et al., 1992). Elsaesser and Foxcroft (1978) observed an immature LH surge in oestradiol benzoate treated 60 day old gilts, while Dial et al. (1984) reported that LH surges were only effectively induced by oestradiol benzoate in gilts older than 100 days of age. Further, between 100 and 190 days of age hourly GnRH infusions elicit a relatively consistent endocrine and ovulatory response (Pressing et al., 1992), whilst studies involving ovariectomised gilts indicate that hypothalamic sensitivity to both positive and negative oestrogen feedback changes between 60 and 160 days of age (Elsaesser and Foxcroft, 1978; Berardinelli et al., 1984). There is also substantial evidence in sheep (Huffman et al., 1987; Ryan et al., 1991), cattle (Day et al., 1984; Dyer et al., 1990); rats (Ramirez and McCann, 1963) and pigs (Elsaesser

et al., 1991) to suggest that inadequate tonic LH secretion, most likely reflecting a suppressed pattern of episodic GnRH release (Elsaesser et al., 1988; Plant, 2002), is the primary inhibitor of ovulation in pre-pubertal gilts. Together, these studies indicate that the final maturation event required for puberty occurs at the hypothalamic level, most likely due to a decline in the sensitivity of GnRH secretion to negative oestrogen feedback (Barb et al., 1988), or a programmed alteration in central nervous system control of GnRH release (Plant et al., 2002; Ojeda et al., 2005).

1.2.4.1 Development of ovarian feedback

During the neonatal and postnatal period, hypophyseal responsiveness to ovarian secreted oestrogens is either weak or non-existent. Ovariectomy of neonatal gilts has no effect on the pattern of LH secretion prior to 80 days of age (Elsaesser et al., 1978; Wise et al., 1981), and GnRH appears to be the primary regulator of gonadotrophin secretion during the neonatal and postnatal period (Colenbrander et al., 1982b). The substantial rise in pituitary concentrations of FSH and LH between the late foetal period and 60 days of age presumably reflects an increase in GnRH stimulated synthesis of gonadotrophins (Elseasser et al., 1988), maturation of central nervous system control of hypothalamic GnRH pulsing (Evans and O'Doherty, 2001), or, as reported in the female rat, increased expression or activity of GnRH receptors (Elseasser et al., 1988; Zapatero-Caballero et al., 2004). Progressive development of negative ovarian feedback in the pre-pubertal female rat occurs in association with a decline in serum concentrations of α-fetaprotein, a glycoprotein known to selectively bind oestradiol and impair the efficiency of oestradiol feedback (Zapatero-Cabellero et al., 2004). However, α-fetaprotein concentrations decline to undetectable levels in 28-day old piglets (Martin et al., 2005), approximately 50 days prior to the development of negative feedback, and a direct causal link between decreasing concentrations of this glycoprotein and the development of oestradiol control of GnRH release is therefore unlikely.

Consequently, the poor response of the neonatal hypothalamus to oestrogen may reflect low expression, or activity, of steroid receptors (Elsaesser and Parvizi, 1979), while conversely, activation of hypothalamic sensitivity to oestrogens may result from an increase in either the expression, or activity, of these receptors (Slevin and Wiseman, 2003).

1.2.4.2 Maturation of positive (stimulatory) feedback

Although a single dose of oestradiol benzoate promotes LH secretion in 1 week old gilts (Elsaesser et al., 1978), subsequent studies (Elsaesser and Foxcroft, 1978) provide clear evidence for an age related maturation of positive oestrogen feedback. The mean time from oestradiol benzoate administration to the onset of the LH surge and the LH peak was significantly shorter in 161 compared to 60 day old gilts: 48.2 versus 63.3 hours and 55.5 versus 76.7 hours, respectively (Elsaesser and Foxcroft, 1978). A similar reduction in the interval from oestradiol benzoate treatment to the onset of the LH surge was observed in the study of Foxcroft et al. (1984), with these authors also observing significantly higher maximal surge levels in 160 compared to 60 day old gilts. Although, inadequate gonadotroph responsiveness to GnRH might explain the 'immature' LH surges generated by 60 day old gilts (Kuneke et al., 1993), pituitary response to GnRH is similar in neonatal and adult female pigs (Elseasser et al., 1974), as well as at 60, 160 and 250 days of age (Elseasser et al., 1988), suggesting that maximum gonadotrophic potential for LH release in response to GnRH is achieved during the post-natal period (Elseasser et al., 1988). Consequently, the inability of 60-day old gilts to generate a mature LH surge is more likely to reflect hypersensitivity of gonadotroph cells to the suppressive effects of oestrogen (Kuneke et al., 1993), or inadequate oestrogen augmentation of pituitary responsiveness to GnRH.

There is convincing evidence that ovarian secretions, particularly oestrogen, play an integral role in maturation of positive feedback control of LH secretion

(Foxcroft et al., 1984; Elsaesser et al., 1998), and in the absence of oestrogen the LH surge mechanism reverts to the immature state (Elsaesser et al., 1998). Ovariectomising gilts at 60 days of age results in an LH response to oestradiol benzoate at 160 days similar to that of intact 60 day old gilts, whereas the LH response at 160 days of age is similar between intact gilts and those that were ovariectomised at 60 days old and implanted with oestradiol (Foxcroft et al., 1984). Further, sensitivity to oestrogen feedback is reduced in long-term (100 days) but not short-term (30 days) ovariectomised sows (Parvizi, 2000), and although evidence in pigs is lacking, studies involving rats suggest that oestradiol enhances the maturation of certain hypothalamic neural systems, including the arcuate nucleus, involved in the control of GnRH secretion (Dyer et al., 1990; Elsaesser et al., 1998). Interestingly, a relatively narrow range of oestradiol concentration also appears to be a requisite for the maintenance of positive oestrogen feedback in sexually mature gilts (Elsaesser et al., 1998). Together, these studies provide indirect evidence that the neural mechanisms mediating the stimulatory effect of oestrogen on GnRH discharge are operational well before the first pre-ovulatory LH surge actually takes place. As a consequence, inactivation of the positive feedback mechanism in pre-pubertal gilts likely reflects insufficient oestrogen concentrations.

1.2.4.3 Maturation of negative (inhibitory) feedback

Negative effects of oestrogen on gonadotrophin secretion become evident between 80 and 135 days of age, with LH concentrations doubling in ovariectomised but not intact gilts (Wise et al., 1981). Similarly, whereas FSH levels declined between 70 and 112 days of age in "sham-operated" gilts, Prunier and Louveau (1997) observed a transient rise in plasma FSH concentrations in ovariectomised gilts between days 84 and 98 post-partum, with concentrations remaining significantly higher until 231 days of age. Despite a similar response to GnRH, the LH content of pituitary cells decreases between 60 and 160 days of age (Elseasser et al., 1988), suggesting that

reduced GnRH pulsing, as opposed to decreased sensitivity of gonadotrophic cells to GnRH stimulation, is the most likely cause of declining LH concentrations during the pre-pubertal period (Plant, 2002). Furthermore, implants of oestradiol-17ß inhibit the post-ovariectomy LH increase in 120 and 150-day old gilts (**Table 1.2.1**; Berardinelli et al., 1984), confirming that oestradiol-17ß exerts a negative feedback effect on pre-pubertal LH secretion, most likely due to a reduction in GnRH pulsing. Whilst oestrogen suppression of activin is known to reduce pituitary FSH synthesis, inhibin, produced in high concentrations by antral follicles, is the most probable cause of declining FSH concentrations during the prepubertal period (Britt et al., 1985; Elsaesser et al., 1988; Hasagawa et al., 1988; Knight, 1996). Equally, an altered pattern of GnRH pulsing may be responsible for the earlier decline in FSH concentrations, since while low frequency GnRH pulsing promotes FSH release, high frequency favours LH secretion (Moenter et al., 2003).

A subtle decrease in the sensitivity of LH secretion to oestrogen inhibition appears to occur with advancing age in pre-pubertal gilts, and whereas sub-physiological concentrations of oestradiol benzoate suppress both frequency and amplitude of LH pulse release in 120 day ovariectomised gilts, only the frequency of LH pulsing was inhibited in 150 day old ovariectomised gilts (Berardinelli et al., 1984). Further, although oestradiol implants elicited a similar reduction in LH secretion on day 2 post-ovariectomy in 120 and 150 day old pre-pubertal gilts, the inhibitory effect is significantly reduced on day 14 post-ovariectomy in 150 but not 120 day old gilts (**Table 1.2.1**). More recently, Elsaesser et al. (1991) also observed a similar reduction in oestradiol induced inhibition of LH secretion, and in conjunction with unchanging patterns of LH release in intact gilts, these authors suggested that suppression of tonic LH release prior to the onset of puberty may also involve non-gonadal, central nervous system inhibition of the GnRH pulse generator. Certainly, pre-pubertal secretion of GnRH is increased following the interruption of the GABAergic neuron system. However, this may simply reflect the reversal of oestrogen

induced inhibition of GABA release (Plant, 2000), and the suppressed pattern of LH secretion in intact gilts compared to their ovariectomised counterparts (Berardinelli et al., 1984; Elsaesser et al., 1991) suggests that the ovary, and its secretions, are integrally involved in the attenuation of LH secretion prior to puberty.

Table 1.2.1 Effect of Oestradiol-17ß implants on mean serum LH concentrations (ng/ml) on day 2, 8 and 14 after overiectomy of 120 and 150 day old pre-pubertal gilts (adapted from Berardinelli et al., 1984)

NOTE:
This table is included on page 16 of the print copy of the thesis held in the University of Adelaide Library.

1.2.5 Puberty attainment

Similar to the follicular phase of the mature oestrous cycle, a marked increase in circulating oestrogen concentrations precedes the pubertal pre-ovulatory LH surge in both gilts (Lutz et al., 1984) and ewe lambs (Ryan et al., 1991). Studies involving ewe lambs (Pelletier et al., 1981; Huffman et al., 1987), heifers (Day et al., 1987, Dyer et al., 1990) and gilts (Lutz et al., 1984), indicate that the period prior to this initial follicular phase is characterised by an increase in the frequency, but not amplitude, of episodic LH release. Although incomplete, current evidence suggests that this increase in episodic LH secretion results from a decline in oestrogen inhibition of LH secretion (Ramirez and McCann,

1963) or an ovarian independent alteration in central nervous system control of the GnRH pulse generator (Plant, 2002; Ojeda et al., 2005).

Based on work involving pre-pubertal female rats, Ramirez and McCann (1963) developed the gonadostat hypothesis for puberty attainment, theorising that a reduction in hypothalamic sensitivity to negative oestrogen feedback allows tonic LH secretion to increase, and is responsible for the first follicular phase and ovulation. In support of this, the ability of oestrogen to inhibit episodic LH release decreases with age in ovariectomised gilts (Berardinelli et al., 1984; Elsaesser et al., 1991), while a pre-pubertal decline in the efficacy with which physiological levels of oestrogen suppress LH secretion has been reported in ovariectomised heifers (Day et al., 1984; Day et al., 1987) as well as in ovariectomised, oestradiol implanted ewe lambs (Foster and Ryan, 1981). Episodic LH secretion appears to be less sensitive to negative oestrogen feedback in post-pubertal gilts (Berardinelli et al., 1984; Elsaesser et al., 1991), and whereas oestradiol implants reduced LH pulse frequency in pre-pubertal 150 day old, ovariectomised gilts, episodic LH release was unaffected in age-matched post-pubertal gilts (Berardinelli et al., 1984). The pre-pubertal decline in hypothalamic sensitivity to negative oestrogen feedback coincides with a reduction in hypothalamic concentrations of oestrogen receptors in both pre-pubertal heifers (Day et al., 1987) and female rats (Kato et al., 1974). Day et al. (1987) reported a reduction in cytosolic concentrations of oestradiol receptors in the medial basal hypothalamus and anterior pituitary, but not in the median eminence or preoptic area, suggesting that sexual development, as opposed to rising oestrogen concentrations, is responsible for this reduction in cytosolic receptor expression (Day et al., 1987). These authors also observed a pre-pubertal decline in pituitary concentrations of oestradiol, but not GnRH, receptors, and proposed that oestradiol might initiate an increase in pituitary responsiveness to GnRH during this period.

Approximately nine weeks after ovariectomy, a progressive rise in LH secretion occurs in both untreated and oestradiol implanted ovariectomised ewe lambs, coinciding with the onset of puberty in their intact counterparts (Foster and Ryan, 1979). Therefore, although a decrease in oestrogen inhibition of episodic LH release certainly precedes the first follicular phase (Lutz et al., 1984; Ryan et al., 1991), an intrinsic change in central nervous system control of GnRH neuron activity might also be responsible for increasing pulsatile LH release during this period (Plant, 2002; Ojeda et al., 2005). Increased frequency of LH pulse release is associated with the reduction of an inhibitory, or addition of a stimulatory, central nervous system input into the GnRH pulse generator (Elsaesser et al., 1998; Plant, 2002; Ojeda et al., 2005). Endogenous opioid peptides inhibit LH secretion in sexually immature female sheep (Ebling et al., 1989), and interact with circulating oestradiol to regulate gonadotrophin secretion in both pre-pubertal heifers (Wolfe et al., 1991; Byerley et al., 1992) and female rats (Meijs-Roelofs and Kramer, 1989). In contrast, opioid peptides do not appear to regulate episodic LH release in pre-pubertal gilts (Barb et al., 1988). Equally, additional studies indicating that endogenous opioid peptides mediate progesterone induced inhibition of GnRH release in sexually mature female pigs (Kraeling and Barb, 1990). Although opioid inhibition of GnRH release diminishes as puberty approaches in heifers (Wolfe et al., 1991) and rodents (Ojeda et al., 2005), endogenous opioid peptides do not appear to prevent puberty onset (Plant, 2002; Ojeda et al., 2005). Equally, although a loss of GABAergic inhibition of GnRH neurone activity occurs in conjunction with the increasing LH secretion (Plant, 2002; Ojeda et al., 2005), this might simply reflect a reduction in negative oestrogen feedback.

The increase in LH pulse frequency that precedes puberty is more likely to result from activation of stimulatory inputs to the GnRH pulse generator, and increased production of excitatory amino acids. Activation of the glutamergic inputs to the GnRH neuronal system advances sexual maturity in female rats, and an increase in glutamate synthesis coincides with the onset of puberty

(Ojeda et al., 2005). In addition to transsynaptic influences, glial cell secreted growth factors, such as insulin-like growth factor-I (IGF-I) and transforming growth factor alpha (TGF-ß), appear to play an important role in the stimulation of GnRH discharge during puberty (Plant, 2002; Ojeda et al., 2005). Data in pre-pubertal rats indicate that IGF-I stimulates GnRH discharge from the median eminence and facilitates pre-pubertal increases in GnRH release (Wilson, 1998), as well as interacting with oestradiol to alter the activity of the noradrenergic system (Quesada and Etgen, 2002).

1.2.6 Proposed sequence of endocrine and ovarian events leading to puberty

In the gilt, the sequence of morphological and physiological changes involved in the attainment of puberty occurs in a specific chronological order (Camous et al., 1985; Slevin and Wiseman, 2003). Pre- and peri-natal maturation of the reproductive axis is characterised by formation of the primordial follicle pool, the appearance of primary and secondary follicles, and the development of hypothalamic control of gonadotrophin release from the anterior pituitary gland (Christenson et al., 1985; Elsaesser, 1982; Parvizi, 2000). Between 40 and 125 days of age, rising FSH concentrations and increased frequency and amplitude of episodic LH release stimulate the formation of the antral follicle pool, resulting in the development of negative oestrogen feedback on the activity of the GnRH pulse generator. By approximately 100 days of age, the individual components of the hypothalamic-pituitary-ovarian axis are operational, with administration of oestradiol benzoate effectively eliciting a pre-ovulatory LH surge, and hourly GnRH infusions stimulating ovulation (Dial et al., 1984; Pressing et al., 1992).

Camous et al. (1985) described the period from approximately 120 days of age until puberty attainment as a "*waiting phase*". During this period, circulating oestrogen concentrations rise (Pressing et al., 1992) and ovarian follicle growth appears to occur in successive waves (Bolamba et al., 1994), with suppressed

activity of the GnRH pulse generator and thus inadequate tonic LH secretion preventing final growth to the pre-ovulatory stage and ovulation (Camous et al., 1985; Elsaesser et al., 1988; Plant, 2002). Similar to studies involving sheep (Ryan et al., 1991), the frequency of episodic release increases, while the amplitude of LH pulsing decreases, approximately 30 days prior to the onset of puberty in gilts (Lutz et al., 1984). This increase in LH pulse frequency stimulates the initiation of the first follicular phase, during which growth of ovarian follicles to the preovulatory stage activates positive oestrogen feedback, stimulating the specific neural pathways that elicit the continuous surge of GnRH discharge responsible for the first pre-ovulatory LH surge and ovulation.

Although a degree of controversy exists, it is probable that a decline in the sensitivity of the GnRH pulse generator to negative oestrogen feedback (Ramirez and McCann, 1963; Lutz et al., 1984; Barb et al., 1998), in combination with a gonad independent reduction in inhibitory inputs and activation of stimulatory inputs to the GnRH neuronal network (Elsaesser et al., 1998; Plant, 2002; Ojeda et al., 2005), is responsible for the increase in the frequency of LH pulse release that precedes puberty in ewe lambs (Pelletier et al., 1981; Huffman et al., 1987) heifers (Day et al., 1987; Moran et al., 1989; Dyer et al., 1990) and gilts (Lutz et al., 1984). Current understanding of the developmental cues responsible for initiating this sequence of neuroendocrine events is limited. However, the available literature strongly supports the existence of an inherent, genetically programmed pubertal clock (Plant, 2002), and Ojeda et al. (2005) suggested that the neuroendocrine control of puberty is in fact regulated by a hierarchically arranged and complex network of genes controlled by a *few highly connected upper-echelon gene hubs*", the activation of which is genetically determined.

It is also logical to assume that puberty will only occur once the gilt has developed sufficiently to support the extremely high energetic costs of

oestrous cycling, gestation and lactation. The regulatory effect of metabolic fuels on the activity of the neuroendocrine reproductive axis suggests that the attainment of puberty is likely to depend on the availability of sufficient energy stores. Further, the signals responsible for activating the onset of reproductive function may result from developmental changes in both nutrient partitioning and metabolic status, and a close association between growth and sexual maturation is well established (Foster and Nagatini, 1999; Messer and I'Anson, 2000; Schneider, 2004; Gamba and Pralong, 2006). Therefore, whilst socio-sexual, seasonal and nutritional factors modulate the timing of puberty, it is likely that they do so by influencing the activity of the GnRH neuronal system and development of the ovary, as opposed to acting as the ultimate cue for pubertal development to occur (Plant, 2002).

1.3 Factors affecting puberty attainment

Although gilts normally attain puberty when they are approximately 200 to 220 days old, age at first oestrus varies enormously, ranging from 105 to 350 days (Hughes, 1982). Controlling gilt age at puberty is extremely beneficial to the productivity of the breeding herd, and early puberty attainment is associated with younger mating ages, a shorter non-productive period prior to breeding herd entry, and a reduction in rearing costs. Equally important, synchronous puberty attainment within gilt cohorts facilitates gilt entry into the breeding herd and increases the efficiency of cull sow replacement (Brooks and Smith, 1980; Aherne and Kirkwood, 1985; Koketsu et al., 1999; Evans and O'Doherty, 2001; Breen et al., 2005). Improved control of puberty requires understanding of the factors affecting reproductive development in the gilt. For the purposes of this review, these factors will be divided into three categories: management cues, specifically boar exposure; environmental cues, namely seasonal and climatic effects; and developmental cues, encompassing the effects of age, weight, body composition, nutrition, metabolic signals, and genotype.

1.3.1 Management cues: boar exposure

Exposing replacement gilts to a reproductively mature boar is an effective method of advancing and synchronising puberty, and is common practice throughout the pig industry (Brooks and Cole, 1970; Hughes and Cole, 1976; Kirkwood and Hughes, 1979; Hughes et al., 1990; Patterson et al., 2002). The efficacy of boar exposure as a stimulus for early puberty attainment, often referred to as the 'boar effect', has been clearly demonstrated (Hughes and Cole, 1976; Kirkwood and Hughes, 1979; Hughes, 1994a) and extensively reviewed (see Hughes et al., 1990). Pearce and Paterson (1992) found that 80% of gilts that commenced boar exposure at 168 days of age attained puberty within 31 days compared to 21% of non-stimulated gilts, and in the study of Paterson et al. (1989) 88% of boar exposed gilts were pubertal by 255 days of age, compared to only 46% of non-exposed gilts. The boar effect is mediated through the synergistic actions of four main types of boar-originating cues (Hughes et al., 1990; Patterson et al., 2002), and the exhibition of a pubertal response depends on the gilt receiving a sufficient level of boar stimulation (Paterson et al., 1989). Variations in gilt response to boar contact are attributed primarily to differences in the stimulus value of the boar, the amount of physical gilt-boar interaction that occurs and the duration and frequency of boar exposure.

1.3.1.1 Boar component stimuli

Current understanding indicates that the combined actions of olfactory, tactile, auditory and visual cues produced by the boar are responsible for accelerating and synchronising puberty in the gilt (Hughes et al., 1990; Patterson et al., 2002). However, removing the olfactory bulbs (bulbectomy) of immature female pigs abolishes the pubertal response to male contact (Table 1.3.1; Kirkwood et al., 1981), demonstrating that boar-originating olfactory cues, or pheromones,

play an integral role in the stimulation of precocious puberty attainment (Izard, 1983; Rekwot et al., 2001; Gelez and Fabre-Nys, 2004).

Table 1.3.1 The effects of the presence or absence of olfactory bulbs on puberty attainment in response to 30 minutes of daily boar exposure commencing at 160 days of age (adapted from Kirkwood et al., 1981)

NOTE:
This table is included on page 23 of the print copy of
the thesis held in the University of Adelaide Library.

Based on the type of response they elicit, pheromones can be divided into two categories: signalling pheromones which *"elicit a specific behavioural response"*; and priming pheromones which elicit *"a chain of physiological events in which endocrine and/or reproductive systems are altered"* (Izard, 1983). The androgen dependant pheromonal steroids, 3α-androstenol and 5α-androstenone, are integrally involved in stimulating receptive behaviour and precocious puberty attainment in gilts (Kirkwood et al., 1981; Hughes et al., 1990). Exposure to either boar odour or warm preputial fluid elicits a standing reflex in previously non-responsive oestrous sows (Signoret, 1970; Signoret, 1972: reviewed by Izard, 1983), and 5α-androstenone, present in boar saliva, urine and preputial fluid, has been identified as one of the signalling pheromones responsible for stimulating this behavioural response (Brennan and Keverne, 2004). High levels of 16-androstene steroids are also released in the frothy saliva secreted by the boar's submaxillary glands in response to sexual arousal (Kirkwood et al., 1981). Surgical removal of boar submaxillary glands (sialectomy) reduces both the volume of frothy saliva produced, and the ability of boars to accelerate puberty attainment (**Table 1.3.2;** Izard, 1983;

Pearce et al., 1988), indicating that priming pheromones, such as 3α-androstenol, present in boar saliva play a fundamental role in the acceleration of gilt puberty (Kirkwood et al., 1981; Pearce and Hughes, 1987; Hughes et al., 1990; Austin et al., 2004).

Table 1.3.2 The attainment of puberty in gilts commencing boar exposure at 165 days of age, and receiving 30 minutes of daily contact with either a sialectomised or an unoperated boar (adapted from Pearce et al., 1988)

Priming pheromones exist as non-volatile, peptide bound ligands (Brennan and Keverne, 2004), and in the pig, the proteins pheromaxein and porcine salivary lipocalin bind and transport priming pheromones from the boar's submaxillary gland to the sensory neurons of the gilt's vomeronasal organ (VNO) (Tegoni et al., 2000; Bakker, 2003; Trinh and Storm, 2004). Transfer of priming pheromones therefore requires the gilt to make contact with boar saliva (Pearce and Paterson, 1992; Austin et al., 2004; Brennan and Keverne, 2004). However, access to the VNO can only occur when the incisive ducts, situated in the roof of the mouth between the second set of incisors, are open (Keverne, 1999). The muscles responsible for opening the incisive ducts are activated by the flehmen reaction, or "yawn-like" movement, observed in boar exposed gilts (Pearce and Paterson, 1992). During flehmen, movement of the gilt's snout and vascular modifications induce the secretion, and active pumping, of mucus into the lumen of the VNO (Tegoni et al., 2000).

It is noteworthy that while a pubertal response to boar contact is dependant on olfactory stimulation (Kirkwood et al., 1981; Hughes et al., 1990), gilt response to isolated boar pheromones is poor (Kirkwood et al., 1983). Equally, compared to non-exposed gilts, exposure to a sialectomized boar significantly decreased gilt age at puberty (**Table 1.3.2**; Pearce and Hughes, 1987), and although fitting gilts with snout masks inhibited gilt access to priming pheromones, puberty attainment was still accelerated in response to physical boar contact (**Table 1.3.3**; Pearce et al., 1992). Together these studies suggest that non-olfactory cues, or olfactory cues present in boar urine or preputial fluid, are involved in the induction of precocious puberty attainment. However, exposing pre-pubertal gilts to long-term androgenised, castrated boars in conjunction with combinations of visual and auditory cues did not promote the exhibition of a pubertal response (**Table 1.3.3**). In contrast, while exposure to synthetic androstene solution also failed to accelerate puberty, the addition of boar urine resulted in a considerable, albeit non-significant, reduction in the interval to puberty (**Table 1.3.4**). Whether the failure of gilts to respond to synthetic androstene solution reflects the absence of the binding proteins known to facilitate the transfer of priming pheromones to the VNO, or the inability of gilts to perceive these priming pheromones is not known (Pearce and Hughes, 1987). Stimulation of the nasal olfactory mucosa by either an odour or pheromone may serve as an olfactory signal for the flehmen reaction, and the apparent additive effect of exposure to boar urine in conjunction with androstenes (**Table 1.3.4**) further supports the hypothesis that air born signalling pheromones indirectly facilitate the transport of priming pheromones to the gilt's VNO. Equally, puberty accelerating priming pheromones are present in the urine and preputial fluid of male mice (Colby and Vendenberg, 1974) and it is plausible that boar urine and/or preputial fluid may also contain priming pheromones.

Table 1.3.3 The effects of fitting gilts with snout masks on the attainment of puberty in response to full physical boar contact commencing at 160 days of age (adapted from Pearce and Paterson, 1989).

NOTE:
This table is included on page 26 of the print copy of the thesis held in the University of Adelaide Library.

Although gilt response to tactile, auditory and visual cues in the absence of olfactory stimuli is poor, exposure to olfactory cues in the absence of physical boar contact, such as during fenceline contact, also significantly reduces the efficacy of boar exposure. Therefore, tactile cues and non-olfactory cues appear to act either additively or synergistically to maximise the stimulus value of the boar (Hughes et al., 1990). Boar-originating non-olfactory cues stimulate acute activation of the gilt's hypothalamo-pituitary adrenal axis resulting in a rapid, brief elevation in plasma cortisol concentrations (Hughes et al., 1990; Turner et al., 1998). Although removal of the adrenal glands can reduce the efficacy of boar contact (Hughes et al. 1990), the acute elevations in cortisol that occur during boar exposure do not appear to play an integral role in boar induced puberty attainment (Turner et al., 1999). It is more likely that physical gilt to boar contact maximises the physical transfer of priming pheromones to the gilt's VNO, while the resultant increase in gilt exposure to non-olfactory stimuli facilitates pheromone transfer by promoting the exhibition of the flehmen reaction.

Table 1.3.4 The effect of visual, auditory, tactile and olfactory stimuli on gilt puberty attainment in experiments one and two (adapted from Pearce and Hughes 1987)

NOTE:
This table is included on page 27 of the print copy of the thesis held in the University of Adelaide Library.

1.3.1.2 Variation in the boar effect: boar stimulus value

Individual boars differ considerably in their ability to stimulate early puberty attainment and elicit a standing reflex in oestrous gilts (Hughes et al., 1990; Hughes, 1994; Chamberlain and Hughes, 1996). Pheromones released by the boar act synergistically with tactile cues to stimulate early puberty attainment, suggesting that between-boar variations in stimulus value reflect differences in the level of pheromone release and/or degree of tactile stimulation provided (Hughes et al., 1990; Chamberlain and Hughes, 1996). Concentrations of boar pheromones increase with age, and the minimal stimulus value of boars less than ten months of age undoubtedly reflects their inability to produce and secrete sufficient quantities of primer pheromones (Kirkwood and Hughes, 1981; Hughes et al., 1990). Variation in the ability of mature boars, namely those older than 10 months of age, to stimulate puberty attainment has also been associated with differences in the level of pheromone release as well as

the sexual motivation or libido of the boar (Hughes et al., 1994; Chamberlain and Hughes, 1996). It is evident that the sexual motivation of the boar has a profound influence on gilt puberty attainment (Hughes, 1994), and exposure to a boar with a high sexual motivation or libido significantly reduces the mean interval to puberty and increases the proportion of gilts attaining puberty within 20 days (**Table 1.3.5;** Hughes, 1994).

Table 1.3.5 Gilt puberty attainment in response to daily contact with boars of a low or high sexual motivation*, commencing at 160 days of age (adapted from Hughes, 1994)

Treatment	Mean days to puberty**	Proportion of gilts pubertal by:		
		Day 20	Day 40	Day 60
No boar contact	48	0.00[a]	0.08[a]	0.35[a]
Daily contact: boar of low sexual motivation	34	0.19[b]	0.62[b]	0.88[b]
Daily contact: boar of high sexual motivation	19	0.59[c]	0.81[b]	0.89[b]

[a,b,c] Superscripts within column indicate significant difference; $P < 0.05$
*Based on 3 times 15 minutes tests described by Hemsworth et al. (1978)
**Refers to gilts reaching puberty by 220 days of age

1.3.1.3 Variation in the 'boar effect': Contact factors

Maximal gilt response requires exposure to the combined actions of olfactory, tactile, visual and auditory cues (**Table 1.3.4**; Pearce and Hughes, 1987). Consequently, it is not surprising that restricting gilt-to-boar contact reduces the efficacy of boar exposure. The provision of full, physical boar contact doubles the proportion of gilts attaining puberty when compared to fenceline boar contact (Pearce and Paterson, 1992), and gilts receiving full physical contact with a mature boar attained puberty approximately 10 days earlier than those receiving fenceline boar contact (Patterson et al., 2002). As well as facilitating

the transfer of priming pheromones, allowing gilts and boars to interact in a reciprocal manner may promote the exhibition of boar courtship behaviours and production of saliva by the submaxillary glands, resulting in greater release of primer pheromones. Further, although data is absent in the pig, studies involving rodent species indicate that priming pheromones in female urine and/or faeces increase testosterone secretion and stimulate a surge of LH release in the male, potentially facilitating the production of male priming pheromones (Bronson and Maruniak, 1975; Lombardi et al., 1976; Maruniak et al., 1978; Johnston and Bronson, 1982; Bakker, 2003; Anand et al., 2004.)

Gilt perception and response to boar component stimuli depends on sufficient contact with the boar, and is influenced primarily by the duration of boar contact, the number of gilts in the exposure group, and the size of the exposure pen (Hughes, 1993). In the study of Caton et al. (1986) the interval to puberty and proportion of gilts responding was similar when gilts received 5, 10 or 30 minutes of boar contact. However, while the timing of puberty attainment was similar for gilts receiving ten or thirty minutes of boar exposure, reducing the duration of boar exposure to two minutes per day significantly increased the interval to puberty (Paterson et al., 1989). In contrast, Hughes (1993) observed an increase in the interval to puberty and a decrease in the proportion of gilts responding when the duration of boar exposure was reduced from 20 to 12.5 minutes per day, but reported no difference in the efficacy of 12.5 or 5 minutes of boar exposure (**Table 1.3.6**). Further, the provision of boar exposure for 30 or 60 minutes daily or on a continuous basis did not improve the pubertal response, suggesting that 20 minutes of daily boar contact optimises the efficacy of boar exposure (Hughes et al., 1990; Hughes, 1994).

Gilts appear to require a minimum period of time to absorb and perceive boar priming pheromones; however, it is also likely that the potential for gilts to avoid boar contact is enhanced when boar exposure is either of a very short duration or takes place in large groups or exposure pens (Paterson et al., 1989;

Hughes, 1993). Reducing the size of the exposure group from 8 to 4 gilts significantly increased the proportion of gilt-to-boar interactions; however, a corresponding increase in the proportion of gilts that responded was not observed. Equally, the size of the exposure pen had little effect on the efficacy of boar contact (Hughes, 1993). Together the results of these studies indicate that although puberty stimulation requires a minimum of 2 – 5 minutes of daily boar contact, optimal gilt response probably occurs when gilts receive 20 minutes of boar exposure per day (Caton et al., 1986; Paterson et al., 1989; Hughes, 1990; Hughes et al., 1993). However, under conditions that allow gilts to avoid direct boar contact, such as large group sizes or the use of a large exposure pen, the duration of boar exposure may need to be extended in order to increase the effectiveness of puberty stimulation. Alternatively, when only short periods of boar exposure are possible, then both group and pen size should be reduced accordingly (reviewed by Hughes et al. 1990).

Table 1.3.6 Effects of duration of boar exposure on gilt pubertal response (adapted from Hughes, 1993)

	Duration of boar contact (minutes per day)		
	20	12.5	5
Number of gilts	46	48	44
Mean days to puberty	20.9[a]	28.4[b]	30.3[b]
Proportion responding within 21 days	0.67[b]	0.34[a]	0.19[a]

[a,b] Superscripts within row indicate significant difference; $P < 0.05$

Maximum gilt response to boar exposure is also dependent on regular reinforcement of the stimulus (Paterson et al. 1989; Philip and Hughes 1995). Continuous ram exposure and hence continuous reinforcement of the gonadotrophin stimulus is required to maintain high rates of LH secretion in spite of the negative feedback effect of rising oestradiol levels, and cessation of ram contact inhibits LH secretion and reduces the ovulatory response (McNeilly

et al., 1982; Martin et al., 1986). Paterson et al. (1988) also demonstrated that maximum puberty stimulation is dependent on gilts receiving boar contact daily until the onset of the first oestrus. Interestingly, the results of more recent studies demonstrate further improvements in the timing of the pubertal response when boar contact occurs several times each day (**Table 1.3.7**). More specifically, increasing the frequency of boar contact from once a day to thrice daily reduced the interval to puberty, and increased the proportion of gilts that attained puberty within 15 days (Hughes and Thorogood 1997) or 20 days (Philip and Hughes 1995) (**Table 1.3.7**).

Table 1.3.7 The effect of frequency of boar contact commencing at 160 days of age on the timing of gilt puberty attainment

		Frequency of daily boar contact			
Reference	Proportion pubertal within:	0	1	2	3
	20 days	0.03	0.06	0.16	0.28
Philip and Hughes	40 days	0.03	0.41	0.61	0.53
(1995)	60 days	0.13	0.72	0.74	0.69
	Mean days to puberty*	42.0	36.8	30.6	23.7
	15 days	0.00	0.10	0.53	-
	30 days	0.00	0.26	0.73	-
Hughes and	45 days	0.07	0.61	0.87	-
Thorogood (1997)	60 days	0.07	0.74	0.87	-
	Mean days to puberty*	45.0	30.7	16.0	

*Only refers to those gilts attaining puberty within the experimental period

It has been proposed that gilts can be divided into three categories based on whether they exhibit a fast-, slow-, or non-response to boar-originating stimuli (Paterson et al. 1989; Philip and Hughes 1995). Furthermore, although increasing the frequency and/or duration of stimulation may result in a more rapid response from gilts in the fast-responder category, and hence a younger mean gilt age at puberty, it may not necessarily change the overall proportion of

gilts that exhibit a pubertal response (**Table 1.3.7**). In support of this, Patterson et al. (2002) demonstrated that although full boar contact reduced mean age at puberty compared to fenceline boar contact, the proportion of gilts attaining puberty within the experimental period was unaffected by the type of boar contact experienced. Consequently, although applying boar contact in such a way as to maximise gilt exposure to boar-originating stimuli, and facilitate the transfer of olfactory cues, is paramount to the timing of gilt response, this does not necessarily result in a corresponding increase in the number of gilts that are able to respond.

1.3.1.4 Gilt response to boar contact

Despite limited data in the pig, there is ample evidence from other mammalian species that priming pheromones released by mature males affect female reproductive status via changes in the activity of GnRH neurons (Rissman, 1996; Rissman et al., 1997; Bakker et al., 2001). Non-volatile, ligand-bound priming pheromones stimulate dendritic receptors of olfactory neurons in the VNO, resulting in neural impulses being transmitted, via the accessory olfactory bulb, to the amygdala, hypothalamus and preoptic area (Kirkwood et al., 1981; Bakker, 2003; Trinh and Storm, 2004). Gonadotrophin releasing hormone fibres are present in the accessory olfactory bulb (Rissman, 1996), and exposure to male pheromones increases GnRH content in the accessory olfactory bulb of female prairie voles (Rissman, 1996). Furthermore, the initial response of prepubertal female mice (Bronson and Desjardin, 1974), prepubertal ewe lambs (**Table 1.3.8**; Knights et al., 2002) and anoestrous ewes (Martin et al., 1986) to the introduction of a reproductively mature male is an alteration in the pattern of LH release.

Based on hormone data derived from a relatively uniform population of pre-pubertal female mice deemed to be peri-ovulatory 72 hours after the start of male contact, Bronson and Desjardins (1974) demonstrated that the initial

response to male contact was an immediate, but brief, surge of LH release. This 1 – 3 hour surge in LH was followed by a 15 – 20 fold increase in circulating oestradiol levels, culminating with the onset of adult-like peri-ovulatory profiles of gonadotrophin release. Similarly, LH secretion increases within 10 minutes of ram introduction (Martin et al., 1980), and compared to non-exposed ewe lambs, both mean LH concentrations and the frequency of LH pulses are significantly higher in ram-exposed animals (**Table 1.3.8**). This male-induced increase in LH secretion promotes ovarian steroidogenesis (Bakker et al., 2001), and within 40 hours of the start of ram exposure follicle development increases in both anoestrous ewes (Atkinson and Williamson, 1985) and ewe lambs (Knights et al., 2002), culminating in an oestrogen induced pre-ovulatory surge of LH release (Atkinson and Williamson, 1985; Rissmen et al., 1997; Bakker et al., 2001; Knights et al., 2002).

Table 1.3.8 Mean concentrations of LH and LH pulse frequency during the period from 12 - 20 hours after ram introduction in ram-exposed ewe lambs and during the equivalent time period in non-exposed ewe lambs (adapted from Knights et al., 2002)

	No ram exposure	Ram exposure
Mean LH (ng/ml)	1.8 ± 0.18^a	2.8 ± 0.14^b
Pulse frequency (pulses/8hours)	2.7 ± 0.80^a	7.7 ± 0.50^b
Mean pulse amplitude (ng/ml)	4.3 ± 1.0	4.8 ± 0.3

[a,b,] Superscripts within row indicates significant differences; $P < 0.05$

The endocrine response of pre-pubertal gilts following the introduction of a boar is less clear; however, similar to the mouse (Bronson and Maruniak, 1975; Bronson and Desjardins, 1974), male-induced puberty attainment in gilts is preceded by a prolonged period of elevated oestradiol concentrations (Esbenshade et al., 1982). In the four gilts that attained puberty, Esbenshade et al. (1982) observed a peak in oestradiol concentration (8.6 pg/ml, range: 7.1 –

13.6 pg/ml) approximately 18 hours prior to ovulation, presumably reflecting increased ovarian follicle growth (**Figure 1.3.1**).

NOTE:
This figure is included on page 34 of the print copy of
the thesis held in the University of Adelaide Library.

Figure 1.3.1 Schematic representations of mean plasma concentrations of oestradiol (pg/ml) and Luteinising Hormone (LH) (ng/ml) from 120 hours before to 24 hours after first observed oestrus in four, 180 day old, boar exposed gilts. Closed squares (■) represent oestradiol and closed triangles (▲) represent LH (adapted from Esbenshade et al., 1982)

Although LH levels remained basal (1-2 ng/ml) until the exhibition of the pre-ovulatory peak (8.7 ng/ml; **Figure 1.3.1**), the apparent lack of an initial surge in LH release, or an increase in LH pulsing, in response to boar exposure likely reflects the infrequent sampling interval used in this study or the variation in the timing of gilt response. Certainly, Kingsbury and Rawlings (1993) did in fact observe an increase in LH pulse frequency (pulses/h) and mean LH levels (ng/ml) during the 6 hours immediately following the start of boar exposure, as well as on the tenth day of continuous boar contact (**Table 1.3.9**). However, it is noteworthy that puberty occurred on average 34 days after the initiation of continuous boar contact, and took place in the absence of continuous elevation in either LH pulse frequency or plasma oestrogen concentrations. Further, alterations in LH pulse frequency were only evident in those gilts exhibiting an ovulatory response (**Table 1.3.9**). However, this is in agreement with studies on

acyclic ewes (Martin et al., 1985), in which ewes that ovulated in response to ram introduction exhibited a distinct increase in the frequency of LH pulsing compared to non-ovulating animals.

Although far from complete, current understanding indicates that olfactory cues, namely priming pheromones (e.g. 3α-androstenol) present in saliva secreted by the boar's submaxillary salivary glands, act in concert with tactile and possibly auditory and visual stimuli, to alter the pattern of LH secretion in the gilt (Hughes et al., 1990; Pearce and Paterson 1992; Kingsbury and Rawlings, 1993). These events actuate an increase in ovarian follicle growth, causing oestrogen concentrations to rise, triggering a cascade of endocrine events equivalent to the follicular phase of the oestrous cycle, and culminating in the onset of oestrus and the first ovulation (Esbenshade et al., 1982; Deligeorgis et al., 1984).

Table 1.3.9. LH pulse frequency and mean LH concentrations in blood serum samples collected from gilts attaining puberty in response to continuous boar contact from 135 to 200 days of age (RESPONDERS), and non-exposed gilts (CONTROLS) (adapted from Kingsbury and Rawlings, 1993)

NOTE:
This table is included on page 35 of the print copy of the thesis held in the University of Adelaide Library.

While data is limited in the gilt, studies involving anoestrous ewes suggest that a relationship exists between the pattern of male induced LH release and the exhibition of an ovulatory response (Martin et al., 1980). Specifically, this increase in LH pulse frequency must be of sufficient magnitude for ovulation to

take place (Martin et al., 1986), and the failure of gilts to respond to boar stimulation appears to reflect an insufficient LH response (Kingsbury and Rawlings, 1993). The frequency and amplitude of tonic LH release is determined by the interplay between the stimulatory effects of GnRH release and the negative feedback effects of oestrogen (Goodman and Karsch, 1980). Limited data suggests that ewes with low frequency LH pulsing coincident with ram introduction fail to ovulate (Martin et al., 1980), while oestradiol injections delay, or suppress, the ovulatory response of anoestrous ewes to ram introduction (Martin et al., 1980). Therefore, as suggested in the sheep (Martin et al., 1988), early puberty attainment in the gilt probably depends on the ability of male-induced alterations in GnRH release to override the negative feedback effects of oestrogen. Consequently, the exhibition of a pubertal response to boar stimulation, and indeed the timing of this response, will be determined by the sensitivity of the GnRH pulse generator to negative oestrogen feedback. Advancing sexual maturity is associated with a reduction in the sensitivity of the GnRH pulse generator to negative oestrogen feedback and increased activity of the GnRH neuronal network. Therefore, the efficacy with which boar contact accelerates puberty attainment will ultimately depend on the sexual maturity, or physiological age, of the gilt (Eastham et al., 1986).

1.3.2 Environmental cues: seasonal and climatic effects

Despite not being distinct seasonal breeders, domestic sows commonly experience a depression in fertility during late summer and early autumn. Referred to as seasonal infertility, and coinciding with the anoestrous period of the European wild boar (Pepin and Mauget, 1989), summer suppression of fertility in the domestic sow is frequently manifested as a delay in puberty attainment and a decrease in farrowing rates (Paterson and Pearce, 1989; Peltoniemi et al., 2005). Changes in photoperiod drive seasonal breeding cycles, and the domestic pig resembles the sheep by being a short-day breeder. Current understanding of the mechanisms underlying seasonal infertility in the

domestic pig is poor; however, in a recent review, Peltoniemi and Virolainen (2005) suggested it was unlikely that the central mechanisms mediating seasonal effects on reproductive activity would differ between pigs and other short-day breeders.

Nocturnal secretion of melatonin by the pineal gland is the underlying mechanism whereby changes in photoperiod synchronise the start of the breeding season (Peltoniemi and Virolainen, 2005). Domestic pigs recognise and respond to changes in photoperiod (Peltoniemi and Virolainen, 2005), and the existence of an endogenous circadian rhythm of melatonin secretion has been demonstrated in domestic gilts (Peltoniemi et al., 2005). Based primarily on studies involving sheep, it is apparent that melatonin has a stimulatory effect on episodic release of GnRH and LH, most likely through the suppression of the negative feedback effects of oestrogen on the GnRH pulse generator. Melatonin production is reduced during exposure to long photoperiods, namely during the summer months, essentially suppressing episodic LH release and arresting reproductive activity and delaying sexual maturation. Conversely, melatonin secretion increases during short photoperiods, eliciting an increase in the activity of the GnRH pulse generator, thus enabling puberty attainment and oestrous cyclicity to occur (Peltoniemi and Virolainen, 2005). Although, Peltoniemi et al. (2005) failed to identify a relationship between individual nocturnal melatonin secretion and seasonal delays in puberty, studies involving sheep demonstrate that exogenous melatonin administration advances first oestrus in ewe lambs. Equally, it is logical that a suppressed pattern of tonic LH would delay sexual maturation.

In the absence of boar contact, the proportion of gilts attaining puberty is significantly reduced in summer compared to winter (Paterson et al., 1989); however, it is apparent that boar exposure can, at least partially, override the suppressive effects of long-day photoperiods on sexual maturation (**Table 1.3.10**). More specifically, when gilts receive daily boar exposure from 174 days

of age onwards, the interval to puberty and the proportion of pubertal gilts appears largely unaffected by the duration of photoperiod (**Table 1.3.10**). However, when boar contact is limited, a significantly lower proportion of gilts attain puberty in summer compared to winter (Paterson et al., 1989), and a reduction in gilt responsiveness to boar stimulation in summer is evident (Paterson et al., 1991). Equally, Hughes (1994) reported a seasonal effect on the frequency of boar contact required to maximise puberty response, supporting the earlier suggestion of Paterson et al. (1989) that the threshold level of boar stimulus required to elicit a rapid pubertal response is higher in summer than in winter.

Table 1.3.10 Puberty attainment in gilts reared under controlled light regimens and with or without boat contact (adapted from Paterson et al., 1991)

	Long-day photoperiod		Short-day photoperiod	
	No Boar (n=37)	Boar (n=29)	No Boar (n=37)	Boar (n=29)
Age at first boar contact (days)	-	174	-	174
Age at slaughter (days)	227	234	227	234
Number of gilts pubertal	1	21	20	18
Proportion gilts pubertal	0.03	0.72	0.54	0.62
Interval to puberty (days)	-	21	-	22

Although, Philip and Hughes (1995) demonstrated that gilt response to boar contact was unaffected by season provided boar exposure was conducted at least once daily, it should be noted that these authors also observed no effect of season on puberty attainment in gilts reared in isolation from boars. Consequently, based on the bulk of the available data, it is apparent that optimising gilt response to boar contact during summer requires an increased level of boar stimulation to overcome the suppressive effects of long-day photoperiods on the activity of the GnRH pulse generator.

1.3.3 Developmental cues: gilt age

Physiological maturity of the hypothalamic-pituitary-ovarian axis determines gilt response to boar contact, and at a constant liveweight, gilt age at start of boar contact has the greatest effect on the timing of puberty attainment (Hughes and Cole, 1976; **Table 1.3.11**). Equally, gilt weight at puberty varies depending on the age at which boar exposure commences (Eastham et al., 1986; Burnett et al., 1988; **Table 1.3.11**). Although gilt response to boar introduction at 90 – 120 days of age is delayed and variable (Kirkwood and Hughes, 1979), the interval to puberty is considerably reduced when the start of boar exposure is delayed from 130 to 160 days of age (**Table 1.3.11**; Hughes, 1976) or 125 to 167 days of age (**Figure 1.3.2A**; Kirkwood and Hughes, 1979). Similarly, Burnett et al. (1988) reported both a marked reduction in the interval to puberty (**Figure 1.3.2B**), and a significant increase in the proportion of gilts attaining puberty, when boar contact started at 170 compared to 130 days of age. Further, gilt age at puberty attainment is actually similar when boar exposure commences at less than 160 days of age, suggesting that gilts introduced to a boar at a young age only initiate a pubertal response once they achieve the required stage of physiological development (Kirkwood and Hughes, 1979).

Based on the available literature, age at first exposure to a mature boar appears to be the most favourable predictor of gilt response (Kirkwood and Hughes, 1979; Hughes, 1982; Eastham et al, 1986; Prunier et al., 1987; Newton and Mahan, 1992), with the hypothalamic-pituitary-ovarian axis sufficiently integrated by 160 to 170 days of age to allow a rapid and synchronous pubertal response (Hughes and Cole, 1976; Eastham et al, 1986; Paterson et al., 1989). Although further delaying the start of boar exposure does result in a slight reduction in the interval to puberty (**Table 1.3.11** and **Figure 1.3.2**), gilt age at puberty is also increased when boar contact commences at greater than 160 days of age (**Table 1.3.11**). Consequently, it is generally agreed that the optimal

response to boar stimulation occurs when gilts are 160 – 170 days old (Hughes and Cole, 1976; Kirkwood and Hughes, 1979; Eastham et al, 1986).

Table 1.3.11 Effects of gilt age at commencement of boar exposure on the interval to puberty, as well as gilt age and liveweight at puberty

| | Gilt age at start of boar contact (days) | | | | | |
| | Hughes (1976) | | | Eastham et al. (1986) | | |
	135	160	190	160	180	200
Liveweight at start of boar contact (kg)	73	73	73	-	-	-
Interval to puberty (days)	34.3[b]	19.0[a]	15.2[a]	11.35	9.40	5.94
Age at puberty (days)	169.4[a]	178.2[a]	205.3[b]	177.3	191.9	206.8
Liveweight at puberty (kg)	97.3	90.4	89.2	69.8[a]	83.0[b]	92.5[c]

[a,b,c] Superscripts within row and source indicate significant differences ($P < 0.05$)
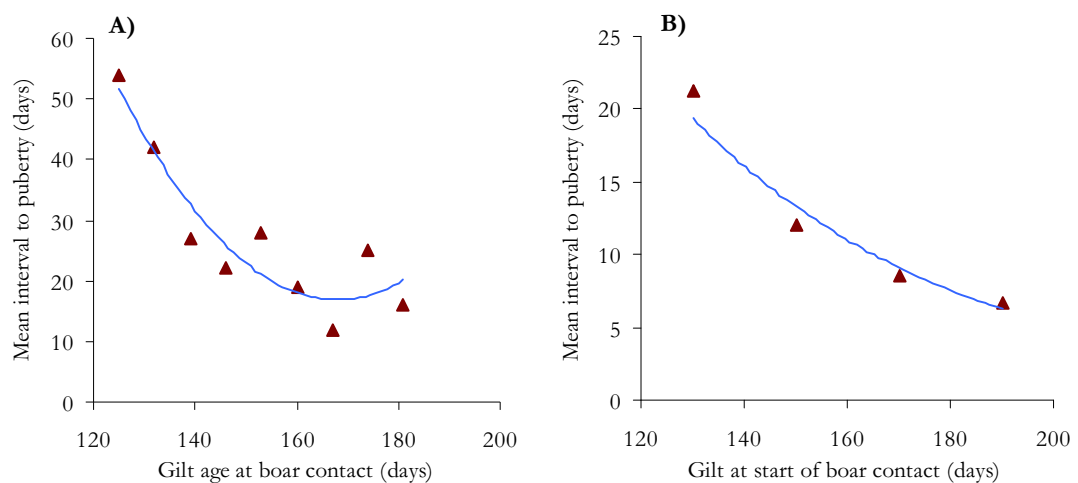
Figure 1.3.2 Effect of gilt age at start of boar contact on the interval to puberty attainment, adapted from A) Kirkwood and Hughes (1979) and B) Burnett et al. (1988).

1.3.4 Developmental cues: weight, body composition and growth rate

Any association between gilt growth and reproductive maturity is likely to be due to the regulatory effects of metabolic status and/or nutrient intake on the reproductive axis (Rozeboom et al., 1995; Foster and Nagatini, 1999). Activity of the reproductive neuroendocrine axis is permitted by the availability of sufficient metabolic fuels (Schneider, 2004), with reproductive processes reduced, or postponed, during periods of extreme growth or when nutrient availability is limited (Cosgrove and Foxcroft, 1996; Wade and Jones, 2004). However, as the animal grows alterations in the partitioning of ingested nutrients and a reduction in basal metabolic rate increase the availability of energy for storage and/or reproductive activity (Foster and Nagatini, 1999). This developmental change in metabolism, mediated by altered levels of metabolites and metabolic hormones, may permit the pre-pubertal increase in the frequency of GnRH release (Hall et al., 1995; Foster and Nagatini, 1999; Messer and l'Anson, 2000).

Puberty does not occur at a specific weight or body composition (Kirkwood and Aherne, 1985; Rozeboom et al., 1995). However, higher rates of growth accelerate reproductive development and increase gilt responsiveness to boar stimuli (Gaughan at el., 1997), supporting at least a permissive role for metabolic signals in puberty attainment (Hall et al., 1995). Age at puberty decreases as liveweight and P2 backfat at start of boar contact increases (King et al., 1989; Gaughan et al., 1997; **Table 1.3.12** and **Table 1.3.13**), and Eliasson et al. (1991) observed a 3 day reduction in age at puberty with each 100 g increase in daily liveweight gain. However, Burnett et al. (1988) reported a decrease in the correlation between gilt growth characteristics and days to puberty with ascending age at boar contact. This suggests a decreasing influence of gilt liveweight and body tissue reserves on sexual maturity with increasing age (Burnett et al., 1988; Hughes et al., 1990), supporting the view that minimum levels of weight or body tissue reserves play a permissive rather

than a stimulatory role in the progression of sexual maturation (Kirkwood and Aherne, 1985).

Table 1.3.12 Effect of gilt liveweight and P2 backfat on age at puberty, when boar contact commenced at 170 days of age (adapted from King et al., 1989)

Table 1.3.13 Effect of liveweight and P2 backfat at 145 days of age on age and liveweight at puberty attainment (adapted from Gaughan et al., 1997)

Although developmental changes in metabolic status are associated with alterations in body composition, a definitive relationship between reproductive development and either protein accretion or fat deposition remains to be established. The timing of puberty has been associated with the point of inflection of the growth curve (Owens et al., 1993), and Cia et al. (1998) suggested that protein mass, as opposed to fatness or ratio of fat to lean, is more likely to regulate reproductive development. In support of this, increased body fat at the same liveweight reduces the ability of gilts to respond to boar contact (Table 1.3.14), suggesting that reproductive development is more

closely related to the rate of protein accretion or the attainment of a minimum protein mass than it is to fat deposition (King et al., 1989). However, when rate of fat deposition is constant, gilt age at puberty appears unrelated to the rate of protein accretion (Patterson et al. 2002). Equally, Eliasson et al. (1991) reported a negative correlation between gilt age at puberty and P2 backfat at 90 kg liveweight, with each 1 mm increase in P2 backfat decreasing age at puberty by 1.6 days. Gilts selected for early puberty attainment were faster growing and fatter compared to their later maturing counterparts. Conversely, compared to their leaner counterparts, gilts with higher backfat at 105 kg liveweight were younger at first oestrus (**Table 1.3.15**; Nelson et al., 1990).

Table 1.3.14 Effect of liveweight and P2 backfat on the timing of puberty attainment in response to boar contact starting at 165 days of age (adapted from King et al., 1989)

NOTE:
This table is included on page 43 of the print copy of the thesis held in the University of Adelaide Library.

Although the data of Nelson et al. (1990) and Eliasson et al. (1991) lend credence to the notion that increasing fat deposition initiates sexual maturation (Foster and Nagatini, 1999), Beltranena et al. (1993) reported that when protein accretion rate is maximal, variations in fat deposition or fat level equivalent to 10 – 16 mm backfat have no effect on reproductive development. Similarly, when gilts are *ad libitum* fed, age at puberty appears to be independent of prepubertal growth rate (Beltranena et al., 1991), and Rozeboom et al. (1995) and Patterson et al. (2002) reported enormous variation in gilt liveweight, body composition and growth rate at puberty. Further, the age at which gilts first

become genetically capable of attaining puberty probably occurs long after the attainment of any permissive threshold levels (Foxcroft et al., 1996), and Beltranena et al., (1993) suggested that, in the absence of nutrient restriction, "*innate genetic variability in LH secretion*" was the most important determinant of reproductive development.

Table 1.3.15 Means for age at puberty, growth rate and backfat of females selected based on either age at puberty or backfat at 105 kg (adapted from Nelson et al., 1990)

	Selection criteria			
	Early puberty$_1$	Late puberty$_1$	High fat$_2$	Low fat$_2$
Age at puberty (days)	185	239	207	220
Average daily gain (kg/day)	0.84	0.75	0.80	0.77
Backfat at 105 kg (mm)	17.3	15.7	19.3	13.5

$_1$ Selected based on age at puberty; $_2$ Selected based on backfat at 105 kg

1.3.5 Developmental cues: nutrition and metabolic signals

Theoretically, nutritionally-induced delays in puberty attainment reflect a chronological delay in the attainment of a metabolic status capable of initiating the physiological chain of events responsible for puberty. However, when nutritionally restricted growth occurs during the later stages of development it becomes difficult to differentiate between growth-induced and nutrition-induced changes in metabolic signals and reproductive activity (Foster and Nagatini, 1999). Although nutritional restriction of pre-pubertal growth delays puberty, Booth et al. (1994) clearly demonstrated that nutritionally induced alterations in gilt metabolic status could affect reproductive function without changing either weight or P2 backfat. Equally, in a study involving post-pubertal gilts, Rozeboom et al. (1993) reported substantial variation in gilt body composition at nutritionally induced anoestrus, concluding that metabolic

signals, as opposed to the attainment of a minimum weight or body composition, initiate the cessation of reproductive activity. Further, feed intake coincident with boar contact, as opposed to either liveweight or P2 backfat at the start of contact, appears to have a greater effect on the timing of the pubertal response (Klindt et al., 2001). Similarly, Schneider and Wade (1987) elegantly demonstrated that whilst body reserves act as a buffer against the suppressive effects of food deprivation on reproduction, activity of the neuroendocrine reproductive axis is controlled by the availability of metabolic fuels rather than any aspect of body size.

The suppressive effect of restrictive feed intake on the release, rather than the synthesis, of both GnRH and LH is well established (Booth et al, 1994; Schillo et al., 1992; Schneider, 2004). Although moderate feed restriction does not appear to affect LH secretion, Prunier et al. (1993) demonstrated that tonic LH release is inhibited in severely feed restricted gilts, supporting earlier reports that underfeeding inhibits LH pulsing in prepubescent rats (Bronson and Heidemann, 1990) and ewe lambs (Schillo, 1992). In feed restricted rats, Bronson and Heidemann (1990) observed a transient increase in LH pulsing after eating, possibly due to a temporary increase in energy availability, and suggested that repetitive LH pulsing would occur in response to re-alimentation. In support of this, dietary repletion induces a rapid increase in episodic LH release in previously feed restricted, pre-pubertal gilts (Booth et al., 1996) and rats (Bronson and Rissman, 1986). Furthermore, pulse like administration of LH stimulates follicle growth and ovulation in feed restricted prepubertal rats and ewe lambs (Bronson and Rissman, 1986; Schillo et al., 1992), confirming that a suppressed pattern of tonic LH release is responsible, at least partially, for delayed puberty attainment in restrictively fed animals. Interestingly, Kirkwood et al. (1978) concluded that restrictively feeding pre-pubertal gilts does not impair the positive feedback effects of oestradiol on LH release; however, the effect of feed restriction on hypothalamus-pituitary response to boar stimuli is unknown.

Feed restriction reduces the ability of the ovary to respond to an oestradiol benzoate induced LH surge (Kirkwood et al., 1987), suggesting that low feed intake retards ovarian follicle growth. Chronic feed restriction significantly reduces follicle growth in prepubertal rats (Lintern-Moore and Everitt, 1978), and Booth et al. (1996) demonstrated that 14 days of feed restriction retarded both ovarian and uterine development in prepubertal gilts, whilst ad-libitum feeding from day 8 to 14 of the study period increased utero-ovarian development (**Table 1.3.16**). Similarly, 12 days of acute nutritional restriction significantly reduce ovarian follicle growth in ruminants (Boland et al., 2001). Although data are unavailable in the gilt, studies involving rodents confirm that extended periods of feed restriction promote atresia of antral follicles and slow the growth of smaller follicles, with retarded folliculogenesis most likely to be responsible for the delayed resumption of oestrous cyclicity observed in dietary repleted rodents despite rapid resumption of LH pulsatility (Messer and l'Anson, 2000). Similarly, Boulanouar et al. (1995) demonstrated no effect of re-alimentation on age at puberty in ewe lambs, with long-term restrictively fed ewe lambs and re-alimented lambs both attaining puberty significantly later compared to their normally fed counterparts. Further, Ronnekleiv et al. (1978) concluded that the inability of the ovary to secrete sufficient oestradiol to elicit a positive feedback effect on GnRH release may be responsible for delayed puberty in restrictively fed rats.

Current understanding of the metabolic signals mediating the effect of changing nutritive status on the activity of the hypothalamic-pituitary-ovarian axis is limited. However, living cells require a continuous supply of oxidisable fuels for biosynthesis and metabolism, and in the absence of any changes in gonadotrophins, short-term changes in nutrient intake can influence folliculogenesis (Messer and l'Anson, 2000; Webb et al., 2004). Equally, activity of the specialised neuronal subpopulations responsible for the regulation of both energy balance and reproductive activity are sensitive to changes in metabolic status (Gamba and Pralong, 2006). Consequently, it is generally

accepted that nutritionally induced changes in reproductive activity occur in response to alterations in the availability of metabolic fuels, changes in circulating concentrations of metabolic hormones, and altered production and release of neurotransmitters/neuropeptides (Foster and Nagatini, 1999; Prunier and Quesnel, 2000; Wade and Jones, 2004). Just as Schneider and Wade (1987) clearly demonstrated that changes in the general availability of metabolic fuels, rather than one specific fuel, elicit alterations in reproductive status in female hamsters, studies involving rhesus monkeys indicate that calorie intake, regardless of the source, regulates LH release (Foster and Nagatini, 1999). Although the underlying mechanisms whereby metabolic fuel availability affect reproductive function remain unclear, it is increasingly evident that a number of circulating factors serve as the metabolic signals affecting the timing of puberty and reproductive activity (Foster and Nagatini, 1999).

Table 1.3.16 Ovarian and uterine development in 145 day old, prepubertal gilts experiencing either 14 days of restrictive feeding (Restricted) or 7 days of restrictive feeding followed by 7 days of *ad-libitum* feeding (Repleted) (Adapted from Booth et al., 1996)

There is substantial evidence to support a regulatory effect of the glucose-insulin system on reproductive function in a variety of species (Booth et al., 1996; Foster and Nagatini, 1999). Exogenous glucose infusions induce a rapid increase in serum insulin levels and partially restore LH pulsing in feed restricted gilts (Tokach et al., 1992; Barb et al., 2001). Equally, Booth et al. (1996) reported a positive correlation between mean plasma LH concentrations and both insulin and glucose, and insulin augments GnRH induced LH release

from cultured pituitary cells (Matamoros et al., 1990). Although plasma insulin and glucose concentrations appear to regulate LH, Schneider and Wade (1987) clearly demonstrated that, provided fatty acid availability was sufficient, oestrous cyclicity was maintained in spite of depleted levels of glucose. Equally, pharmacological suppression of insulin does not prevent feed-induced increases in episodic LH secretion in male monkeys, suggesting that neither insulin nor glucose provide the critical link between reproduction and gonadotrophin secretion (Cameron, 1996). However, a direct effect of glucose on ovarian follicle growth is probable (Boland et al., 2001), and insulin appears to reduce atresia of medium follicles, thereby promoting ovarian follicle growth and increasing ovulation rate in pigs, whilst short-term reductions in insulin availability dramatically reduce both follicle growth and intrafollicular concentrations of IGF-I (Matamoros et al., 1990).

A reduction in circulating IGF-I concentrations occurs during periods of negative energy balance (Simmen et al., 1998). Barb et al. (2001) proposed that IGF-I might act as the signal linking energy balance with reproductive activity and growth processes. Equally, Booth et al. (1996) proposed that IGF-I might act as a long-term mediator of the effects of energy status on reproduction. Cosgrove et al. (1992) suggested a facilitative role for IGF-I in follicle growth, and based on the data of Booth et al. (1996), there appears to be an association between low plasma IGF-I concentrations and retarded follicle growth. In addition to a direct effect on folliculogenesis, IGF-I also modulates pituitary LH release in the pig (Whitley et al., 1995), and stimulates the release of GnRH from the median eminence of pre-pubertal rats in vitro (Pine et al., 2006). Hiney et al. (1996) proposed that pre-pubertal elevations in growth hormone are responsible for peri-pubertal increases in liver IGF-I gene expression, and a dramatic increase in peripheral IGF-I concentrations precedes the onset of puberty in rodent and ruminant species. Exogenous IGF-I restores age at puberty in IGF-I deficient rats (Pine et al., 2006), and studies involving ovariectomised, prepubertal rats indicate that the stimulatory effect of IGF-I on LH release is dependent on

oestradiol, with oestradiol stimulating the expression of IGF-I receptors in the hypothalamus and median eminence. Although Patterson et al. (2002) was unable to identify a relationship between the timing of the pubertal response to boar contact and peripheral IGF-I concentrations at start of boar contact, it is plausible that in all the gilts sampled, IGF-I levels were in excess of any permissive threshold level required to facilitate the release of either GnRH or LH.

Synthesised by adipose tissue and secreted into the blood stream, leptin functions as a circulating signal of metabolic status and is positively correlated with adiposity (Barb et al., 2005). Fasting rats for 48 hours reduces both episodic LH release and peripheral leptin concentrations, with exogenous leptin administration reversing the inhibitory effect of restrictive feeding on LH pulsing (Foster and Nagatini, 1999). An indirect effect of leptin on the neuroendocrine reproductive axis has been proposed, with leptin promoting the availability of glucose and free fatty acids (Schneider, 2004), as well as acting indirectly to influence the activity of GnRH neurons (Cunningham et al., 1999). Functional leptin and insulin receptors are expressed on the neuropeptide Y neurones present in the arcuate nucleus region of the hypothalamus. Neuropeptide Y plays an integral role in control of GnRH release, augmenting the stimulatory effects of oestrogen on GnRH release, but inhibiting GnRH release in castrated animals (Gamba and Pralong, 2006). Therefore, assuming a stimulatory effect of leptin on neuropeptide Y release, metabolically induced elevations in peripheral leptin, in conjunction with rising secretion of ovarian oestradiol, could act as the metabolic signal responsible for puberty attainment. However, nutritionally-induced restoration of LH pulsing in previously feed restricted ewes occurs in the absence of any measurable change in peripheral leptin (Schneider, 2004), and studies in prepubertal gilts (Barb et al., 2001) indicate that the acute effects of energy depletion on episodic LH release occur independently of any change in circulating leptin levels. Despite indications that administering leptin to pre-pubertal rodents can partially counteract the delaying effect of dietary

restriction on puberty, recent studies suggest that leptin is merely one of a cohort of permissive factors required for first oestrus to occur (reviewed by Zeiba et al., 2005). More specifically, leptin does not appear to trigger puberty in heifers or female mice (Bronson, 2001; Zeiba et al., 2005). Equally, Barb et al. (2005) suggested a permissive effect of leptin on the activity of the porcine reproductive axis, supporting the earlier conclusion of Patterson et al. (2002) that gilt response to boar stimulation is not related to plasma leptin concentrations coincident with the start of boar contact.

1.3.6 Developmental cues: genetics and genotype

The age at which gilts attain puberty varies enormously between breeds and genotypes (Hughes, 1982; Evans and O'Doherty, 2001), and at a constant age, genotype affects the ability of gilts to respond to exogenous hormones (Tilton et al., 1995). Further, although a permissive, rather than a causative, role for growth in the timing of puberty has been established, there is evidence to support an association between genetic capacity for growth and gilt age at puberty. Kerr and Cameron (1997) proposed that the attainment of a genotype specific minimum level of fat deposition and protein accretion determined age at puberty, and within genotypes, faster growing gilts attain puberty at a younger age compared to their slower growing counterparts (Kirkwood and Aherne, 1985; Eliasson et al., 1991). Partial support for the suggestion that selecting for increased growth rate results in a decrease in pubertal age is provided by the study of Nelson et al. (1990), in which gilts selected based on high backfat at 105kg liveweight, grew faster and reached puberty at a younger age than their leaner counterparts (**Table 1.3.15**). Equally, gilts born from sows selected for early puberty experienced their first oestrus at an earlier age and with more backfat compared to the offspring of sows selected for late puberty (Hixon et al., 1987). Despite limited data supporting a direct link between fat deposition and age at puberty, leaner gilts do appear to cycle at an older age, and Nelson et al. (1990) suggested that selection criteria based solely on

extreme leanness would have a detrimental effect on reproductive performance.

During the past 20 to 30 years, rising production costs and increased consumer demand for leaner cuts of meat, have resulted in faster growth rates and increased lean percentage forming the base criteria for selection of breeding stock (Gaughan et al., 1995). The suggestion that selection for increased carcass lean results in animals with a greater mature bodyweight has been widely accepted (Kirkwood and Aherne, 1985; Evans and O'Doherty, 2001). Whilst the past twenty years have certainly seen a considerable increase in sow liveweight and decrease in backfat (**Table 1.3.17**), the consequences of current selection criteria on sexual maturation and reproductive performance have yet to be fully explored (Gaughan et al., 1995). Based on the assumption that sexual maturity coincides with the point of inflection of the growth curve, it has been proposed that gilts selected for decreased fatness are less physiologically mature at a given weight than their unselected, fatter counterparts (Gaughan et al., 1995; Whittemore, 1996). Equally, heifers from faster growing breeds with larger mature body weight are considerably older at puberty compared to heifers from slower growing, smaller breeds, and in terms of fat deposition, lean pig genotypes certainly appear to be later maturing (Kirkwood and Aherne, 1985). Consequently, there is general agreement that selection for increased lean growth, and thus higher mature weight, is also a selection against early puberty attainment (Kirkwood and Aherne, 1985; Evans and O'Doherty, 2001; Hughes and Varley, 2003). Simply stated, at a given age, current, genetically lean genotypes are at a lower proportion of their mature weight, and hence physiologically less mature compared to older, fatter genotypes (Kirkwood and Aherne, 1985; Evans and O'Doherty 2001). However, Whittemore (1996) suggested that mature weight would occur at the same age regardless of how heavy that mature weight is, concluding that weight, but not age, at puberty will be higher in faster growing genotypes, and therefore selection for increased lean tissue accretion would be unlikely to delay sexual maturation.

Table 1.3.17 Changes in weight and backfat at weaning of parity one and three sows used in United Kingdom nutrition experiments between 1983 and 1993 (adapted from Edwards, 1998)

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1.4 Factors affecting first litter size

Productivity of the breeding sow depends, at least in part, on the number of piglets born per litter, and increasing the number of pigs weaned per sow and per year improves the profitability of the breeding herd (Stein et al., 1990; van der Lende, 1994; Le Cozler et al., 1998; Tummaruk et al., 2001). Due to the high prevalence of premature culling, early parity sows constitute a large proportion of modern breeding herds (Hughes and Varley, 2003), with breeding herd profitability increasingly dependent on litter size at first farrowing. The number of piglets born is a function of ovulation rate, fertilisation rate and the number of embryos surviving to term (prenatal survival rate). Under normal conditions, losses of potential piglets due to fertilisation failure are small, with fertilisation rate generally accepted to be between 95% and 100% (Lambert et al., 1991; van der Lende et al., 1994). Therefore, the number of ova shed at the oestrous of mating and the extent of pre-natal mortality are the primary determinants of gilt prolificacy. Most of pre-natal mortalities occur before day 35 of pregnancy, with approximately 20% to 30% of embryos lost during this period (**Figure 1.4.1;** Pope and First, 1985; van der Lende and Schoenmaker, 1990; Lambert et al., 1991; Ashworth and Pickard, 1998). This chapter will summarise current understanding of the regulation of ovarian follicle growth and oocyte developmental competence, the control of ovulation and the timing of embryo

loss in the pig, with particular emphasis placed on the influence of pre-mating nutrition and management on first litter size.

1.4.1 *Regulation of ovarian follicle growth and oocyte developmental competence*

The fundamental function of the ovarian follicle unit is to protect and nourish the growing oocyte, thereby ensuring the ovulation of an oocyte capable of successfully undergoing fertilization and completing the pre-implantation stages of embryogenesis (Eppig, 2001; Britt et al., 2004). Only 0.1% of growing follicles will reach the ovulatory stage and successfully shed their oocyte, with the remainder undergoing the degenerative process known as follicle atresia that results from the programmed death of the follicle somatic cells and oocyte (for reviews see Britt et al., 2004; Manabe et al., 2004; Quirk et al., 2004). The avoidance of atresia and successful completion of folliculogenesis depends on the presence of specific survival factors responsible for promoting and coordinating growth of the follicle-oocyte complex, as well as protecting the follicle cells from apoptosis (Quirk et al., 2004). This section will briefly describe the morphological development of the follicle-oocyte complex and intra-ovarian control of follicle growth, as well as summarise the role of pituitary secreted gonadotrophins and metabolic influences during follicle growth.
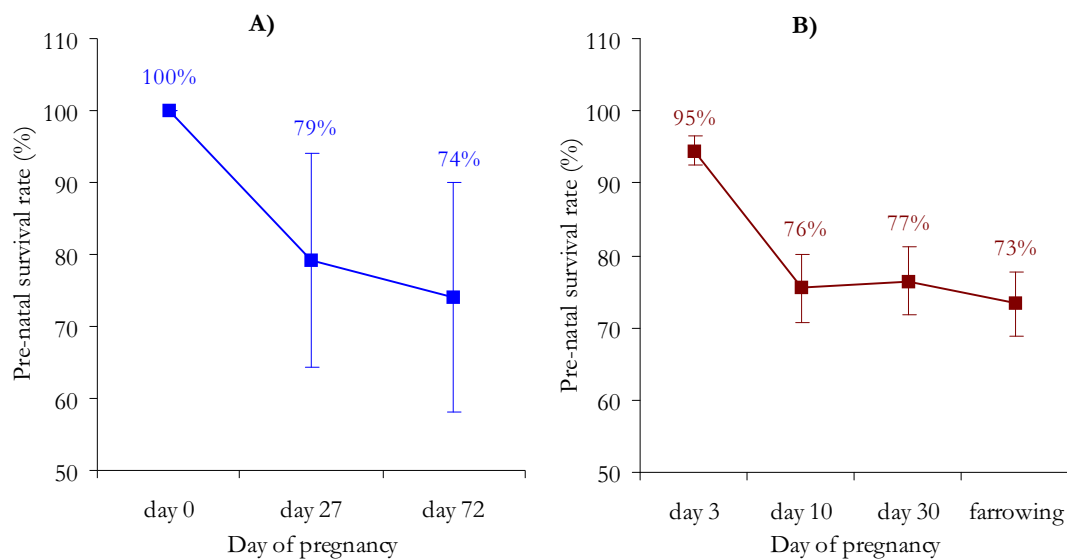
Figure 1.4.1 Timing of pre-natal mortality throughout pregnancy (mean ± S.E.M) (adapted from A) van der Lende and Schoenmaker., 1990 and B) Lambert et al., 1991).

1.4.1.1 Growth and development of the antral follicle

In the pig, it takes approximately 84 days from the re-activation of primordial follicle growth to the formation of the antral cavity, equating to a growth rate of approximately 0.0035 mm per day (**Table 1.4.1**). In brief, the transition from the primordial to the primary follicle stage is the first step in the re-activation of follicular maturation, and is the point at which the follicle becomes committed to further growth and development (McGee and Hseuh, 2000; Findley et al., 2002). Progression from the primary to the secondary, or preantral, stage of development is characterised by gradual proliferation of the granulosa cells, the formation of small fluid filled cavities between adjacent granulosa cells and the development of a morphologically recognizable thecal cell layer surrounding the follicle (Guraya, 1985; Itahana et al. 1996; Hunter, 2000). Preantral growth culminates with the acquisition of two or three granulosa cell layers, differentiation of the thecal layer and the gradual development of the antral cavity (Foxcroft and Hunter, 1985; Mao et al, 2002). More specifically, as the granulosa cells multiply the production of follicular fluid and the number of

extracellular spaces increase, and at a diameter of approximately 0.4 mm these small pockets of fluid combine to form the antral cavity or antrum, (Foxcroft and Hunter, 1985; Guraya, 1985; Morbeck et al., 1992).

Table 1.4.1 Time spent in specific follicle size categories from the primary to early antral stage of development (adapted from Morbeck et al., 1992)

Following the formation of the antral cavity, the rate at which the follicle grows increases exponentially. It takes approximately 20 days for the antral follicle to reach its ovulatory size of 8 to 10 mm (**Table 1.4.1**), and between 3 and 10 mm the developing follicle grows at the rate of 1.14 mm per day. This period of rapid growth reflects dramatic expansion of the antrum as well as rapid proliferation of the granulosa cells population (Grant et al., 1989; Morbeck et al., 1992). Growth of the antral cavity matches the growth of the follicle, and development of the follicle depends on the synchronised maturation of the follicular cells, the oocyte and the follicular fluid. The follicular fluid contains a complex mix of carbohydrates, proteins, amino acids, enzymes, gonadotrophins, steroids, as well both inhibitors and stimulators of both follicle

cell and oocyte function. These chemical, physical and endocrine components change as the follicle matures, playing an integral role in the regulation of steroidogenic activity in the follicle, maturation of the oocyte, as well as during ovulation and pick-up of the egg by the oviduct (reviewed by Guraya, 1985; Gore-Langton and Armstrong, 1988; Greenwald and Terranova, 1988).

1.4.1.2 Intra-ovarian control of follicle growth and development

Growth and differentiation of the ovarian follicle is orchestrated by factors secreted by the somatic cells and oocyte. Functional feedback loops between the granulosa cells and the oocyte, as well as between the theca and granulosa cells appear to promote cell growth throughout the various stages of follicular development (Carabatsos et al., 1998; Driancourt et al., 2000; Nilsson et al, 2001; Nilsson and Skinner, 2002; Fortune, 2003; Hunter et al., 2004). Athough poorly documented in the pig, many of the growth factors identified in mice, rats and cattle, are likely to be involved in porcine follicle growth (reviewed by Hunter, 2000; Knights and Glister, 2003). Granulosa cell secreted Kit ligand and Leukaemia inhibitory factor (LIF) promote oocyte growth (Driancourt et al., 2000; Nilsson et al., 2001), whilst oocyte secreted substances, such as basic fibroblast growth factor (bFGF) and growth differentiation factor – 9 (GDF-9), regulate granulosa cell mitosis and differentiation, as well as steroidogenesis and the formation of LH receptors (Hunter, 2000; McGee and Hseuh, 2000). Cumulus-oocyte expressed factors also regulate the proliferation and differentiation of the thecal layer (Gore-Langton and Armstrong, 1988; Driancourt et al., 2000), as well as thecal steroid production (Carabatsos et al, 1998; Driancourt et al, 2000).

The avascular nature of the follicle means that the oocyte-cumulus complex secreted factors involved in the regulation of granulosa cell proliferation and differentiation diffuse through the follicle. As the volume of the antral cavity increases, large sections of the membrana granulosa become increasingly

distal to the oocyte-cumulus complex, and are essentially out of reach of oocyte-cumulus expressed factors. Granulosa cell apoptosis, and subsequent follicle atresia and degeneration, is related to insufficient exposure to essential nutrients and growth factors. Consequently, continued granulosa and theca cell proliferation and differentiation becomes increasingly reliant on stimulation from endocrine factors, with the avoidance of atresia and further growth dependent on blood-born metabolites, metabolic hormones and gonadotrophins (Gore-Langton and Armstrong, 1988).

1.4.1.3 Gonadotrophin control of follicle growth and ovarian steroidogenesis

The gonadotrophins, FSH and LH, play a central role in the formation and maturation of the antral follicle. Acting via specific membrane bound receptors, the effects of FSH and LH are mediated through cAMP induced post-receptor signalling (Hillier et al, 1994). This signalling stimulates production of a number of factors, such as Kit Ligand, keratinocyte growth factor (KGF) and inhibin (Hillier et al, 1994; Parrott and Skinner, 1998; Driancourt et al, 2000), which are believed to regulate the proliferation and differentiation of granulosa and theca cells, as well as steroidogenesis. FSH receptor (FSHR) mRNA is expressed exclusively in the granulosa cells of growing follicles (Liu et al., 1998), and the development of functional FSH receptors coincides with the differentiation of the thecal layer (Foxcroft and Hunter, 1985). The addition of FSH to *in vitro* culture medium reduces granulosa cell apoptosis, accelerates growth of porcine preantral follicles and promotes antrum formation (Mao et al., 2002).

It is generally accepted that FSH and LH coordinate the interactions between the membrana granulosa and theca interna responsible for the biosynthesis of ovarian steroids, and that the "two-cell, two-gonadotrophin" model, proposed over twenty years ago is applicable to the pig (Foxcroft and Hunter 1985; Guraya, 1985; Gore-Langton and Armstrong, 1988; Greenwald and Terranova, 1988). The conversion of cholesterol and pregnenolone into progesterone

occurs primarily in the granulosa cells (Foxcroft and Hunter, 1985). However, the steroidogenic enzyme required for androgen synthesis, P450 17α-hydroxylase (P450$^{17\alpha}$), is restricted to the thecal layer of 2 to 9 mm porcine follicles, and conversion of progesterone into androgen substrates (androstenedione or testosterone) occurs solely within theca interna cells. In contrast, P450 aromatase (P450^{arom}), the enzyme responsible for aromatising androgens to form oestrogens, is present in both the theca and granulosa cells of porcine follicles (Guthrie et al., 1994; Shores and Hunter, 1999; Findlay et al, 2001). Thecal oestrogen production increases as the follicle matures (reviewed by Gore-Langton and Armstrong, 1988), and as suggested by Shores and Hunter (1999), this source of oestrogen might be required for stimulation of the pre-ovulatory LH surge.

Although progesterone biosynthesis and androgen aromatisation is responsive to both FSH and LH, conversion of progesterone to androgens is regulated solely by the actions of LH. As a consequence, oestrogen synthesis is limited during the late preantral and early antral stages of development due to the follicle's limited ability to produce androgen substrate (Guyura, 1985; Foxcroft and Hunter, 1985; Gore-Langton and Armstrong, 1988; Drummond and Findley, 1999). However, as well as stimulating aromatase activity and subsequent oestrogen synthesis, FSH enhances cellular expression of its own binding sites, as well as those of LH and cAMP (Greenwald and Terranova, 1988; Hillier et al, 1994). Increased exposure to FSH also causes increased inhibin secretion by the granulosa cells, and this protein has been shown to increase thecal sensitivity to LH. In this way, thecal androgen synthesis increases, leading to an increase in oestrogen synthesis, as well as an increase in FSH-stimulated aromatase activity. In addition, androgen has been reported to augment FSH induction of aromatase production via an androgen–receptor mediated mechanism (reviewed by Hillier et al, 1994), and a facilitating, but not obligatory, effect of oestrogens on the actions of FSH and LH has been suggested (Hillier et al., 1994). As the follicle grows, oestrogen production increases (**Table 1.4.2**;

Ainsworth et al., 1980; Gore-Langton and Armstrong, 1988), with follicular fluid concentrations of oestrogen positively related to granulosa and theca expression of P450$^{17\alpha}$, P450^{arom}, and LHR mRNA (Liu et al., 2000). Acting via specific receptors, oestradiol up-regulates FSH and LH receptor expression by granulosa cells, as well as facilitating the effects of FSH, LH and prolactin on granulosa cell proliferation and differentiation, including the promotion of cAMP binding sites and gap junction formation (Ainsworth et al., 1980; Drummond and Findley, 1999). Importantly, oestradiol also promotes progesterone production and aromatase activity, stimulates IGF-I synthesis, and inhibits granulosa cell apoptosis.

Table 1.4.2 Concentrations of progesterone, androstenedione and oestradiol (mean ± SEM) in follicular fluid of different sized follicles following PMSG treatment to induce follicle growth (adapted from Ainsworth et al., 1980)

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This table is included on page 59 of the print copy of the thesis held in the University of Adelaide Library.

However, whilst FSH enhances granulosa cell expression of FSH receptor (FSHR) mRNA in 1 - 2 mm follicles (Sites et al., 1994), follicle growth from 2 to 4 mm is associated with the down regulation of FSHR mRNA expression. Expression of FSHR mRNA is below the detectable limit in 6 and 8 mm follicles (Liu et al., 2000), and Murphy and Dobias (1999) concluded that FSH actually reduces expression of its own receptors in 5 – 6 mm follicles. Concurrent with declining FSHR mRNA expression, LH receptor (LHR) mRNA increases. More specifically, LHR mRNA expression is significantly higher in the granulosa and theca cells of large (> 6 mm) compared to small (< 4 mm) porcine follicles (Yuan et al., 1996; Liu et al., 2000).

Following FSH stimulated expression of LH receptors by the granulosa cell, LH is also able to stimulate all FSH induced cellular responses, including aromatase activity (Filicori et al, 2003). In this way, FSH stimulated antral follicle growth and development becomes increasingly dependant on LH, and following adequate granulosa cell expression of LH receptors, further follicle growth and steroidogenic activity is mediated solely by LH (Filicore et al, 2003). These observations have led to the suggestion that the "two-cell, 2-gonadotrophin" model of follicle steroidogenesis is only truly applicable in small antral follicles (Filicore et al., 2003). The *in vivo* data of Driancourt et al. (1995) supports this, and using an LH specific GnRH antagonist these authors demonstrated that while antral follicles of 2 to 4 mm required FSH support, follicle growth beyond 4 mm was dependant on episodic LH release.

1.4.1.4 Metabolic influences on follicle growth and development

There is strong evidence to support a central role for the insulin-related regulatory system during follicle growth and development (reviewed by Giudice, 1992; Poretsky et al., 1999; Prunier and Quesnel, 2000). Insulin, acting via receptors present in granulosa and theca cells, promotes transmembrane glucose transport, stimulates granulosa cell proliferation *in vitro* and acts synergistically with LH to promote ovarian follicle growth. More precisely, insulin promotes LH receptor (LH-R) expression, regulates the expression of steroidogenic enzymes, and facilitates the stimulatory effects of FSH and LH on steroidogenesis (reviewed by Poretsky et al., 1999). Equally, insulin stimulates preantral follicle growth *in vitro*, with high insulin doses supporting growth of preantral hamster follicles and promoting the proliferation of rat granulosa cells (Yu and Roy, 1999; Kezele et al, 2002; Fortune, 2003). It is also likely that insulin stimulates receptors on the oocyte of pigs (Quesnel, 1999) and rats (Kezele et al, 2002), but whether it directly affects oocyte viability or is involved in the regulation of the paracrine and/or autocrine factors essential for oocyte and follicle cell growth has yet to be established. However, a possible stimulatory

effect of insulin on the production of oocyte-expressed growth factors could be hypothesised, as could a role in the mediation of the oocyte response to growth stimuli received from the surrounding pre-granulosa cells.

In a wide variety of species, including cattle, rodents and pigs, changes in the intrafollicular IGF-I system play an integral role in the orchestration of ovarian follicle growth (reviewed by Giudice, 1992; Poretsky et al., 1999; Pruner and Quesnel, 2002). The IGF system includes the mitogenic peptides IGF-I and IGF-II, the IGF-binding proteins (IGFBP) –1 through – 6, which regulate IGF availability, as well as cell bound receptors. Synthesised primarily in the liver, but also in the ovary, IGF-1 influences a number of intra-ovarian biosynthetic processes. A stimulatory effect of IGF-I on granulosa cell proliferation, aromatase activity and progesterone biosynthesis is well established (Giudice, 1992; Poretsky et al., 1999; Spicer et al., 2002), and Mao et al. (2004) confirmed that IGF-I promotes antrum formation by porcine follicles *in vitro.* The available literature supports a synergistic relationship between the IGF system and gonadotrophins during ovarian follicle growth and development. Acting in conjunction with oestradiol, FSH stimulates IGF-I synthesis by porcine granulosa cells in vitro (Hammond et al., 1988), as well as promoting the stimulatory effect of insulin and IGF-I on granulosa cell proliferation (Spicer et al., 2002). Conversely, IGF-I facilitates FSH stimulation of FSHR mRNA expression, as well as stimulating oestradiol production by granulosa cells, and acts in synergy with FSH to regulate aromatase activity in bovine granulosa cells (Spicer et al., 2002).

Although generally accepted to be a potent mitogen and differentiation factor during somatic cell growth and development (Giudice, 1992; Spicer et al., 2002), correlations between follicular fluid concentrations of IGF-I and porcine follicle growth are weak (Howard and Ford, 1992). Liu et al. (2000) reported similar expression of IGF-I mRNA in follicular fluid collected from 2, 4, 6 and 8 mm follicles; however, Yuan et al. (1996) reported significantly higher IGF-I

mRNA in granulosa cells from large antral compared to preantral follicles, with follicle growth also associated with changes in expression of IGFBP. The role of IGFBP as modulators of IGF-I activity in developing follicles is well accepted (Giudice, 1992; Poretsky, 1999). Specifically, IGFBP affect the binding affinity of IGF-1 for its receptors (Howard and Ford, 1992), with IGFBP-2 and -4 reducing, and IGFBP-3 increasing, IGF-I bioavailability (Poretsky et al., 1999). Expression of IGFBP-2 and -4 decreases with follicle growth, whereas theca, but not granulosa, cell expression of IGFBP-3 mRNA and follicular fluid concentrations of IGFBP-3 are higher in antral compared to preantral porcine follicles (Liu et al., 2000; Wandji et al., 2000). Although an inhibitory effect of FSH on granulosa cell IGFBP-3 production is evident in vitro (Poretsky et al., 1999), the IGFBP-3 present in follicular fluid is predominantly of extra-follicular origin, entering the follicle from the surrounding vasculature (Wandji et al., 2000), and Howard and Ford (1992) proposed a stimulatory effect of IGFBP-3 on IGF-1 induced cell proliferation.

1.4.1.5 Morphological aspects of oocyte development and maturation

As early as day 40 post conception, oogonia enter into meiotic division. This process halts at the diplotene stage of the first prophase, with the oogonia remaining in a state of arrested meiotic division, the germinal vesicle stage (**Figure 1.4.2**). Simultaneously with the arrest of meiotic division, primary oocytes are surrounded by a layer of pre-granulosa cells and enclosed within an intact basal lamina, thus forming the primordial follicle (**Figure 1.4.2**; Elseasser, 1982; Picton, 2000; Fair, 2002; McCoard et al, 2003). Formation of the primordial follicle pool is complete during the peri-natal period, providing a finite supply of oocytes from which the female draws throughout her reproductive life (Kezele et al, 2002). Concurrent with re-activation of follicle growth, and the acquisition of a complete ring of cuboidal granulosa cells, the previously non-growing, but metabolically active, 'primordial' oocyte enters into a period of

extensive growth (Moor et al., 1990; Parrott and Skinner, 1998; Hunter, 2000; Braw-Tal et al., 2002).

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Figure 1.4.2 Summary of mammalian oocyte meiosis: (1 - 4) prophase stages of first meiotic division, which in most mammals occur during foetal life; (5) dictyate stage; (6) germinal vesicle stage (GV), meiotic process is arrested at this stage (first meiotic arrest); (7 - 11) resumption of meiosis and completion of first maturation division; (11) metaphase II (MII), this is the second meiotic arrest, in which state the oocyte is normally ovulated; (12 - 14) second meiotic division, which normally occurs in the oviduct, following sperm penetration (taken from Guraya, 2000).

An integral role for oocyte-granulosa cell communication during oocyte growth and maturation is well established (Hunter, 2000). Oocyte growth is characterised by a dramatic increase in RNA and protein synthesis, the accumulation of lipid droplets and multivesicular bodies, formation of the zona pellucida, and a 200-fold increase in oocyte volume. During the growth phase, porcine oocytes increase in diameter from 30 to 120 µm, and progressively acquire the ability to resume meiosis and maintain a stable metaphase II (**Table**

1.4.3). The ability to undergo germinal vesicle breakdown and reach metaphase I is acquired earlier in the growth phase than the ability to reach metaphase II, with the capacity to progress to metaphase II, meiotic competence, only acquired once the oocyte approaches its full size (**Table 1.4.3;** Motlik and Fulka, 1986; Moor et al., 1990; Hyttel et al., 1997; Hunter, 2000; Van den Hurk and Zhao, 2005).

Table 1.4.3 Relationship between the size of the oocyte and its ability to undergo Germinal Vesicle Breakdown (GVBD) and reach the Metaphase I (MI) or Metaphase II (MII) stages of the meiotic cycle (adapted from Motlik and Fulka, 1986)

Follicle size	Mean oocyte diameter	Proportion oocytes undergoing GVBD	Proportion of oocyte at:	
			MI	MII
0.3 - 0.7 mm	100 µm	59	4	0
1.8 – 2.2 mm	115 µm	97	31	49
5.0 – 6.0 mm	120 µm	100	24	76

Oocyte growth is complete in small antral follicles of approximately 2.2 mm, and transcripts and proteins synthesised during the growth phase are sequestered and stored for use during the pre-implantation stages of embryo development (Hunter, 2000). However, the cessation of oocyte growth and acquisition of meiotic competence does not signify complete developmental competence (Moor et al., 1990). The ability of oocytes to progress to metaphase II and develop to the blastocyst stage of embryo development following *in vitro* maturation and fertilisation increases with follicle growth beyond 3 mm (**Table 1.4.4;** Marchal et al., 2002). During the antral stages of follicle growth, and following luteolysis, the specific transcription factors vital for the resumption of meiosis, fertilisation and early stages of embryo development accumulate in the cytoplasm of the oocyte (Hyttel et al., 1997; Krisher, 2004). Successful completion of the first six to nine days of embryonic

development and activation of the embryonic genome, thus completing the maternal to zygote transition, depends on these maternal transcription factors. The majority of early embryo failure, at least *in vitro*, occurs coincident with the activation of the embryonic genome, suggesting that developmentally incompetent oocytes fail to accumulate the appropriate transcription factors during folliculogenesis (reviewed by Moor et al., 1990; Hunter, 2000; Krisher, 2004; Van den Hurk and Zhao, 2005; Sirard et al., 2006). Defects during cytoplasmic maturation also result in sperm chromatic condensation and polyspermy, whilst Sirard et al. (2006) proposed that the ability to develop to the blastocyst stage of embryonic development is the most accurate indicator of oocyte developmental competence, or 'normalcy', and is largely dependent on the follicular environment in which the oocyte develops.

Table 1.4.4 Relationship between follicle size, meiotic progression and blastocyst formation *in vitro* (adapted from Marchal et al., 2002)

Follicle size	Proportion of matured oocytes at:		Blastocysts / MII[2]	Blastocysts / 2PN[3]
	GV to pre-MI	MII		
< 3 mm	54[b]	44[a]	7[a]	23[a]
3 – 5 mm	20[a]	77[b]	19[b]	72[b]
> 5mm	14[a]	86[b]	27[b]	83[b]

[ab] Superscripts within row indicate significant difference; $P < 0.05$
[1] GV = germinal vesicle; MI = metaphase I; MII = metaphase II
[2] Proportion of oocytes at MII that developed to the blastocyst stage by day 8.
[3] Proportion of oocytes undergoing normal fertilisation that developed to the blastocyst stage by day 8

1.4.1.6 Intra-follicular steroids and oocyte developmental competence

Concurrent with the acquisition of oocyte developmental competence, the oocyte is exposed to sequential changes in follicular fluid steroid concentrations (Dode and Graves, 2002; Marchal et al., 2002), and *in vitro* indicate that cAMP induced inhibition of meiotic progression is promoted by

various steroid hormones, including progesterone, testosterone and oestradiol, (Tsafriri et al., 2005). However, a direct or indirect effect of changes in the intra-follicular steroid milieu on oocyte maturation has yet to be firmly established. Premature exposure to elevated oestradiol concentrations prior to oocyte retrieval has a detrimental effect on the ability of oocytes to mature *in vitro* (reviewed by Moor et al., 1990), and the data of Grupen et al. (2003) provide tentative support for a relationship between intra-follicular steroid concentrations *in vivo* and oocyte developmental competence. Equally, the established relationship between porcine follicle diameter and both follicular fluid steroid concentrations (**Table 1.4.4**) and oocyte maturation *in vitro* (**Table 1.4.4** and **Table 1.4.5**) provides circumstantial evidence to support the involvement of steroidal factors in the acquisition of oocyte developmental competence. However, Huynh et al. (2004) demonstrated that oocytes recovered from aromatase knockout mice were able to progress to metaphase II, be fertilised and successfully complete the early stages of embryo development, concluding that oestrogen is not required for the acquisition of oocyte developmental competence in mice.

The role of ovarian steroids during the resumption of meiosis and final preparation of the oocyte for fertilisation and early embryo development is also controversial. Dode and Graves (2002) demonstrated that the ability of oocytes to progress to metaphase II and complete fertilisation and pronuclear formation was unaffected by the addition of various combinations of ovarian steroids to the culture medium. However, these authors also demonstrated that cumulus-oophorus complexes secrete both progesterone and oestradiol during nuclear maturation, and suggested that steroid production by the cumulus cell was sufficient to meet the oocyte's requirement for the completion of nuclear or cytoplasmic maturation. However, maturing porcine oocytes in conditioned media derived from large compared to small follicles promotes nuclear maturation (Ding and Foxcroft, 1994). Equally, in an earlier study, these authors reported a correlation between the follicular fluid steroid milieu during *in vitro*

maturation and male pronuclear formation of sperm penetrated oocytes. More specifically, male pronuclear maturation was positively correlated with follicular fluid concentrations of progesterone and oestradiol, as well as the ratios of progesterone and oestrogen to testosterone (Ding and Foxcroft, 1992). Further evidence that factors present in the follicular fluid of large follicles promote oocyte maturation is provided by the more recent study of Algriany et al. (2004). Specifically, compared to cumulus-oocyte complexes (COC) matured in the presence of follicular fluid obtained from large (5 - 8 mm) follicles, culturing COC with follicular fluid derived from small (2 – 4 mm) follicles significantly reduced the proportion of oocytes able to progress to metaphase II and subsequently develop to the blastocyst stage following in vitro fertilisation (Table 1.4.5).

Table 1.4.5 Nuclear maturation rates and blastocyst formation rates of sow COC cultured in follicular fluid derived from small (SFF) or large (LFF) follicles and with or without FSH (adapted from Algriany et al., 2004).

Treatment	FF steroid concentrations		Proportion of matured oocytes[2] at		Blastocysts / cleaved[3]
	$E2_1$ (ng/ml)	$P4_1$ (ng/ml)	GV_1	MII_1	
Control	-	-	27^b	51^a	23^a
SFF	10	72	31^b	55^a	23^a
LFF	84	248	15^a	72^b	31^b

[ab] Superscripts within column indicate significant difference; $P < 0.05$
[1] E2 = oestradiol; P4 = progesterone; GV = germinal vesicle; MII = metaphase II
[2] Oocytes aspirated from 2 – 5 mm follicles
[3] Proportion of cleaved embryos that developed to the blastocyst stage

Interestingly, Gandolfi et al. (1997) demonstrated that the developmental competence of bovine oocytes aspirated from 2 – 5 mm follicles is related to the gross morphology of the ovary from which they were obtained (Table 1.4.6). Specifically, nuclear maturation rates, blastocyst formation rates and blastocyst cell number were significantly lower for oocytes aspirated from ovaries with

less than ten 2-5 mm follicles, compared to those aspirated from ovaries with more than ten 2 – 5 mm follicles (**Table 1.4.6**). Although these authors did not measure intra-follicular concentrations of steroids, they suggested that this difference in developmental competence was reflective of alterations in the levels of gonadotrophins to which the ovaries had been exposed *in vivo*. Equally, Algriany et al. (2004) demonstrated that FSH potentiates the stimulatory effect of follicular fluid on oocyte maturation *in vitro*.

Table 1.4.6 Nuclear maturation rate, fertilisation rate and blastocyst rate of oocytes aspirated from 2 – 5 mm follicles on ovaries with either < 10 follicles with a diameter of 2 – 5 mm or > 10 follicles with a diameter of 2 – 5 mm (adapted from Gandolfi et al., 1997).

Number of 2 – 5 mm follicles[1]	Nuclear maturation rate (%)	Fertilisation rate (%)	Blastocyst rate (%)	Blastocyst cell number
< 10	81.0[b]	73.9	22.9[b]	104.1[b]
> 10	61.6[a]	65.9	5.5[a]	66.0[a]

[ab] Superscripts within column indicate significant difference; $P < 0.05$
[1] No follicle greater than 10 mm in diameter

1.4.2 Follicle selection and ovulation

The endocrine, intra-follicular and cellular processes involved in the growth of ovarian follicle through to the ovulatory stage have been extensively reviewed (Foxcroft and Hunter, 1985; Cardenas and Pope, 2002; Hunter et al., 2004; Knox, 2005). Before the onset of puberty and during the luteal phase of the oestrous cycle, a proliferating pool of 50 – 100 "recruited" antral follicles measuring between 1 and 6 mm in diameter is present on the surface of the ovary (Foxcroft and Hunter, 1985; Hunter et al., 2004; Knox, 2005). Many of the follicles within the proliferating pool are at different stages of growth, with recruitment and atresia appearing to occur synchronously within small cohorts of follicles. In this way, a heterogeneous pool of growing follicles is created,

from which the ovulatory follicles are selected during the 5 – 7 day follicular phase (Foxcroft and Hunter, 1985; Bolamba et al., 1994; Guthrie et al., 1995; Knox, 2005).

Five days before ovulation, approximately 40 to 50 follicles are present on both ovaries, with follicles evenly distributed between the 2.0 – 3.9 mm and 4.0 to 5.9 mm size categories. However, the majority of these follicles undergo atresia and degenerate (Guthrie et al., 1995), and during the five days preceding ovulation, Grant et al. (1989) reported a decline in the number of 2.0 – 3.9 mm follicles and a corresponding increase in the number of 4.0 – 8.0 mm follicles. The data presented by Clarke et al. (1992) and Ryan et al. (1994) portray a similar pattern of follicle growth (**Table 1.4.7**), with mean follicle diameter increasing from 3.99 mm on day 16 to 8.80 mm on day 21 (Hunter and Weisak, 1990). The appearance of large (> 6 mm) follicles coincides with a dramatic increase in atresia rates amongst small (1 – 2 mm) and medium (3 – 6 mm) follicles. Specifically, incidences of atresia amongst small and medium follicles increase from 22% to 80% after day 16 of the oestrous cycle (Guthrie et al., 1995), and studies involving exogenous hormone treatments indicate that day 18 of the oestrous cycle is the point at which selection of follicles from the proliferating pool ceases (reviewed by Knox, 2005). This growth of large follicles, and degeneration of small and medium follicles, results in the formation of a pool of 12 to 20 ovulatory follicles on day 21 of the oestrous cycle (Foxcroft and Hunter, 1985; Grant et al., 1989; Hunter and Weisak, 1990; Cardenas and Pope, 2002; Hunter et al., 2004).

Table 1.4.7 Mean number of small (1 – 2 mm), medium (3 – 6 mm) and large (7 – 10 mm) follicles present on the right ovary of gilts on days 13, 15, 17 and 19 of the oestrous cycle (adapted from Clark et al., 1982).

Day of oestrous cycle	Number of follicles			
	Small (1 – 2 mm)	Medium (3 – 6 mm)	Large (7 – 10 mm)	Total
13	23.2	22.0	0.0	45.2
15	28.4	23.0	0.0	51.4
17	33.6	14.6	2.5	49.2
19	14.0	10.8	6.0	24.6

The endocrine and intra-follicular mechanisms responsible for follicle selection remain to be clearly established. However, current understanding suggests that only those follicles that are able to make the switch from FSH to LH dependence will avoid atresia and ovulate. The pattern of episodic LH release changes on days 13 – 14 of the oestrous cycle, characterised by a switch from high amplitude, low frequency pulsing to high frequency, low amplitude pulsing (Flowers et al. 1991). This increased frequency of pulsatile LH release has been suggested as one probable stimulus for the commencement of follicle selection (Knox, 2005), with declining FSH concentrations between days 13 and 19 of the oestrous cycle causing the rapid atresia of small and medium follicles and inhibiting further recruitment (Foxcroft and Hunter, 1985; Cardenas and Pope, 2002). Guthrie et al. (1995) reported an inverse relationship between FSH concentrations and incidences of follicle atresia, and it is probable that only the most mature follicles present on day 16 of the oestrous cycle will be selected for ovulation (Foxcroft and Hunter, 1985). Aromatase activity, oestrogen biosynthesis and LH binding capacity appear to be higher in selected follicles, with follicle growth between day 16 to 20 associated with 4 fold and 5 fold increase in granulosa and theca cell binding of labelled hCG respectively (Foxcroft and Hunter, 1985; Hunter and Weisak, 1990; Cardenas and Pope, 2002). Flowers et al. (1991) reported a temporal relationship between LH pulses

and oestradiol secretion by the ovary, suggesting that the high frequency, low amplitude pattern of LH pulsing provides the stimulus for increased oestradiol biosynthesis during the late follicular phase. Follicular fluid concentrations of oestradiol increase from 22 to 236 ng/ml between days 16 and 20 of the oestrous cycle, with the oestrogenic activity of the most mature follicle stimulating the commencement of the pre-ovulatory LH surge (Foxcroft and Hunter, 1985; Hunter and Weisak, 1990). The avoidance of atresia and 'selection' into the ovulatory pool is restricted to those smaller follicles with sufficiently high somatic cell expression of LH receptors to respond to the preovulatory LH surge (Foxcroft and Hunter, 1985; Hunter and Weisak, 1990; Knox, 2005).

Activation of the LH surge typically occurs on day 18 of the oestrous cycle, with the size of the ovulatory pool determined by the number of smaller follicles that are sufficiently mature to respond to the LH surge (Hunter and Weisak, 1990). On day 16 of the oestrous cycle, asynchrony is evident within the proliferating follicle pool, with variation in follicle maturity coincident with the initiation of the LH surge resulting in the formation of a morphologically and biochemically heterogeneous pool of ovulatory follicles (Hunter and Weisak, 1990; Hunter et al., 2004). Grant et al. (1989) observed considerable variation in follicle diameter, granulosa cell number and follicular fluid concentrations of steroids within the 14 largest follicles present on day 20 of the oestrous cycle (**Table 1.4.8**). Importantly, this disparity in follicle morphology appears to continue throughout the peri-ovulatory period, affecting follicle luteinisation and subsequent luteal function, as well as the maturity of the shed oocytes (Hunter and Weisak, 1990).

Table 1.4.8 Individual follicle characteristics in the 14 largest follicles present on the ovary of one gilt on day 20 of the oestrous cycle (adapted from Grant et al., 1989)

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│   This table is included on page 72 of the print copy of │
│     the thesis held in the University of Adelaide Library.│
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1.4.3 Embryo losses during gestation

Approximately 30% of viable embryos are lost during the first 30-35 days of gestation, with the majority of embryos dying between days 5 and 18 of pregnancy (Pope et al., 1990; Xie et al., 1990; Geisert and Schmitt, 2002). A number of factors are believed to affect embryo mortality rates before and during implantation (days 5 – 18 of pregnancy) and these have been well reviewed by Ashworth and Pickard (1998). Essentially, most embryos are lost at this stage because they are either less developed than their littermates before ovulation, were fertilised later than their littermates or were exposed to a sub-optimal uterine environment during their first two weeks of life. A number of recent reviews have discussed foetal loss in the gilt, and current understanding of the factors responsible for the loss of foetuses between days 30 - 35 and term have been detailed by Vallet et al. (2002) and Ford (2003), and will not be described in this review. Consequently, this section will detail only the most recent information relating to heterogeneity within the pre-ovulatory follicle pool, as well as synchrony of development between litter-mate embryos and between the embryo and the uterus.

While growth of the conceptus is programmed through innate developmental cues (Geisert and Yelich, 1997), growth factors and nutrients provided by the uterus are also vital for embryonic development (Vallet et al., 2002). In particular, the timing of changes in uterine secretions is critical for conceptus survival (Soede et al., 1999), as asynchrony between the conceptus and the uterine environment is detrimental for development and survival of embryos. Naturally occurring variation in development between litter-mate embryos, combined with altered patterns of uterine secretion in response to nutritional and endocrine signals, are the primary causes of asynchronous development (Vallett et al., 2002). As discussed previously, follicle selection occurs over a protracted period of 5 – 7 days, resulting in the formation of a heterogeneous pre-ovulatory population characterised by differences in follicle diameter, varying concentration of steroids in the follicular fluid, and oocytes in a wide range of meiotic stages (**Table 1.4.9**; Hunter and Weisak, 1990). The actual pattern of pre-ovulatory follicular development and oocyte meiotic maturation within animals is skewed, such that most follicles and oocytes are at a similar stage of development to each other, but there is a small population (ca. 30%) that are less developed. Similarly, time of ovulation is skewed with approximately 70% of follicles ovulating over a short period of time, with the remaining 30% of oocytes released over a more protracted period (reviewed by Pope et al., 1990). This disparity in follicle development and oocyte maturity has been identified as the principal cause of asynchronous development between litter-mate embryos during the first 12 days of gestation, with later ovulated oocytes becoming the least developed embryos (Hunter and Weisak, 1990; Pope et al., 1990; Xie et al., 1990).

During days 10 to 12 of gestation, pig blastocysts synthesise and secrete increasing amounts of oestrogen (Pope et al., 1990), stimulating endometrial secretion of proteins and growth factors involved in conceptus elongation and implantation (Geisert and Yelich, 1997). However, uterine secretions that facilitate elongation and implantation of the more developed embryos may not

be conducive to the continued development of the least developed embryos, resulting in their elimination (Pusateri et al., 1990). Less developed blastocysts may not be protected against the embryo-toxic effects of factors such as retinol and uteroferrin that are released into the uterine lumen in response to oestrogen secreted by the conceptus (Vallet et al., 1996; Geisert and Yelich, 1997). Alternatively the attachment of less developed blastocysts may be prevented by changes in uterine surface protein (Soede et al., 1999).

Table 1.4.9 Stage of meiosis of oocytes obtained from individual gilts at either 27 or 30 hours after the onset of oestrus (adapted from Pope et al., 1990)

Hours after onset of oestrus	Stage of Meiosis			
	Germinal vesicle	Germinal vesicle breakdown	Anaphase 1	
			Metaphase 1 to Telophase 1	Metaphase 2
27	3	9		
27	4	1	15	
27			5	11
27			1	1	9
27			3		16
27			4	2	8
30			2	2	12
30			1	2	9
30			1		13
30			1		16
30			2		11

1.4.4 Effects of dietary intake on litter size

Studies involving a wide variety of mammalian species, including pigs, cattle, and sheep, demonstrate that changes in dietary intake profoundly affect the fertility and fecundity of breeding females (Prunier and Quesnel, 2000; Martin et

al., 2004; Webb et al., 2004). In the pig, it has long been known that ovulation rate is affected by feed intake prior to mating, and that embryo development and survival are sensitive to changes in dietary intake during the peri- and post-ovulatory period (Brooks and Cole, 1974; Ashworth et al., 1999). However, there is now strong evidence that changes in dietary intake prior to mating also affect the developmental competence of the oocytes shed as well as subsequent embryo survival (Webb et al., 2003; Hunter et al., 2005). Consequently, this section will detail current understanding of the effects on nutritional status before and after mating on potential litter size, as well as the mediatory mechanisms involved.

1.4.4.1 Pre-mating dietary intake and follicle growth

The size of the proliferating follicle pool coincident with follicle selection has been identified as one determinant of ovulation rate in the pig (Hunter and Weisak, 1990). An effect of nutritional status on follicle growth has been established; however, there is a paucity of data describing the effects of nutritional status on the size and composition of the proliferating pool in the pig (Prunier and Quesnel, 2000). Using prepubertal gilts, Dufour et al. (1985) demonstrated that feed intake affected the morphology of the proliferating antral pool; however, this effect was evident in only one of the genotypes used. Low lactation feed intake reduces the proportion of 1 – 2.9 mm follicles present in the proliferating antral follicle pool at weaning (**Figure 1.4.3**; Quesnel et al., 1998), and increasing feed intake during the last five days of the luteal phase, increased the ratio of medium to small follicles present on day 15 (Dailey et al., 1972). Restrictive feeding during the last 7 days of a progestagen induced luteal phase reduced the growth of small and medium follicles (Hazeleger et al., 2005), and Cosgrove et al. (1992) and Booth et al. (1996) demonstrated that five days of re-alimentation following a seven day period of restrictive feeding increased follicle growth in prepubertal gilts.

Figure 1.4.3 Proportion of surface antral follicles within three size categories (< 1.0 mm, 1.0 - 2.9 mm, 3 – 5 mm) at weaning of sows receiving either a high (■) or a low (■) lactation feed intake during lactation. [ab] Superscripts within follicle size categories indicate significant difference (adapted from Quesnel et al., 1998)

Further evidence for a nutritional effect on ovarian follicle growth is provided by studies involving rodents and ruminants (Lintern-Moore and Everitt, 1978; Boland et al., 2001). Just as restrictively feeding pre-pubescent rodents reduces follicle growth (Lintern-Moore and Everitt, 1978), Spicer et al. (1991) demonstrated a significant reduction in the growth of large follicles in pre-pubertal heifers fed to either maintain or lose weight for 70 days. Similarly, restricting the feed intake of dairy cattle during the first three days of the oestrous cycle significantly reduces the recruitment of small follicles during the first follicular wave, and restrictively feeding heifers for 17 days reduces the number of small (1– 4 mm), but not medium (5 – 8 mm), follicles (Armstrong et al. 2002). Reducing the dietary intake of dairy heifers also retards the growth of large follicles (Spicer et al., 1991; Armstrong et al., 2001), and restrictively feeding gilts between days 14 and 18 of the oestrous cycle significantly reduces the number, but not the diameter, of large (> 5mm) follicles present on the ovary on day 19 (Quesnel et al., 2000). Interestingly, nutritional promotion of the recruitment of small antral follicles also increases the number of ovulations in response to super-ovulatory hormone treatments in dairy cattle (Webb et al.,

2004), supporting the view that the size of the proliferating pool affects ovulation rate (Hunter and Weisak, 1990).

1.4.4.2 Dietary intake and ovulation rate

A number of studies and reviews have detailed the effects of pre-mating feed intake on the final stages of follicle growth and ovulation rate (eg Brooks and Cole, 1974; Cosgrove and Foxcroft, 1996; Prunier and Quesnel, 2000). In an early review, Brooks and Cole (1974) reported that gilts fed ad libitum during the period immediately preceding puberty shed an average of 1.2 more ova at the pubertal oestrous compared to their restrictively fed counterparts. More recently, Beltranena et al (1991) demonstrated that ad libitum feeding resulted in a numerical, but not significant, increase in pubertal ovulation rate, specifically compared to their restrictively fed counterparts ad libitum fed gilts shed one more ova at their pubertal ovulation (12.1 versus 11.1). In contrast, ad libitum feeding during the first oestrous cycle significantly increased ovulation rate at the second oestrous compared to restrictive feeding (14.7 versus 12.0; P < 0.05). However, feed restriction during the luteal phase of the oestrous cycle does not affect ovulation rate when gilts received a high level of feeding from day 16 through to oestrous (**Table 1.4.10;** Almeida et al., 2000; Novak et al., 2003). Equally, in a comprehensive review of the available literature, Priunier and Quenel (2000), concluded that while ovulation rates decrease by an average of 4.5 ova in response to feed restriction during both the luteal and follicular phases, they are unaffected by reduced feed intake during the luteal phase alone. Taken together, these studies indicate that nutritional effects on ovulation rate reflect alterations in the follicle's final stages of growth, with nutritional status during follicle selection appearing to be a critical determinant of ovulation rate (Prunier and Quesnel, 2000).

Table 1.4.10 Effect of restricted feed intake during two periods of the luteal phase (day 1 –7 versus day 8 – 15) on subsequent ovulation and embryo survival in post-pubertal gilts (adapted from Almeida et al., 2000)

NOTE:
This table is included on page 78 of the print copy of the thesis held in the University of Adelaide Library.

1.4.4.3 Post-mating feed intake and embryo survival

It has been suggested that peri-ovulatory progesterone concentrations mediate nutritional effects on early embryo survival (Jindal et al., 1996 and 1997; Almeida et al., 2000). This is supported by two recent studies. The first demonstrated that a higher plane of feeding (2 times maintenance) from day 1 of gestation delayed the increase in progesterone concentration, and increased embryonic mortality by 20% up to day 28 of pregnancy, compared to gilts receiving 1.5 times maintenance (Jindal et al., 1996). The second study indicated that treating gilts with six intra-muscular injections of progesterone at 12-hourly intervals between 24 and 108 hours after oestrus detection reversed the detrimental effects of higher plane feeding on embryo survival (Jindal et al., 1997). It was concluded that reducing feed intake from day 1 of pregnancy (Day 0 refers to the 24 hour period after the first detection of oestrus) was the most beneficial in terms of increased embryo survival, and that feed intake around the time of ovulation alters the pattern of progesterone secretion due to variations in follicle luteinization and progesterone secretion. Further, changes in peripheral progesterone concentration may reflect altered metabolic clearance rate. Soede et al. (1999) demonstrated a link between feed intake during early pregnancy and both embryo development and uterine protein

secretion. In a separate study, progesterone treatment on days 2 and 3 of the oestrous cycle or pregnancy resulted in an earlier rise in the secretion of total protein, uteroferrin and retinol binding protein (Vallet et al., 1998). Together, these studies suggest that changes in progesterone concentration, induced by feeding level or exogenous administration, affect embryo survival via affects on the uterine secretion of proteins, such as uteroferrin and retinol binding protein.

1.4.4.4 Pre-mating nutrition, oocyte quality and embryo survival

There is increasing evidence that pre-mating nutrition affects embryo survival (Hunter et al., 2004). Indeed the recent studies of Ashworth et al. (1999) indicate that nutrition before mating may in fact have a greater impact on embryo survival than feed intake after mating (**Table 1.4.11**). This supports the finding of Almeida et al. (2000) that moderate feed restriction between days 8 and 15 of the oestrous cycle reduced embryo survival (**Table 1.4.10**) in association with a significant reduction in peripheral progesterone concentrations 24 and 72 hours after first detection of oestrous. Therefore, in addition to the effects of feeding level around the time of ovulation on the uterine environment and luteal function after ovulation, nutritional effects on the physiological development of recruited and pre-ovulatory follicles may also alter progesterone production and/or secretion (Mao and Foxcroft, 1998), as well as affecting the quality of the oocyte released.

The effect of nutrition before mating on embryo survival appears to be partially mediated by changes in follicle steroidogenesis and oocyte maturation (Zak et al., 1997b; Ferguson et al., 2003), which influence the developmental competence of the oocytes released at ovulation. This is supported by two studies involving primiparous sows in which the feeding regime during the previous lactation that resulted in decreased embryo survival rates in the subsequent gestation also reduced the ability of oocytes obtained from pre-ovulatory follicles to reach metaphase II when matured in vitro (Zak et al., 1997a

and b). Specifically, a greater proportion of abattoir derived, pre-pubertal oocytes were able to reach metaphase II when matured in the presence of follicular fluid obtained from sows that were fed ad-libitum between days 21 and 28 of lactation compared to those matured with follicular fluid from sows that were fed restrictively during the same period (Zak et al., 1997b). However, using the dietary model of Almeida et al. (2000), Novak et al. (2003) showed no effect of dietary restriction during the luteal phase on the ability of collected zygotes to develop to the blastocyst stage after 144 hours of culture *in vitro*. Conversely, using the same dietary treatments as Ashworth et al (1999), Ferguson et al. (2003) demonstrated that gilts on a high feeding level throughout the oestrous cycle before ovulation possessed a greater proportion of oocytes in the presumptive ovulatory pool that were able to reach metaphase II compared to oocytes obtained from maintenance fed gilts (**Table 1.4.12**).

Table 1.4.11 Effect of feed intake, before and after mating, on ovulation rate, embryo number and embryo survival on day 12 of gestation in Meishan gilts (adapted from Ashworth et al., 1999)

NOTE:
This table is included on page 80 of the print copy of
the thesis held in the University of Adelaide Library.

Table 1.4.12 Effect of feed intake during the luteal and follicular phase on oocyte nuclear maturation and follicular fluid concentration of oestradiol on day 19 of the oestrous cycle (adapted from Ferguson et al., 2003)

NOTE:
This table is included on page 81 of the print copy of the thesis held in the University of Adelaide Library.

Concentrations of oestradiol were also higher in the follicular fluid of gilts on a high feeding plane (**Table 1.4.12**; Ferguson et al., 2003), whilst O'Callaghan et al. (2000) observed a significant increase in intra-follicular progesterone concentrations in severely feed restricted ewes. An association between the steroid content of follicular fluid and the ability of oocytes from pre-pubertal gilts to reach metaphase II *in vitro* has also been established (Grupen et al., 2003). Together, these studies indicate that factors within the follicular fluid are involved in the regulation of meiotic competence of oocytes, and that nutritionally induced alterations in oocyte quality may be mediated, at least in part, by changes in the intra-follicular environment.

1.4.4.5 Dietary intake and ovarian activity

As previously discussed, activity of the reproductive neuroendocrine axis is sensitive to changes in the availability of metabolic fuels, and it has been established that a number of circulating metabolic hormones and metabolites mediate nutritional effects on the activity of the hypothalamic-pituitary-ovarian axis (Foster and Nagatini, 1999; Prunier and Quesnel, 2000; Wade and Jones, 2004). Feed restriction inhibits episodic LH release (Booth et al., 1992; Schillo et al., 1992; Prunier et al., 1993), and the relationship between nutritionally

induced elevations in LH pulse frequency and ovulation rate is well established (Cox, 1997). Further, maintenance feeding during the luteal and follicular phase increases peripheral oestradiol concentrations and decreases the frequency of LH pulsing in cycling gilts (**Table 1.4.13**). Ferguson et al. (2003) also reported an inverse relationship between mean oestradiol concentrations and the frequency of LH pulsing in gilts on a high feeding level, suggesting that high feed intake before mating increases portal blood flow, effectively reducing negative feedback at the hypothalamus. This increase in LH pulse frequency promotes thecal androgen production and subsequent intra-follicular oestradiol metabolism, and consequently the size of the pre-ovulatory follicle pool is increased (Armstrong et al., 2002; Ferguson et al., 2003).

Table 1.4.13 Effect of feed intake during the luteal and follicular phase on plasma IGF-1 concentrations and LH pulse frequency (adapted from Ferguson et al., 2003)

NOTE:
This table is included on page 82 of the print copy of
the thesis held in the University of Adelaide Library.

Dietary effects on ovulation rate also occur in the absence of changes in the pattern of episodic LH release (Cox, 1997). Severely restricting the feed intake of cyclic gilts for ten days inhibits the growth of follicles beyond 2 mm without affecting circulating gonadotrophin levels (Quesnel et al., 2000), and nutritionally induced alterations in metabolic hormones appear to act directly at the ovary to alter follicle growth and development (Cosgrove et al., 1993; Hunter et al., 2004; Hazeleger et al., 2005). The insulin-related regulatory system plays an integral role during follicle growth and development, and both

insulin and IGF-I promote granulosa cell proliferation and formation of the antral cavity, as well as facilitating the stimulatory effects of gonadotrophins on follicular steroidogenesis and growth. Further, the available literature strongly supports the view that glucose and the insulin-related regulatory system act as signals mediating the effects of nutrition on ovarian activity.

A rise in insulin concentration takes place concomitant with dietary induced increases in ovulation rate, and administering insulin to high or normally fed gilts increases ovulation rate (Cox, 1997). Using ovarian artery infusions, Downing et al. (1999) reported a direct affect of glucose and insulin on follicle growth in ewes, and insulin appears to reduce incidences of follicle atresia in restrictively fed gilts (Matamoros et al., 1990), as well as promoting the growth of small ($\leq$ 3mm) follicles in normally fed gilts (Matamoros et al. 1991). However, injecting normally fed gilts with insulin has little effect on the growth of follicles beyond 4 mm (Matamoros et al., 1991), and the negative effects of feed restriction during the follicle phase on the number of large follicles present on day 19 of the oestrous cycle are not reversed by insulin injections (Quesnel et al., 2000). Equally, long-lasting injections of insulin do not increase ovulation rate or embryo survival in gilts that were restrictively fed during days 8 – 15 of the oestrous cycle (Almeida et al., 2001).

However, dietary regimes that increase the size of the presumptive ovulatory pool also increase plasma IGF-I concentrations (**Table 1.4.13**), and low plasma IGF-I concentrations have been associated with retarded follicle growth in gilts (Booth et al., 1996). A positive correlation between plasma IGF-I concentrations and oocyte nuclear maturation rates has been reported (Ferguson et al., 2003), and restricting lactation feed intake reduces both plasma and intra-follicular IGF-I levels (Prunier et al., 1998). Insulin depletion of diabetic gilts increases follicle atresia and decreases intrafollicular IGF-1 concentrations (Prunier and Quesnel, 2000), and exogenous insulin administration doubles intrafollicular IGF-I concentrations in medium (4 – 6 mm) follicles, but does not affect

steroidogenesis (Matamoros et al., 1991). Although Spicer et al. (1991) reported no effect of restrictive feeding on ovarian IGF-I levels, subsequent studies involving cattle (Armstrong et al., 2002) and pigs (Prunier and Quesnel, 2000) suggest that metabolic effects on follicular steroidogenesis are mediated by changes in the bioavailability of intra-follicular insulin-like growth factors. High energy diets appear to increase the bioavailability of IGF-I in small (1 – 4 mm) follicles due to a reduction in intrafollicular expression of IGFBP-2 and –4 in small bovine follicles (Armstrong et al., 2001). Further, systemic levels of IGFBP-3, a binding protein known to promote IGF-I bioavailability, are positively correlated with dietary intake in cattle (Armstrong et al., 2002), and the IGFBP-3 present in follicular fluid is predominantly of extra-follicular origin (Wandji et al., 2000).

A stimulatory effect of IGF-I, both on its own and in synergy with FSH, on progesterone synthesis and aromatase activity is well established (Poretsky et al., 1999), and Armstrong et al. (2002) demonstrated a 3.7 fold increase in oestradiol production from the granulosa cells of small follicles obtained from cattle on a high nutritional plane. As well as increasing the number of small (1 – 2.5 mm) follicles present on the ovaries of cyclic ewes, four weeks of high feed intake also appears to promote aromatase expression in these follicles, as determined by a decrease in the intra-follicular ratio of oestradiol to testosterone (Rhind and McNeilly, 1998). Furthermore, dietary induced alterations in porcine oocyte developmental competence are accompanied by changes in steroidogenesis and intrafollicular oestradiol production (Ferguson et al., 2003), and reduced oocyte competence in pre-pubertal calf oocytes has been related to decreased aromatase activity of the follicle cell wall (Driancourt et al., 2001). In summary, it is evident that nutritional effects on ovarian follicle growth reflect the direct actions of metabolic hormones, and that these hormones elicit changes in ovarian steroidogenesis and sensitivity to gonadotrophins, ultimately effecting follicle recruitment and selection as well as oocyte developmental competence.

1.4.5 Effects of age and oestrus at first mating on litter size

Despite the equivocalness of the literature, a presumptive strategy of first mating gilts at their second or third oestrus has been widely adopted by the pork industry (Martinat-Botte et al., 1985; Whittemore, 1996). Reports on the benefits of delaying first mating are not only contradictory but frequently fail to separate the confounding effects of sexual or physiological age (i.e. number of oestrus cycles experienced) from those of chronological age (i.e. days). Delayed first mating has been associated with higher ovulation rates (Warnick et al., 1951; Archibong et al., 1987), improved oocyte quality (Koenig and Stormshak, 1993; Herrick et al., 2003), and decreased incidences of abnormal embryo development (Menino et al., 1989; Archibong et al., 1992). Although delaying first mating beyond the first (pubertal) oestrous is also associated with a gradual increase in first litter size, a correspondent increase in both gilt age and weight at first mating also takes place (**Table 1.4.14**; MacPherson et al., 1977; Young and King, 1981). Equally, although Young and King (1981) reported a single piglet increase in first litter size for gilts mated at their third compared to their first oestrus (10.6 versus 9.6), these gilts were also approximately 44 days older (224 versus 180 days old). Conversely, by stimulating gilts to attain puberty at two different ages and mating them at their second oestrus, Brooks and Smith (1980) demonstrated that first litter size increased by one piglet when age at mating increased from 198 to 237 days of age. Consequently, it remains unclear whether advancing sexual age or chronological age has a greater effect on first litter size (Archibong et al., 1992; Brooks and Smith, 1992).

Kirkwood and Aherne (1985) concluded that ovulation rates will increase with each oestrous period when gilts reach puberty at a young age (150 – 210 days). However, Young et al. (1990a) reported no affect of oestrus at mating on first litter size when puberty occurred at 168 days of age (**Table 1.4.14**), and litter size is similar when gilts are mated at their second or third oestrus, but the

same age (215 days old) (Grigoriadis et al., 2001). Further studies also indicate that sow productivity over multiple parities is largely unaffected by oestrus number, age or weight at first mating (Brooks and Smith, 1980; Young et al., 1990a and 1990b). MacPherson et al. (1977) concluded that the benefits of delaying first mating beyond the pubertal oestrus were marginal, while more recently Aumaitre et al. (2000) reported a decrease in annual breeding herd productivity when gilts were mated at their second or third oestrus.

Table 1.4.14 Effect of mating gilts at their first, second or third oestrus on age and liveweight at mating as well as litter size and born alive at first farrowing

Reference	Measurement	Oestrus at mating		
		First	Second	Third
1	Age at mating (days)	189	204	237
	Liveweight at mating (kg)	88	98	115
	Litter size at first farrowing	7.1	8.4	9.8
	Born alive at first farrowing	7.0	8.3	9.6
2	Age at mating (days)	168	182	205
	Liveweight at mating (kg)	108	119	134
	P2 backfat at mating (mm)	20	22	24
	Litter size at first farrowing	9.3	10.4	9.5
	Born alive at first farrowing	9.0	9.7	8.8

1. MacPherson et al. (1977); 2. Young et al. (1990a)

1.5 Purpose and Scope of the Investigation

As previously stated, appropriate management of replacement gilts is a key determinant of sow lifetime productivity, yet incidences of gilt reproductive disorders and failure remain unacceptably high, with 12.4% of all premature culls attributed to anoestrus and pregnancy failure in gilts. In order to address this problem, it is necessary to consider whether current gilt management strategies developed for genotypes of 20 – 30 years ago are suitable for today's

heavier yet leaner genotypes. Further, it is essential that we understand the mechanisms that control puberty attainment and determine litter size. Nutrient availability and growth have an enormous impact on sexual activity and reproductive processes in the female pig. Furthermore, the low fat content and strong inherent drive toward lean accretion typical of current genotypes, strongly suggests that the timing of puberty attainment and first litter size of today's gilts will be highly sensitive to changes in nutritional management during the rearing period.

The objectives of this study were therefore to address the following three issues relating to the development of appropriate gilt management strategies:

1. The effects of age at first boar contact on the timing of puberty attainment and the effects of oestrus at first mating on potential litter size
2. The effects of growth rate during the pre and peri pubertal period on ovarian maturity, specifically follicle growth and oocyte developmental competence
3. The effects of growth rate during the pre and peri pubertal period on the timing of puberty in response to boar stimulation and potential litter size

Studies were conducted at The University of Adelaide, Roseworthy, South Australia, with approval from the animal ethics committee The University of Adelaide.

Chapter 2   Increasing the age of gilts at first boar contact improves the timing and synchrony of the pubertal response but does not effect potential litter size

2.1 Introduction

Gilt age at mating is an important determinant of breeding herd efficiency, and largely depends on age at puberty (Koketsu et al, 1999*)*. Female pigs may attain puberty between 102 and 350 days of age. Reducing this variation by controlling the onset of puberty is extremely beneficial to the productivity of a breeding herd (Hughes, 1982). Early age at puberty is associated with younger mating ages and a shorter non-productive period prior to breeding herd entry, while synchronous puberty attainment within cohorts of gilts facilitates entry into the breeding herd (Brooks and Smith, 1980; Aherne and Kirkwood, 1985; Evans and O'Doherty, 2001). Failure to attain puberty or exhibit regular oestrous cycles is a common problem associated with replacement gilts (Whittemore, 1996). Lucia et al. (2000) reported that nearly 20% of premature culling of females from the breeding herd occurs at parity 0, with 65% of these culls attributed to reproductive disorders or failure. Considering the high cost associated with rearing replacement gilts, it is appropriate to investigate whether current gilt management strategies can be revised to better suit current genotypes.

Repeated studies have shown that daily physical contact with a mature boar (the 'boar effect') is an effective method of stimulating precocious puberty attainment in replacement gilts (Hughes and Cole, 1976; Kirkwood and Hughes, 1979). The boar effect is mediated through the synergistic actions of visual, tactile, olfactory and auditory stimuli (Patterson et al, 2002). Age at start of boar contact is the most favourable determinant of gilt response (Hughes and Varley, 1980; Eastham et al, 1986). Further, it has previously been reported that optimal timing and synchrony of puberty attainment occurs when gilts are 160 days old at commencement of boar contact (Hughes and Cole, 1976; Kirkwood and Hughes, 1979; Eastham et al, 1986). However, the higher rates of lean growth, reduced body fat content, and greater mature weights typical of current genotypes, mean that gilts are now heavier yet leaner when puberty stimulation

and mating take place. More importantly, they are also at a lower proportion of their mature weight and therefore physiologically less mature. Consequently, a number of recent reviews have suggested that selection for improved production traits may also be a selection against early puberty attainment (Whittemore, 1996; Edwards, 1998; Evans and O'Doherty, 2001; Slevin and Wiseman, 2003).

Despite the equivocal nature of the literature, a presumptive strategy of mating gilts at their second or third oestrus has been widely adopted by the pork industry (Whittemore, 1996). Delaying first mating has been associated with higher ovulation rates (Archibong et al, 1987), decreased embryo mortality (Menino et al, 1989, Archibong et al, 1992), and a tendency toward larger first litter sizes (Young and King, 1981). In contrast, in a number of studies neither oestrus number nor age at first mating had any affect on first litter size or sow productivity over multiple parities (Brooks and Smith, 1980; Young et al, 1990a; Young et al, 1990b), and Aumaitre et al (2000) reported a decrease in annual breeding herd productivity when gilts were mated at their second or third oestrus. In addition to the contradictory results described, these studies did not separate the confounding effects of sexual and physiological age (i.e. number of oestrous cycles) from those of chronological age (i.e. days), and consequently the relative importance of these two factors remains unclear (Brooks and Smith, 1980).

The present study had two objectives; first, to investigate the effect of gilt age at start of boar contact on the rate of puberty attainment; and second, to investigate the effect of gilt age and oestrous cycle at mating on potential litter size on day 20 post conception.
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2.2 Methods

This experiment was conducted at the University of Adelaide, Roseworthy Campus, South Australia, with approval from the animal ethics committees of

Primary Industries and Resources South Australia and the University of Adelaide. The experimental design was a 3 x 2 factorial, incorporating three gilt ages at the start of boar exposure (161 days, 182 days and 203 days) and artificial insemination (AI) at either the first (pubertal) or second (first post-pubertal) oestrus, and was conducted in two blocks. Block one ran from March until July (autumn/winter) 2003; and block two ran from July until December (winter/spring) 2003.

2.2.1 Animals, housing and feeding

At approximately 126 days of age, one hundred and ninety two Large White/Landrace crossbred gilts were weighed, stratified according to weight and randomly allocated to one of six treatment groups (n = 16 gilts/treatment/block). When litter of birth for the gilts was known, littermates were allocated across treatments as evenly as possible. Gilts were housed in grower pens from selection through to their first insemination, and penned in groups of eight, with a space allowance of 1.2 square metres per gilt. The grower sheds contained no male pigs, and were fitted with adjustable side blinds. Following their first AI gilts were moved to individual stalls, where they remained until slaughter. From 126 days of age until their second AI gilts received 3 kg of a female finisher diet while from their second AI through to slaughter gilts received 2.3 kg/day of a dry sow diet (**Table 2.2.1**).

2.2.2 Boar contact and oestrous detection

From selection at 126 days of age until the commencement of boar exposure gilts had no contact with male pigs. From 140 days of age through to mating, gilts were checked daily, in their pens and without boar contact, for vulval swelling and reddening as well as signs of behavioural oestrus. Gilts exhibiting a standing reflex (**Figure 2.2.1B**) in response to the manual application of pressure to the gilt's back (the "back pressure test"; Lamberson et al., 1996)

prior to the age at which boar exposure was due to commence were removed from the trial. Boar exposure began at one of three ages; 161, 182 or 203 days of age, with gilts artificially inseminated at either the pubertal (1st) or second (2nd) oestrus. Consequently, the treatments were 161/1st, 161/2nd, 182/1st, 182/2nd, 203/1st and 203/2nd.

Table 2.2.1 Diet specifications, and timing of feeding each diet during each phase, from piglet creep to dry sow ration

Name of diet	Timing of feeding	DE pigs (MJ)	Protein (%)	Fat (%)	g available lysine/MJ
Creep	20 - 42 days old	15.7	23.8	9.7	0.9
Weaner	43 - 70 days old	14.7	23.2	4.3	0.8
Female porker	70 - 98 days old	13.8	20.1	3.5	0.7
Female grower	99 - 126 days old	13.5	18.7	3.3	0.7
Female finisher	127 days old plus	13.0	15.5	3.3	0.6
Dry sow	Mated gilts	13.0	14.0	2.9	0.5
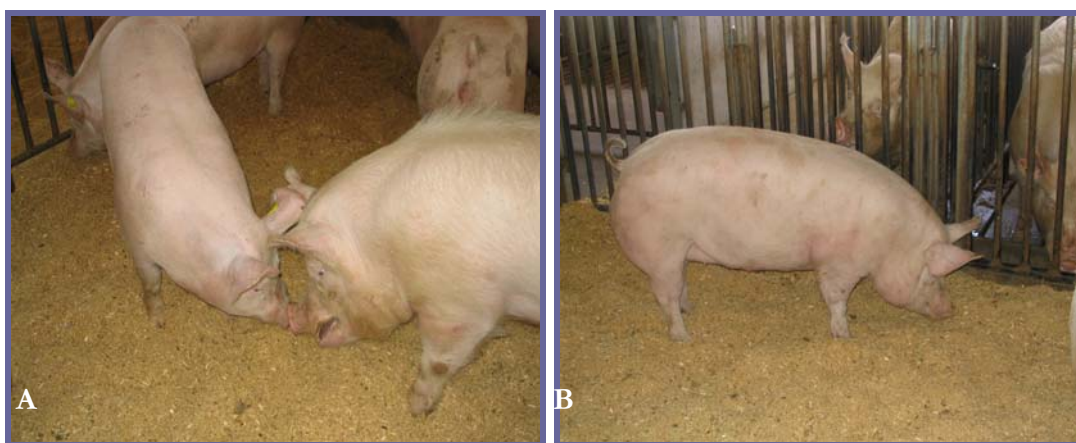
Figure 2.2.1 Supervised full contact with a vasectomized boar (A), and a gilt exhibiting a standing reflex (B)

Each group of gilts, as penned, were taken daily to a detection-mating area (DMA), where they received twenty minutes of supervised full contact with a

vasectomized boar (Figure 2.2-1 A). Boar exposures began at 09:00 hours. Three vasectomized boars, greater than 10 months of age, were used in rotation. The DMA was situated in a separate building approximately 100m from the grower rooms in which gilts were housed, and consisted of four pens measuring 3 m by 3.5 m and lined on two sides by inward facing boar pens. The attainment of puberty was defined as the first signs of a standing reflex (**Figure 2.2.1**), either in response to the back pressure test, or mounting by the boar, and the timing of the pubertal oestrus was recorded for all gilts. Days-to-puberty refers to the number of days from the start of boar exposure to the start of the pubertal oestrus. Gilts not detected in oestrus by 35 days after commencement of boar exposure were slaughtered to confirm pre-pubertal status, and those gilts with prepubertal ovaries were ascribed a nominal days-to-puberty of 40 days (Siswadi and Hughes, 1995). Oestrous cycle length was expressed as the number of days from the start of the pubertal oestrus to the start of the second (first post-pubertal) oestrus. Gilts that did not return to oestrus within 35 days of the pubertal oestrus were not investigated further, but their data on age at puberty were included in the analysis.

2.2.3 Artificial Inseminations

All gilts received two artificial inseminations (AIs), once at detection of oestrus and again 24 hours later. All AIs took place in the DMA, with fence-line contact with a boar during the procedure. Inseminations were performed as per standard industry practice using disposable spirette catheters, with each insemination consisting of an 80 ml dose of fresh, extended semen (3×10^9 spermatozoa per inseminate; $\leq$ 4 days old). Semen used for this experiment was purchased from a commercial artificial insemination collection centre (SABOR Pty. Ltd, Clare, South Australia).

2.2.4 Animal Measurements

2.2.4.1 Gilt liveweight and backfat measurements

Gilts were weighed and P2 backfat depth was measured at 126, 161, 182, and 203 days of age, and also at the start of the pubertal oestrus, at mating and at slaughter. P2 backfat depth was measured over the last rib, 65 mm from the vertebrae, by an experienced operator using a hand-held digital, backfat indicator (Renco Lean-meter ®).

2.2.4.2 Reproductive parameters: ovulation rate and embryo measurements

Gilts were slaughtered at a commercial abattoir 22.8 $\pm$ 0.4 days after their first mating (mean $\pm$ S.E.M.) and the reproductive tract of each gilt recovered. The ovaries were weighed and where necessary dissected to determine the number of corpora lutea. The number of corpora lutea was taken to represent the number of oocytes ovulated at the oestrus of AI. The uterus was trimmed of mesentery and dissected, the number of viable and non-viable embryos recorded, embryo crown-to-rump length measured, and the distance between embryos was also measured. Embryos were described as viable or non-viable based on their gross morphology and crown-to-rump length; embryos were classified as non-viable if their crown-to-rump length was more than two standard deviations less than the mean for that gilt (Virolainen et al., 2004). The total number of viable embryos observed in both uterine horns was expressed as total embryo number. Embryo survival was calculated based on the total number of viable embryos, and expressed as a percentage of the number of corpora lutea observed in both ovaries.

2.2.4.3 Statistical Analysis

Values in the text are expressed as mean $\pm$ standard error. A one-way analysis of variance (ANOVA) with blocks, was used to study the effects of gilt age at start of boar exposure on days-to-puberty, and age, liveweight and P2 backfat depth at puberty. A two-way ANOVA, with blocks, was used to examine the effects of age at start of boar exposure and mating oestrus on age, liveweight and P2 backfat depth at mating, as well as ovulation rate, embryo number and embryo survival. Between treatment differences were examined using least significant difference. Correlations between variates were determined using general linear regression. All analyses were performed using Genstat, 6th Edition (Committee of the Statistics Department, Rothamsted Experimental Station, Harpenden).

2.3   Results

2.3.1   General Results

Of the 192 gilts allocated to the experiment, a total of 24 gilts were removed from the analyses. Sixteen gilts were removed as a result of ill health or locomotive problems, and eight gilts were removed for exhibiting a standing reflex prior to the commencement of boar exposure (1, 1, 2 and 4 gilts in the 182/1st, 182/2nd, 203/1st and 203/2nd treatment groups, respectively). Consequently, the data presented below relates to 30, 30, 30, 24, 29, and 25 gilts from treatment groups 161/1st, 161/2nd, 182/1st, 182/2nd, 203/1st, and 203/2nd, respectively.

As expected, gilt liveweight at start of boar exposure increased with age at start of boar exposure; gilts weighed 99.4 $\pm$ 1.2, 114.9 $\pm$ 1.3, and 132.5 $\pm$ 1.5 kg at 161, 182 and 203 days of age (P < 0.01). P2 backfat at start of boar exposure was the same at 161 and 182 days of age, but was significantly higher at 203

days of age (13.4 ± 0.4 and 13.8 ± 0.3 mm versus 15.6 ± 0.4 mm at 161, 182 and 203 days respectively (P < 0.01).

2.3.2 Puberty attainment

Mean days to puberty were significantly higher (P < 0.01) when boar contact commenced at 161 days of age compared to either 182 or 203 days of age: 18.9 ± 1.5 days versus 10.6 ± 1.2 and 8.3 ± 0.9 days respectively (**Table 2.3.1**). Gilt age at puberty attainment increased significantly (P < 0.01) as age at start of boar contact increased (**Table 2.3.1**); gilts were 179.5 ± 1.6, 191.9 ± 1.2, and 210.6 ± 0.9 days old when boar exposure commenced at 161, 182 and 203 days of age respectively.

Table 2.3.1 Days from start of boar exposure to puberty attainment, age, liveweight and P2 backfat at puberty for gilts that started boar exposure at either 161, 182 or 203 days of age

Age at start of boar exposure	Days to puberty$_1$*	Age at puberty* (days)	Liveweight at puberty (kg)	P2 backfat at puberty (mm)
161 days of age (n = 60)	18.9[a] ± 1.56	179.5[a] ± 1.56	109.2[a] ± 1.42	15.1[ab] ± 0.48
182 days of age (n = 54)	10.6[b] ± 1.20	191.9[b] ± 1.23	122.1[b] ± 1.62	14.1[a] ± 0.31
203 days of age (n = 54)	8.3[b] ± 0.86	210.6[c] ± 0.90	138.8[c] ± 1.68	16.4[b] ± 0.43

[a,b,c] Means in column are significantly different (P < 0.01)

* Gilts not attaining puberty by day 35 of boar exposure were ascribed a nominal days-to-puberty of 40 days

[1] Interval from initial exposure to a vasectomized boar until exhibition of a standing reflex

The effect of age at start of boar exposure on the timing and synchrony of puberty attainment is described in **Figure 2.3.1**. The pattern of puberty onset

was similar when boar exposure commenced at 182 and 203 days. However, gilts commencing boar contact at 161 days of age exhibited greater variation in the timing of the pubertal oestrus. A significantly smaller percentage ($P < 0.01$) of gilts starting boar exposure at 161 days of age had reached their pubertal oestrus by day 10 and by day 20 of boar exposure compared to gilts commencing boar exposure at 182 and 203 days of age; 24%, 67% and 70% of gilts attained puberty by day 10, and 70%, 81% and 93% of gilts attaining puberty by day 20 when boar exposure commenced at 161, 182 and 203 days of age, respectively (**Figure 2.3.1**). The number of gilts that failed to attain puberty within the 35 days of boar exposure was 11, 1 and 0 when boar exposure commenced at 161, 182 and 203 days of age, respectively. At slaughter, these animals were deemed to be pre-pubertal based on the absence of corpora lutea and corpora albicantia. One gilt in the 203/2nd treatment group failed to return to oestrus within 35 days of her pubertal oestrus.

2.3.3 Potential litter size

The effect of age at start of boar exposure and mating oestrus on gilt age, liveweight and P2 backfat at mating is described in **Table 2.3.2**. There was no interaction between gilt age at start of boar exposure and mating oestrus on ovulation rate, embryo number or embryo survival. The data presented in **Table 2.3.3** indicate ovulation rate, embryo number and embryo survival were not significantly affected by treatment ($P > 0.05$). However, there was a tendency for both ovulation rate and embryo number to increase when gilts where mated at the second oestrus (by 0.6 ova and 1.0 embryo; P =0.095 and 0.058, respectively). No relationship between age at mating and either ovulation rate or embryo number was observed.

For gilts commencing boar exposure at 161 days of age, there was a positive, significant (P < 0.05) correlation between gilt liveweight at entry into the

breeding herd (126 days of age) and ovulation rate ($r^2 = 0.22$) and embryo number ($r^2 = 0.11$). Similarly, ovulation rate and embryo number were positively correlated with liveweight at mating: $r^2 = 0.21$ and 0.13, respectively ($P < 0.05$). No significant correlations between liveweight and either ovulation rate or embryo number were observed with gilts that commenced boar exposure at 182 or 203 days of age ($P > 0.05$).
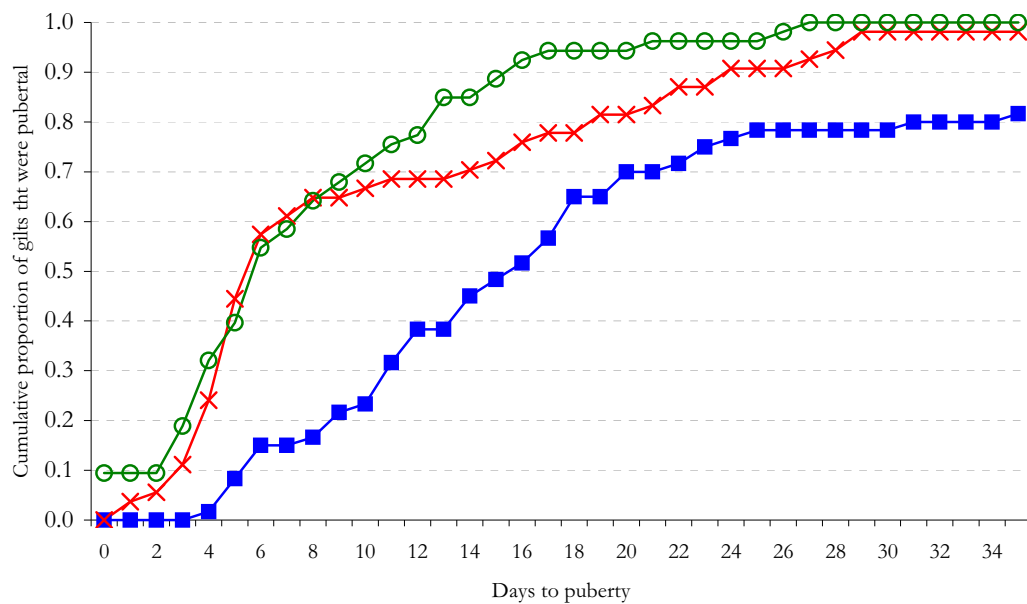
Figure 2.3.1 Cumulative proportion of gilts attaining puberty in response to daily contact with a vasectomized boar when boar exposure commenced at either 161, 182 or 203 days of age. Closed squares (■) represent 161 days of age at start of boar contact, crosses (x) 182 days of age at start of boar contact, and open circles (○) 203 days of age at start of boar contact.

Table 2.3.2 Age, liveweight and P2 backfat at mating for gilts that started boar exposure at either 161, 182 or 203 days of age and were mated at either the first (pubertal) or second oestrus

Age at start of boar exposure	Age at mating (days)			Liveweight at mating (kg)			P2 backfat at mating (mm)		
	Mating oestrus		Pooled across mating oestrus	Mating oestrus		Pooled across mating oestrus	Mating oestrus		Pooled across mating oestrus
	1st	2nd		1st	2nd		1st	2nd	
161 days	174.2	195.7	185.0[a]	104.3	127.0	115.6[a]	15.3	15.6	15.5[ab]
182 days	189.3	215.5	202.3[b]	118.8	143.2	131.0[b]	13.9	16.0	15.0[a]
203 days	209.1	232.7	220.9[c]	138.3	154.5	146.4[c]	16.5	16.9	16.7[b]
Pooled across age at start of boar exposure	190.9[a]	214.6[b]		120.4[a]	141.6[b]		15.2	16.2	
Pooled S.E.M			1.3			2.5			0.5

a, b, c means in column are significantly different (P<0.01); a,b means in row are significantly different (P<0.01)

Table 2.3.3 Ovulation rate, embryo number, and embryo survival at approximately day 20 post-mating for gilts starting boar contact at either 161, 182 or 203 days of age and first mated at either the first (pubertal) or second oestrus

Age at start of boar exposure	Ovulation rate			Number of embryos			Embryo survival (%)		
	Mating oestrus		Pooled across mating oestrus	Mating oestrus		Pooled across mating oestrus	Mating oestrus		Pooled across mating oestrus
	1st	2nd		1st	2nd		1st	2nd	
161 days	14.8	15.5	15.1	11.8	11.8	11.8	80.1	76.6	78.4
182 days	14.6	15.7	15.1	10.7	12.0	11.3	72.5	76.0	74.3
203 days	15.3	15.5	15.4	11.5	13.2	12.4	76.7	86.7	81.7
Pooled across age at start of boar exposure	14.9	15.5		11.3	12.3		76.5	79.8	
Pooled S.E.M			0.5			0.7			3.6

2.4 Discussion

Overall, the current data indicate that puberty is attained more rapidly, and with greater synchrony within cohorts of gilts when first boar exposure commences at an age of at least 182 days. This is an important result, as it contradicts previous reports in the literature indicating that optimal puberty response to boar stimulation occurs in 160-day old gilts, and after 160 days of age gilts are equally responsive to the stimuli of boars (Hughes and Cole, 1976; Eastham et al, 1986, Paterson, 1989). The present data also support previous reports that regular, daily boar contact is an effective method of stimulating precocious puberty attainment in replacement gilts (Hughes and Cole, 1976; Patterson et al, 2002a), and that gilt age at first boar exposure is a critical determinant of the timing and frequency of the pubertal response (Hughes and Cole, 1976; Kirkwood and Hughes, 1979; Eastham et al, 1986).

The gilts used in the present study were approximately 40% heavier at any given age than their counterparts used in studies conducted about two decades earlier (eg Kirkwood and Hughes, 1979; Eastham et al, 1986), supporting previous suggestions that modern gilts are heavier at first breeding than their counterparts of 20 – 30 years ago (Hughes and Varley, 2003). The increased lean growth rates and reduced body fat content typical of current genotypes have been associated with a considerable increase in mature bodyweight (Edwards, 1998). Consequently, it has been suggested that when measured on a chronological timescale, the peak of the protein growth curve will occur later (Slevin and Wiseman, 2003), causing the shift towards increased lipid accretion to also occur at an older age. It is therefore likely that the gilts used in the present study were not only at a lower proportion of their mature weight at a given age, but also had a very different body composition compared to the smaller, fatter genotypes of 20 – 30 years ago. Kirkwood and Aherne (1985) proposed that minimum threshold levels for liveweight and body tissue reserves act in a permissive manner by allowing rather than actively triggering,

the onset of puberty. Although Foxcroft et al. (1996) suggested that puberty occurs long after these permissive levels have been reached, it could be proposed that current genotypes achieve these minimum threshold levels for body tissue reserves at an older age. This may result in a chronological delay in the integration of the components of the hypothalamic-pituitary-ovarian axis that govern puberty attainment.

Faster gilt response to boar stimulation is indicative of a more developed hypothalamic-pituitary-ovarian axis (Kirkwood and Hughes, 1979). Earlier studies concluded that the components of the reproductive mechanism involved in puberty onset were sufficiently integrated by 160 days of age to allow a rapid and synchronous pubertal response (Hughes and Cole, 1976; Eastham et al, 1986; Paterson, 1989). However, the current study indicates that physiological maturation continues between the ages of 161 and 182 days of age. Furthermore, the increased synchrony of puberty attainment observed in gilts commencing boar exposure at 182 days of age suggests less variability in sexual maturity between contemporary females compared to gilts that started boar exposure at 161 days of age. Consequently, the current data indicate physiological maturation occurs later than with previous genotypes, and is consistent with recent suggestions that current, lean genotypes are later maturing (Whittemore, 1996; Evans and O'Doherty, 2001).

The second objective of the current study was to compare the effects of first mating gilts at the pubertal or second oestrus on potential litter size, namely ovulation rate and embryo number at approximately day 20 of gestation. It is evident, as suggested by Brooks and Smith (1980), that the relative importance of chronological age and sexual age (i.e. number of oestrous cycles experienced) of the gilt as determinants of first litter size remains unclear. It has previously been reported that ovulation rate and first litter size increase with advancing sexual age (eg Archibong et al, 1992), whereas other studies suggest that chronological age at first mating may have a greater effect on reproductive

performance than does oestrus number (eg Brooks and Smith 1980; Paterson and Lindsey 1980). In the present study, there was a tendency for potential litter size to increase when gilts were mated at the second oestrus. A numerical, but not significant, increase in both ovulation rate (0.6 ova) and embryo number (1.0 embryo) was associated with delaying mating until the second oestrus (P = 0.095 and 0.058, respectively). The current data therefore supports the finding of Young et al. (1990b) that first litter size is marginally, but not significantly, improved when first mating is delayed until the second oestrus. However, compared to reproductive performance at the third oestrus, the pubertal oestrus has been associated with inferior oocyte quality (Herrick et al., 2003) and higher incidences of abnormal embryo development (Menino et al., 1989; Archibong et al., 1992). Recently, Bagg et al. (2004) also concluded that improved oocyte competence may depend on the completion of a number of oestrous cycles. It is therefore possible that delaying first mating until the third oestrus may have further improved embryo number.

Paterson and Lindsay (1980) suggested that stimulating gilts to attain puberty at a young age might be associated with low reproductive performance. This was not supported in the present study, as no relationship was found between potential litter size and the age of gilts at start of boar exposure, puberty attainment or mating. In fact ovulation rates observed in this study were high for all groups, with gilts shedding approximately three more ova than reported in earlier studies (eg Paterson and Lindsay, 1980; Brooks and Smith, 1980; Archibong et al, 1992).

The current data indicated a faster gilt response to boar contact when first boar exposure occurred at 182 or 203 days of age as opposed to at 161 days of age, suggesting that the hypothalamic-pituitary-ovarian axis of the younger gilts was less mature. Further the reproductive performance (eg embryo number and/or ovulation rate) of the youngest gilts at mating, namely those that commenced boar exposure at 161 days of age was positively related to their liveweight at

the time of selection for the study as well as at mating. This would suggest that the reproductive performance of young and therefore less sexually mature gilts might be more responsive to differences in nutritional signals than older gilts. Nutritional status has a considerable influence on reproductive development and performance in gilts, affecting all levels of the hypothalamic pituitary-ovarian axis and resulting in changes in ovarian follicle dynamics, ovulation rate, oocyte quality and embryo number (Hunter et al, 2004; Webb et al, 2004). Importantly, Prunier et al. (1993) demonstrated that the pubertal response was improved, and that circulating gonadotrophin and metabolic hormone levels were altered, in well-fed compared to restrictively fed gilts. Consequently, the current data and previous studies collectively suggest that if management practices continue to promote early-breeding strategies, then improving gilt reproductive performance may require an increased emphasis on pre-pubertal nutrition and growth characteristics to enhance sexual development and maturation.

In conclusion, the current data confirm that daily contact with a vasectomized boar is an effective method of inducing precocious puberty attainment in gilts. Further, the present study suggests that optimal response to boar stimulation occurs when gilts are 182 days of age or older. This is three weeks later than previously reported in the literature, and may be indicative of a delay in sexual maturity, probably due to the increased lean growth potential and larger mature size of current genotypes. In addition, the current data demonstrated a numerical, but not significant, improvement in potential litter size when first mating was delayed until the second oestrus. If gilts are to continue to be mated at a young age, then more emphasis in research is required to develop pre-pubertal nutritional strategies that promote earlier sexual development by advancing the maturation of the hypothalamic-pituitary-ovarian axis.

Chapter 3   Impact of prepubertal growth rate on ovarian follicle populations and oocyte competence of 161- and 175-day old, non-cycling gilts

3.1 Introduction

Reproductive processes in the female pig are influenced by the availability of sufficient metabolic fuels (Schneider, 2004), and hence appropriate nutritional management of the pre-pubertal gilt facilitates gilt entry into the breeding herd and maximises subsequent reproductive performance (Evans and O'Doherty, 2001). Recent studies suggest that within genotypes, the rate of sexual maturation, and hence puberty attainment, is sensitive to altered nutritional status during the prepubertal period (Beltranena et al., 1991; Prunier et al., 1993). Higher rates of prepubertal growth accelerate reproductive development of the gilt (Gaughan et al., 1997), whilst restricting pre-pubertal feed intake and liveweight gain can significantly delay puberty onset in replacement gilts (Friend et al., 1981; Van Lunen et al., 1987; Beltranena et al., 1993). While information regarding the influence of growth rate and nutrition on ovarian development in pre-pubertal gilts is limited, data derived from weaned sows (Quesnel et al., 1998) provides evidence for a causal relationship between nutrition and follicular development and the timing of oestrus. Studies involving nutritionally restricted pre-pubertal rats and ewe lambs indicate that delayed puberty attainment is associated with a suppressed pattern of tonic Luteinising Hormone (LH) release and retarded ovarian follicle growth (Bronson, 1986; Schillo, 1992).

Recent evidence also indicates that reducing the pre-mating feed intake of cycling gilts to maintenance levels alters the physiological development of recruited and pre-ovulatory follicles, impairing the developmental competence of the oocytes shed at ovulation (Ferguson et al., 2003). This response appears to be mediated, at least in part, by alterations in the intra-follicular environment, such as alterations in follicular fluid steroid concentrations (Ding and Foxcroft, 1992; Zak et al., 1997; Ferguson et al., 2003; Grupen et al., 2003). An association between nutritionally induced alterations in ovarian function and

circulating, plasma concentrations of both IGF-I and oestradiol has also been reported (Ferguson et al., 2003). Although the negative impacts of maintenance feeding during the final stages of follicle growth on oocyte quality are convincing, the effects on oocyte developmental competence of more moderate restriction of feed intake which allows animal growth to occur but is less than ad libitum have not been established. Consequently, the objective of the current study was to test two hypotheses: (i) that moderate restriction of pre-and peri-pubertal liveweight gain impairs ovarian development and oocyte quality in peri-pubertal gilts; (ii) that long-term nutritional restriction during the pre-pubertal period will result in carry-over effects on ovarian and intra-follicular characteristics, regardless of nutritional intake during the peri-pubertal period. This was achieved by studying the effects of restrictive feeding during two distinct periods within the prepubertal period (70 to 161 days of age and 161 to 175 days of age) on the dynamics of the antral follicle population and oocyte developmental competence.

3.2   Materials and Methods

This study was conducted at the University of Adelaide's Roseworthy Campus. Animal trials were conducted with approval from the Animal Ethics Committees of Primary Industries and Resources South Australia and the University of Adelaide. The experimental design was a 2 x 2 factorial, incorporating two target liveweights at 161 days of age (LIGHT versus HEAVY) and two target rates of daily liveweight gain between 161 and 175 days of age (LOW versus HIGH) with ovaries collected at two gilt ages (161 or 175 days of age). This study was conducted in two blocks. Block one was conducted from the 30th March 2004 until 13th July 2004 (autumn/winter) (n = 24 gilts/treatment group) and block two ran from 7th December 2004 until 23rd March 2005 (summer/autumn) (n = 24 gilts/treatment group). All the oocytes from 161-day old LIGHT and HEAVY gilts from block one used to assess nuclear maturation

rates were accidentally destroyed prior to assessment, and hence the treatments were repeated with 12 gilts per group in order to collect 'replacement' oocytes at day 161. This repeat block was carried out between the 18th August 2004 and 15th November 2004 (spring), and will be referred to as block 1R.

3.2.1 Animals

At approximately 70 days of age (selection), 120 Large White/Landrace crossbred gilts were weighed, stratified according to weight and allocated to either the LIGHT or HEAVY feeding treatment (n = 60 gilts/treatment). There were 24 gilts per treatment in blocks one and two; and 12 gilts per treatment in the 'repeat' block (block 1R) that was conducted to replace lost samples. Mean liveweight at selection was 27.6 ± 0.4 kg. Gilts in the LIGHT treatment group were fed to attain a liveweight of 70 kg at 161 days of age, whilst those in the HEAVY treatment group were fed to attain a liveweight of 100 kg. When litter of birth for the gilts was known, litter sisters were allocated across treatments as evenly as possible.

In all blocks, gilts were weighed at approximately 161 days of age and within treatment (LIGHT or HEAVY) were stratified according to weight and allocated to one of three groups (Table 3.2.1). In blocks one and two, one third of gilts in each treatment were transported to the abattoir and reproductive tracts collected for the 161 day-old measurements (n = 8 gilts/treatment/block). The remaining 64 gilts were allocated to gain liveweight at a rate of either 0.5 kg per day (LOW) or 1.0 kg per day (HIGH) between 161 and 175 days of age (n = 8 gilts/treatment/block). In block 1R, reproductive tracts were collected from all gilts at 161 days of age (n = 12 gilts/treatment).

3.2.2 Housing and Feeding

From selection at 70 days of age until 126 days of age, gilts were housed in grower rooms (**Figure 3.2.1**) with a space allowance of 1.2 square metres per gilt. At 126 days of age, gilts were moved to finisher rooms, where they were housed in their original groups with a space allowance of 1.3 square metres per gilt, and remained until transported to an abattoir for slaughter. Both grower and finisher rooms were fitted with adjustable side-blinds, and gilts were exposed to a natural lighting regime. From selection until slaughter, gilts had no contact with male pigs.

Table 3.2.1 The number of gilts in each treatment group and experimental block at each measurement point (161 or 175 days of age)

	Number of gilts per block		
	Block 1	Block 1R	Block 2
Day 161 reproductive tract collection			
LIGHT	$8^{(1)}$	$12^{(2)}$	8
HEAVY	$8^{(1)}$	$12^{(2)}$	8
Day 175 reproductive tract collection			
LIGHT-LOW	8	-	8
LIGHT-HIGH	8	-	8
HEAVY-LOW	8	-	8
HEAVY-HIGH	8	-	8

[1] Oocytes for nuclear maturation assessment lost
[2] Replacement samples for those lost from block 1A

At all times, gilts received the standard diets used by the Roseworthy piggery, with the specifications of these diets changing according to gilt age (**Table**

2.2.1). From weaning until selection at 70 days of age gilts had ad libitum access to feed. Following selection, gilts were fed once daily, between 8 and 9 am, with the ration broadcast on the floor of the pen. The daily feed requirements to achieve the two target liveweights at 161 days of age (70 kg; LIGHT versus 100 kg; HEAVY) and the two target rates of liveweight gain between 161 and 175 days of age (0.5 kg/day; LOW versus 1.0 kg/day; HIGH) were from "Feeding Standards for Australian Livestock: Pigs" (SCA; Tables 1.9 – 1.14; p151-156). Using these tables, liveweight gain per MJ DE intake was calculated for gilts weighing from 20 to 150 kg, and the amount of feed required to attain the target growth rates for each treatment group was then calculated. Between 70 and 161 days of age, mean daily feeding levels were 2.5 or 3.3 times maintenance for LIGHT and HEAVY gilts, whilst between 161 and 175 days of age, LOW and HIGH gilts received 2.2 or 3.0 times their maintenance requirements. As gilts were group fed, feeding level per pen was calculated based on the mean gilt weight per treatment multiplied by the number of gilts per pen. Gilts were weighed weekly, and the daily feed intake required to attain the target rates of liveweight gain was adjusted based on the mean liveweight per treatment.

Figure 3.2.1 Grower room, pens with a space allowance of 1.2m² per gilt

3.2.3 Animal measurements

3.2.3.1 Gilt liveweight, backfat and maximum muscle depth measurements

Gilts were weighed prior to being fed, and P2 backfat and maximum eye muscle depth (MMD) were measured over the last rib 65 mm down from the vertebrae by an experienced commercial operator using a 3.5 MHz linear probe (Ausonics Impact). Gilts were initially weighed at 70 days of age and at weekly intervals thereafter until slaughter at either 161 or 175 days of age. P2 backfat and (MMD) was measured at approximately 90, 155 and 170 days of age.

3.2.3.2 Collection of blood samples

Preprandial blood samples were collected at approximately 160 and 173 days of age. Blood samples were collected by jugular venipuncture into 9 ml EDTA-coated collection tubes (Vacuette®, Griener Labortechnik, Austria). Blood samples were maintained in ice, and within an hour of collection were centrifuged for fifteen minutes at 3000 rpm. Plasma was stored in 2 ml tubes at $-20°C$ for later analysis.

3.2.3.3 Collection of ovaries and follicle cells

All gilts were slaughtered at a commercial abattoir, and the reproductive tract of each animal was recovered within 20 minutes of exsanguination. Ovaries were removed from the rest of the reproductive tract, placed in individual bags containing saline supplemented with penicillin G (100 IU/ml) and streptomycin sulphate (100 mg/ml), and maintained at a temperature of $35 - 37$ °C during transport to the laboratory.

Upon arrival at the laboratory, ovaries were washed thoroughly in pre-warmed (37 °C) saline, and maintained at 37 °C during processing. Ovaries from each gilt were weighed, and antral follicles were aspirated according to three size categories: 1 – 2.9 mm in diameter (small); 3 – 6 mm in diameter (medium); and > 6 mm in diameter (large), and pooled for each size class and treatment. The time from ovary collection to follicle aspiration was 2 – 3 hours, and follicles were aspirated using an 18-gauge needle inserted in a 9 ml vacutainer tube, through which a constant suction (50 ml/min) was applied. During aspiration, the number of follicles within each of the three size categories was recorded for individual gilts. All aspirates were allowed to sediment for approximately thirty minutes at 37 °C, after which the sediment was transferred to a petri dish. Using a dissecting microscope, cumulus oocyte complexes (COCs) with $\geq$ 2 uniform layers of compact cumulus cells and containing oocytes with an even cytoplasm, were recovered for in-vitro maturation (IVM) (described in Section 3.1.4.2). For all pooled samples the aspirant remaining in the vacutainer tube was centrifuged for 20 minutes at 3000 rpm, and the resulting supernatant (follicular fluid) stored at –20 °C for later analysis.

3.2.3.4 Collection of uterine horns and liver

Following the removal of the ovaries, the uterus from each gilt was placed in an individually-labelled bag, and transported to the laboratory on ice. Upon arrival at the laboratory, the uterine body/horns were trimmed of mesentery and weighed. In block two only, individual liver weights were also measured.

3.2.4 Oocyte developmental competence

3.2.4.1 Media and chemicals

All chemicals and reagents were purchased from Sigma-Aldrich (Australia), unless otherwise specified. The basic oocyte maturation media (bOMM) consisted of Medium 199 (Gibco, Australia) (with Earle's salts, L-glutamine, and 2,200 mg/ml sodium bicarbonate) supplemented with 0.1 mg/ml sodium pyruvate, 0.076 mg/ml penicillin-G, 0.05 mg/ml streptomycin sulphate, 1 μl/ml cysteamine, and 1 mg/ml bovine serum albumin (BSA). The bOMM was filtered through a sterile 0.22 μm pore filter (Millipore, Australia), and allowed to equilibrate overnight at 38.5°C in a humidified atmosphere of 6% CO_2 in air. On the morning of oocyte collection, 1 μl/ml insulin (8.2 mg/ml), 1 μl/ml epidermal growth factor (10 mg/ml), 1 μl/ml follicle stimulating hormone (1.5 mg/ml: Folltropin-V; Vetrepharm, Camperdown, NSW, Australia) and 1 μl/ml luteinizing hormone (0.2 mg/ml: LH; Lutropin-V; Vetrepharm, London, Ontario, Canada) were added to the bOMM.

Hepes-buffered tissue culture medium -199 plus 0.1% bovine serum albumin (BSA) (HTCM /BSA) was filtered through a sterile 0.22 μm pore filter. Droplets for washing COCs were prepared on the morning of oocyte collection and allowed to warm under mineral oil for ≥ 3hours at 38.5°C in a humidified atmosphere of 6 % CO_2 in air. Embryo-tested mineral oil was used to cover all wash and in-vitro maturation (IVM) droplets.

Prior to parthenogenetic activation oocytes were denuded and washed in HTCM/BSA, and then held in 1ml droplets of tyrode-albumin-lactate-pyruvate-polyvinyl-alcohol (TALP-PVA: **Table 3.2.2**.) medium, supplemented with 1.85 μl/ml of 10mM Na-lactate, 0.012 mg/ml of 0.10 mM Na-pyruvate, 0.796 mg/ml of 2.0 mM caffeine-sodium benzoate, 0.656 mg/ml of 3.0 mM Ca-lactate$_2$, and

3.0 mg/ml BSA. Oocytes were activated in 5 μM ionomycin in TALP-PVA, followed by North Carolina State University media 23 (NCSU-23)/6-dimethylaminopurine (6DMAP)/cytoclasinB, which consisted of 3.5 ml NCSU-23 (**Table 3.2.2**); supplemented with 0.146 mg/ml of 1.0mM glutamine, 0.875 mg/ml of 7.0 mM taurine, 0.545 mg/ml of 5.0 hypotaurine and 4 mg/ml BSA), 1.12 mg of 2.0 mM 6DMAP, and 3.5 μl of cytoclasinB). The final culture medium was NCSU-23.

Table 3.2.2 Components of stock solutions

Component	(mg/ml)	
	TALP-PVA[1]	NCSU23 medium[2]
Poly-vinyl alcohol (PVA)	1.0	6.354
Penicillin-G	0.075	0.075
Streptomycin-sulphate	0.050	0.050
Phenol red	0.002	
D-glucose	4.0	1.000
NaCl	6.664	
KCl	0.240	0.356
$CaCl_2.2H_2O$	0.684	0.250
$MgCl_2.6H_2O$	0.1	
$NaH_2PO_4.2H_2O$	0.054	
$NaHCO_3$	2.1	2.106
$MgSO_4.7H_2O$	0.293	
KH_2PO_4	0.162	

[1] Tyrode-albumin-lactate-pyruvate-polyvinyl-alcohol

3.2.4.2 In vitro maturation of oocytes

Recovered COCs were washed three times in 600 μl droplets of HTCM/BSA under mineral oil, once in a 600 μl droplet of OMM, and then transferred to 500

μl of OMM (50 COCs per well) in a 4 well NUNC dish. COC's were incubated for 44 – 46 hours at 38.5°C in a humidified atmosphere of 6 % CO_2 in air. Due to a lack of numbers in the large follicle size category, only COC's from the small and medium sized follicles classes were selected for *in vitro* maturation.

3.2.4.3 Parthenogenetic activation

At the end of IVM, all COC's were transferred to 100 μl droplets of HTCM /BSA, treated with 4 μl hyaluronidase (25 mg/ml) for 1 minute, and gently aspirated with a small-bore glass pipette to remove the cumulus cells. In block one and block 1R, *in vitro* matured oocytes were randomly selected for activation from each group; however, due to insufficient numbers, none of the matured oocytes from the LIGHT – medium group were selected for activation. In block two, oocytes were selected based on the presence of a polar body following visual assessment using a stereomicroscope. Only *in vitro* matured oocytes from the medium-sized follicles obtained from 175-day old LIGHT-LOW, LIGHT-HIGH, HEAVY-LOW and HEAVY-HIGH gilts were selected for activation. The number of in-vitro matured oocytes selected for activation is described in **Table 3.2.3**.

Selected oocytes were incubated in 5 μM ionomycin in TALP-PVA for precisely 5 minutes (protected from light, at 37°C). The oocytes were then washed 3 times in NCSU-23/6DMAP/cyctoclasinB, and incubated for 3 hours in 50 μl droplets of NCSU/6DMAP/cytoclasinB covered with mineral oil, at 38.5°C in a humidified atmosphere of 6% CO_2 in air. At the end of the 3 hour incubation oocytes were transferred through 2 washes of NCSU-23, to a final 50 μl incubation droplet of NCSU-23 culture medium (10 - 15 oocytes per droplet), covered with mineral oil, and incubated at 38.5°C in a humidified atmosphere of 6% CO_2. On day 2 of culture, embryo cleavage was assessed, and on day 5 of culture 10% foetal calf serum (FCS) was added to the culture droplets. On day 7

of culture the total number of embryos that cleaved and their developmental stage was recorded for all groups. Activation data are expressed as the proportion of activated oocytes that cleaved, and the proportion of cleaved activated oocytes that developed beyond the 2-cell stage and to the blastocyst stage.

Table 3.2.3 Number of *in vitro* matured oocytes from each treatment group that were selected for activation or fixed to assess nuclear maturation

Treatment	Follicle size	Number of in vitro matured oocytes that were activated			Number of in vitro matured oocytes that were fixed and assessed for stage of nuclear maturation		
		BL. 1[1]	BL. 1R[1]	BL. 2[1]	BL. 1[1]	BL. 1R[1]	BL. 2[1]$_1$
161 days of age							
LIGHT	Small	30	61	56	-	25	108
LIGHT	Medium	-	46	52	-	46	56
HEAVY	Small	40	58	50	-	42	84
HEAVY	Medium	45	31	48	-	53	55
175 days of age							
LIGHT – LOW	Medium	28	-	38	14	-	17
LIGHT – HIGH	Medium	36	-	55	24	-	35
HEAVY – LOW	Medium	41	-	63	25	-	26
HEAVY - HIGH	Medium	46	-	48	50	-	12

[1] BL. 1, BL. 1R and BL. 2 refers to block one, the 'repeat' block and block 2, respectively
$_1$ Includes oocytes that possessed a polar body and were selected for activation

3.2.4.4 Oocyte nuclear maturation

At the end of IVM, all denuded oocytes that were not selected for parthenogenetic activation were fixed in 1:3 absolute ethanol: acetic acid for ≥ 48 hours at room temperature. Fixed oocytes were mounted on a slide, stained with orcein (1% orcein in 45% acetic acid) for 60 minutes, and destained (glycerol: acetic acid: water, 1:1:3). Chromatin configuration of the oocytes was examined under a phase-contrast microscope at 400x magnification. Meiotic stage of oocytes was classified as described by Ye et al. (2002): germinal vesicle I (GVI); germinal vesicle breakdown (GVBD), including germinal vesicle II (GVII), germinal vesicle III (GVIII), germinal vesicle IV (GVIV), and diakinesis (D); metaphase I (MI) (which included anaphase I (AI)); and metaphase II (MII) (which included telophase I (TI)). The number of oocytes from each group that were fixed is presented in **Table 3.2.3.** In the first block, all the oocytes from LIGHT and HEAVY gilts that were not selected for activation were accidentally destroyed; hence no data was obtained from these samples. In block two, the number of *in vitro* matured oocytes that possessed a polar body, and were selected for activation, were also included in the total number of oocytes reaching MII. Oocyte IVM data is expressed as the proportion of oocytes in each meiotic stage following IVM to the total number of oocytes assessed.

3.2.5 Hormone assays

All steroid hormone assays were conducted at a commercial diagnostics laboratory (Repromed), using RIA kits (Diagnostic System Laboratories, Webster, TX) in accordance with the manufacturers' instructions. The validity of the assays was assessed based on the intra- and inter-assay coefficients of variations of the controls (20 per run of 60 samples). Assays were only accepted as valid when the intra- and inter-assay coefficients of variation of the controls were within the values described in the manufacturers' instructions.

The intra- and inter-assay coefficients of variation were 6.4% and 2.4%, respectively, for the progesterone kit; 4.3% and 6.3%, respectively, for the androstenedione kit; 7.7% and 10.5%, respectively, for the testosterone kit; and 5.3% and 8.1%, respectively, for the oestradiol kit. The minimum detection limit for the progesterone, testosterone, androstenedione and oestradiol assays was 0.318 nmol/l, 0.0636 nmol/l, 0.159 nmol/l, and 0.01739 nmol/l, respectively. For each steroid assayed all samples were analysed in singleton.

Insulin-like growth factor-I (IGF-I) assays were performed at the School of Animal Biology, The University of Western Australia. Plasma concentrations of IGF-1 were measured in duplicate by double-antibody RIA described by Gluckman et al. (1983) after 100 μl of plasma was cleared of binding proteins using the acid-ethanol cryoprecipitation method described by Brier et al. (1991). In block 1, the IGF-1 assay included six replicates of two control samples containing 72 ng/ml and 4.7 ng/ml and which were used to estimate the intra-assay coefficients of variation of 5.7% and 7.4%. In blocks 2 and 3, the IGF-1 assay included six replicates of two control samples containing 71.5 ng/ml and 5.6 ng/ml and which were used to estimate the intra-assay coefficients of variation of 5.7% and 9.0%.

3.2.5.1 Statistical analysis

Data are expressed as mean $\pm$ SEM unless otherwise indicated in the text. All variables were tested for differences between groups at 161 days of age using a one-way analysis of variance (ANOVA) with blocking. All variables were tested for differences between groups at 175 days of age using a two-way ANOVA with blocking. The proportion of surface antral follicles per size class to the total number of surface antral follicles greater than 1 mm was transformed by arcsin square root transformation prior to analysis. A general analysis of variance (ANOVA) with blocks built in was used to study the effects of "target liveweight

at 161 days of age", "target average liveweight gain between 161 and 175 days of age" and follicle size on the arcsin square root transformed oocyte IVM data and activation data, as well as the absolute concentration, and molar ratios, of steroids in the follicular fluid. Differences between treatments were examined using least significant difference. Correlations between variates were determined using a general linear regression approach. All analyses were performed using Genstat, 6th Edition (Committee of the Statistics Department, Rothamsted Experimental Station, Harpenden).

3.3 Results

Of the 120 gilts allocated to this experiment, a total of eight gilts were removed from the analyses. Two LIGHT gilts from the 161 day reproductive tract collection group and one LIGHT-HIGH gilt were removed as a result of ill health. The ovaries of five gilts, two from the LIGHT-Low, and one each from the LIGHT-HIGH, HEAVY-LOW and HEAVY-HIGH treatment groups were lost or damaged during collection at the abattoir, and their reproductive data therefore excluded. Consequently, the data presented below relate to 26 and 28 gilts from the LIGHT and HEAVY treatment groups, and 14, 14, 15, and 15 gilts from the LIGHT-LOW, LIGHT-HIGH, HEAVY-LOW and HEAVY-HIGH treatment groups, respectively.

3.3.1 Growth Characteristics

As intended, gilts with a LIGHT target liveweight at 161 days of age had a significantly lower ($P < 0.01$) average daily liveweight gain (ADG) between 70 and 161 days of age compared to gilts with a HEAVY target liveweight: 0.55 ± 0.01 versus 0.85 ± 0.01 kg per day (**Table 3.3.1**). At 161 days of age, LIGHT gilts were significantly lighter ($P < 0.01$) and had significantly lower ($P < 0.01$) P2 backfat and MMD than HEAVY gilts (**Table 3.3.1**).

As a result of the imposed dietary treatments, ADG from 161 to 175 days of age was significantly higher ($P < 0.01$) for HIGH compared to LOW gilts (1.22 ± 0.04 versus 0.51 ± 0.03 kg / day). At 175 days of age, LIGHT gilts had significantly lower ($P < 0.01$) liveweights compared to HEAVY gilts (89.1 ± 1.71 versus 113.9 ± 1.71 kg), and LOW gilts were significantly lighter than HIGH gilts (96.6 ± 2.57 versus 106.4 ± 2.79 kg; **Table 3.3.2**). Liveweight at 175 days of age was 84.2 ± 1.85, 94.0 ± 2.36, 109.0 ± 1.86 and 118.8 ± 2.32 kg for LIGHT-LOW, LIGHT-HIGH, HEAVY-LOW and HEAVY-HIGH gilts, respectively. P2 backfat and

MMD at 170 days of age was unaffected by target ADG from 161 to 175 days of age; however, LIGHT gilts had significantly lower (P < 0.01) P2 backfat and MMD compared to HEAVY gilts: 7.4 ± 0.20 versus 11.2 ± 0.33 mm, and 44.9 ± 0.78 versus 51.6 ± 0.69 mm, respectively (**Table 3.3.2**).

Table 3.3.1 Liveweight, average daily liveweight gain, P2 backfat and MMD for gilts fed to attain either 70 kg (LIGHT) or 100 kg (HEAVY) at 161 days of age

	Target LW category at 161 days of age		*Pooled SEM*
	LIGHT	HEAVY	
Actual LW at 161 days of age (kg)	74.8[a]	102.2[b]	*0.96*
ADG: 70 - 161 days of age (kg per day)	0.55[a]	0.85[b]	*0.01*
P2 backfat at 155 days of age (mm)	7.2[a]	10.2[b]	*0.17*
MMD at 155 days of age (mm)	43.8[a]	47.9[b]	*0.44*

[ab] Superscripts within row indicate significant difference; P < 0.01

Table 3.3.2 Growth characteristics of gilts fed to attain a LIGHT or HEAVY target liveweight at 161 days of age and a LOW or HIGH target average daily liveweight gain from 161 to 175 days of age

	Target LW at 161 days of age		Target ADG from 161 to 175 days of age		*Pooled SEM*
	LIGHT	HEAVY	LOW	HIGH	
LW at 175 days of age (kg)	89.1[a]	113.9[b]	96.6[a]	106.4[b]	*2.09*
P2 backfat at 170 days of age (mm)	7.4[a]	11.2[b]	9.3	9.3	*0.36*
MMD at 170 days of age (mm)	44.9[a]	51.6[b]	47.8	48.7	*1.01*

[a,b,] Superscripts within row, and main effect, indicate significant difference; P < 0.01

In block two, liver weight, expressed as a percentage of total body weight, was significantly higher (P < 0.01) for LIGHT compared to HEAVY gilts at 161 days

old (1.94 ± 1.40 versus 14.6 ± 1.35 g/kg). At 175 days of age, there was also a significant (P < 0.01) interaction between "target LW at 161 days of age" and "target ADG from 161 to 175 days of age": liver weight was 1.67[a] ± 0.48, 2.03[c] ± 0.25, 1.80[b] ± 0.50, and 1.97[c] ± 0.51 kg for LIGHT-LOW, LIGHT-HIGH, HEAVY-LOW and HEAVY-HIGH gilts, respectively, where different superscripts indicate significant difference.

3.3.2 Plasma hormone concentrations

At 160 days of age, LIGHT gilts had significantly lower (P < 0.01) plasma concentrations of IGF-I and oestradiol (E2) compared to HEAVY gilts: 27.2 ± 1.47 versus 60.4 ± 1.91 ng/ml and 3.1 ± 0.44 versus 5.35 ± 0.40 pg/ml (**Figure 3.3.1**).
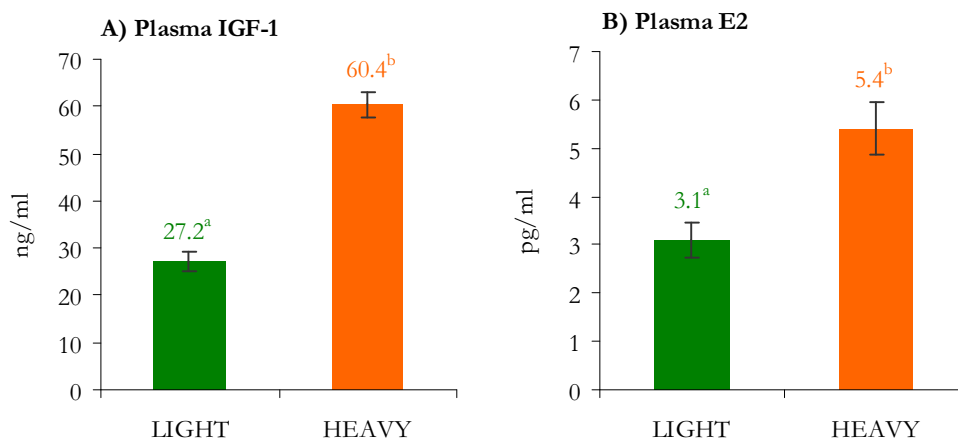
Figure 3.3.1 Concentrations of A) IGF-I and B) Oestradiol in plasma samples collected from 160 day old, LIGHT and HEAVY gilts. [ab] Superscripts indicate significant difference (P < 0.01).

Table 3.3.3 Concentrations of IGF-I and E2 in plasma collected from 173 day-old LIGHT versus HEAVY and Low versus High gilts

Target LW at 161 days of age	Plasma IGF-I (ng/ml)			Plasma Oestradiol (pg/ml)		
	Target ADG from 161-175 days of age		Pooled across target ADG	Target ADG from 161-175 days of age		Pooled across target ADG
	LOW	HIGH		LOW	HIGH	
LIGHT	26.8	40.6	33.7[c]	3.6	4.1	3.9[c]
HEAVY	38.2	60.8	49.5[d]	7.1	5.7	6.4[d]
Pooled across target LW	32.5[a]	50.7[b]		5.3	4.9	
Pooled SEM			*3.26*			*0.89*

[ab] Superscripts within row indicate significant difference; P < 0.01
[cd] Superscripts within column indicate significant difference; P < 0.01

At 170 days of age, plasma IGF-I concentrations were significantly lower (P < 0.01) for LIGHT compared to HEAVY gilts (33.7 ± 2.33 versus 49.5 ± 3.36 ng/ml), and for LOW compared to HIGH gilts (32.5 ± 2.23 versus 50.7 ± 3.26 ng/ml; **Table 3.3.3**). Oestradiol concentrations were significantly lower (P < 0.01) in the plasma collected from LIGHT compared to HEAVY gilts (3.9 ± 0.63 versus 6.4 ± 0.73 pg/ml), but similar for LOW and HIGH gilts (5.3 ± 1.00 and 4.9 ± 1.06 pg / ml).

3.3.3 *Reproductive characteristics*

All gilts were considered to be prepubertal at the time of ovary collection due to the observed absence of corpora lutea and corpora albicantia.

3.3.3.1 Ovarian follicle populations of 161 day old gilts

There was no effect of gilt liveweight at 161 days of age (LIGHT versus HEAVY) on the mean number of surface antral follicles greater than 1 mm in diameter, or on the mean number of small (1 - 2.9mm) follicles (**Table 3.3.4**). However, the ovaries of LIGHT gilts possessed significantly fewer (P < 0.05) medium (3 – 6 mm) follicles than those of HEAVY gilts (19.9 ± 3.77 versus 30.4 ± 2.70 follicles), and tended (P = 0.06) to have fewer large (> 6mm) follicles than those of HEAVY gilts (0.0 ± 0.00 versus 1.2 ± 0.62 follicles; **Table 3.3.4**).

Table 3.3.4 Number of surface antral follicles with a diameter > 1 mm, 1 - 2.9 mm (small), 3 - 6 mm (medium), or > 6 mm (large), present on the ovaries of 161 day old, LIGHT and HEAVY gilts

Number of surface antral follicles with a diameter:	Target LW at 161 days of age		*Pooled SEM*
	LIGHT	HEAVY	
1 – 2.9 mm (small)	82.7	74.5	*6.53*
3 – 6 mm (medium)	19.9[a]	30.4[b]	*3.18*
> 6mm (large)	0.0*	1.2*	*0.45*
> 1mm (total)	102.6	106.1	*6.79*

Superscripts within row indicate significant difference; [a,b] P < 0.05; * P = 0.06

The ovaries of LIGHT gilts possessed a significantly higher (P < 0.05) proportion of follicles in the small size category compared to those of HEAVY gilts (0.80 ± 0.04 versus 0.65 ± 0.03), and a significantly lower proportion of follicles in the medium sized category (0.20 ± 0.04 versus 0.32 ± 0.03). The proportion of large follicles was unaffected by treatment; nil ± 0.00 and 0.03 ± 0.02 for LIGHT and HEAVY gilts, respectively. For HEAVY gilts there was a significant, positive correlation (R^2 = 0.17; P < 0.01) between ADG from 70 to 161 days of age and the number of 3 – 6 mm follicles; however, for LIGHT gilts the number of 3 – 6 mm follicles was not related to ADG (**Figure 3.3.2**). The ovaries and

uterine horns of LIGHT gilts were significantly lighter (P < 0.01) than those of HEAVY gilts: 8.72 ± 0.44 versus 6.11 ± 0.25 g and 72.9 ± 5.60 versus 129.4 ± 12.31 g, respectively.
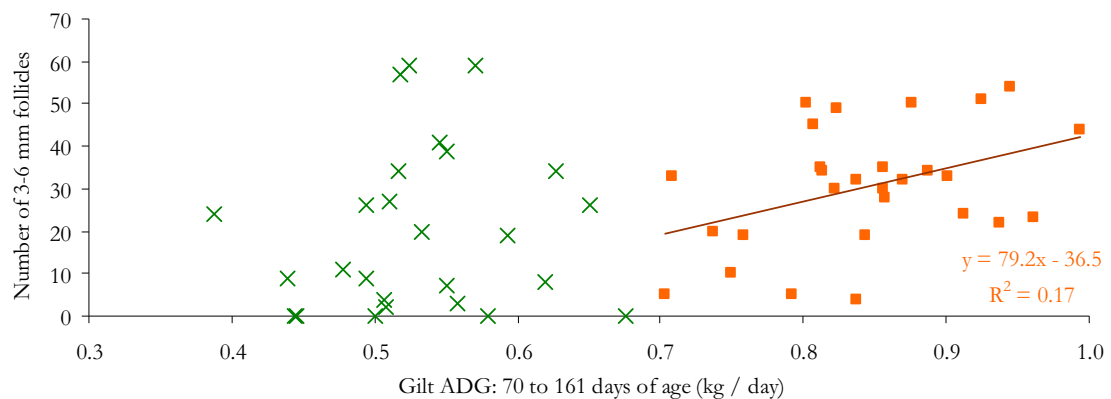
Figure 3.3.2 Relationship between ADG from 70 to 161 days of age and the number of 3 – 6 mm follicles present on the ovaries of LIGHT (×) and HEAVY (■) 161 day old, non-cycling gilts. The fitted line and equation relate to HEAVY gilts only

3.3.3.2 Ovarian follicle populations of 175 day old gilts

The total number of follicles greater than 1 mm in diameter was significantly higher (P < 0.05) on the ovaries of Low compared to High gilts (116.1 ± 5.0 versus 95.1 ± 8.0), but was similar for LIGHT and HEAVY gilts (**Table 3.3.5**). The number of small (1 – 2.9 mm) ovarian follicles was similar for LIGHT and HEAVY gilts (77.2 ± 6.78 and 75.6 ± 8.12), and the ovaries of LOW gilts possessed significantly more (P < 0.01) small follicles compared to HIGH gilts (92.8 ± 8.35 versus 59.5 ± 5.24; **Table 3.3.5**). The ovaries of LIGHT gilts possessed significantly fewer (P < 0.05) medium (3 – 6 mm) follicles compared to HEAVY gilts (25.1 ± 2.59 versus 32.5 ± 2.86), whilst the ovaries of LOW gilts possessed significantly fewer (P < 0.01) medium follicles compared to HIGH gilts (23.3 ± 2.65 versus 34.3 ± 2.60; **Table 3.3.5**). The number of large ovarian

follicles (> 6 mm) was similar for LIGHT and HEAVY gilts, but tended (P = 0.06) to be lower for LOW compared to HIGH gilts (**Table 3.3.5**).

Table 3.3.5 Number of surface antral follicles with a diameter 1- 2.9 mm (small), 3 - 6 mm (medium), > 6 mm (large) or > 1 mm present on the ovaries of LIGHT versus HEAVY and LOW versus High non-cycling gilts at 175 days of age

	Number of antral follicles with a diameter:			
	1 - 2.9 mm	3 – 6 mm	> 6 mm	> 1 mm
LIGHT-LOW	95.2	21.1	0.0	116.3
LIGHT-HIGH	59.2	29.1	0.1	88.4
HEAVY-LOW	90.4	25.6	0.0	116.0
HEAVY-HIGH	60.7	39.5	1.7	101.9
Pooled SEM	*9.10*	*3.36*	*0.48*	*8.78*
Pooled across target LW at 161 days				
LIGHT	77.2	25.1[a]	0.0	102.3
HEAVY	75.6	32.5[b]	0.8	108.9
Pooled SEM	*6.43*	*2.38*	*0.34*	*6.21*
Pooled across target ADG: 161-175 days				
LOW	92.8[b]	23.3[a]	0.0	116.1[b]
HIGH	59.9[a]	32.5[b]	0.9	93.3[a]
Pooled SEM	*6.43*	*2.38*	*0.34*	*6.21*

[ab] Within column and within main effect indicate significant difference; P < 0.05

The ovaries of LIGHT gilts were significantly lighter (P < 0.01) than those of HEAVY gilts (7.1 ± 0.25 versus 8.2 ± 0.39), and there was a tendency (P = 0.06) for LOW gilts to have lighter ovaries than HIGH gilts (7.3 ± 0.32 versus 8.0 ± 0.36). Uterine weight was significantly lower (P < 0.01) for LIGHT compared

to HEAVY gilts (100.5 ± 8.86 versus 122.8 ± 10.27 g) and for LOW compared to HIGH gilts (90.2 ± 7.39 versus 133.1 ± 9.07 g); and there was a significant interaction (P < 0.05) between "target LW at 161 days of age" and "target ADG between 161 and 175 days of age". Uterine weight was 86.9[a] ± 9.42, 93.4[ab] ± 11.62, 114.1[b] ± 13.36 and 152.1[c] ± 8.39 g for LIGHT-LOW, HEAVY-LOW, LIGHT-HIGH, and HEAVY-HIGH gilts, respectively, where different superscripts indicate significant difference.

Table 3.3.6 Proportion of surface antral follicles with a diameter of 1 - 2.9 mm, 3 – 6 mm or > 6mm to the total number of surface antral follicles greater than 1 mm in diameter present on the ovaries of LIGHT versus HEAVY and LOW versus High non-cycling gilts at 175 days of age

	Proportion of antral follicles with a diameter:		
	1 – 2.9 mm	3 – 6 mm	> 6 mm
LIGHT-LOW	0.80	0.20	0.00
LIGHT-HIGH	0.65	0.35	0.00
HEAVY-LOW	0.73	0.27	0.00
HEAVY-HIGH	0.57	0.42	0.01
Pooled SEM	*0.037*	*0.037*	*0.004*
Pooled across target LW at 161 days			
LIGHT	0.73[b]	0.27*	0.00[a]
HEAVY	0.65[a]	0.34*	0.01[b]
Pooled SEM	*0.026*	*0.026*	*0.003*
Pooled across target ADG: 161-175 days			
LOW	0.77[b]	0.23[a]	0.00
HIGH	0.61[a]	0.38[b]	0.01[b]
Pooled SEM	*0.026*	*0.026*	*0.003*

[ab] Within column, and within main effect, indicate significant difference; P < 0.05.
*P = 0.08

The proportion of follicles in the small category was significantly higher (P < 0.05) on the ovaries of LIGHT compared to HEAVY gilts (0.73 ± 0.03 versus 0.65 ± 0.03), and was significantly higher (P < 0.01) on the ovaries of LOW compared to HIGH gilts (0.77 ± 0.03 versus 0.61 ± 0.03) (**Table 3.3.6**). Conversely, the proportion of follicles in the medium (3 – 6 mm) category tended to be lower (P = 0.08) on the ovaries of LIGHT compared to HEAVY gilts (0.27 ± 0.03 versus 0.34 ± 0.03), and was significantly lower (P < 0.01) on the ovaries of LOW compared to HIGH gilts (0.23 ± 0.03 versus 0.38 ± 0.03).

3.3.3.3 Developmental competence of oocytes obtained from 161 day old gilts

There was no effect of gilt liveweight at 161 days of age or follicle size on the proportion of oocytes at the GV or GVBD stages of meiosis after IVM (**Table 3.3.7**). Gilt liveweight at 161 days of age had no effect on the proportion of oocytes at the MI stage of meiosis after IVM (**Table 3.3.7**). A significantly higher (P < 0.05) proportion of oocytes from small follicles were at MI compared to oocytes from medium follicles (0.41 versus 0.23), and a significantly lower proportion of oocytes from small follicles were at MII (0.50 versus 0.75; **Table 3.3.7**). A significantly lower (P < 0.05) proportion of oocytes from LIGHT-161 gilts were at MII following IVM compared to oocytes from HEAVY -161 gilts: 0.57 versus 0.68 (**Table 3.3.7**). There was no significant effect of gilt liveweight at 161 days of age on the proportion of activated oocytes that cleaved, or the proportion of cleaved oocytes that developed beyond the two-cell stage, or to the blastocyst stage. However, a significantly (P < 0.05) lower proportion of activated oocytes from small follicles cleaved compared to those from medium follicles (**Table 3.3.8**).

Table 3.3.7 Meiotic stage of in vitro matured oocytes obtained from small and medium sized ovarian follicles of 161 day old LIGHT versus HEAVY gilts

Meiotic Stage$_1$	Target LW at 161 days of age		Follicle size		Pooled SEM
	LIGHT	HEAVY	Small	Medium	
Proportion GV	0.035	0.005	0.030	0.010	*0.031*
Proportion GVBD	0.058	0.008	0.056	0.010	*0.055*
Proportion MI	0.34	0.31	0.41[b]	0.23[a]	*0.040*
Proportion MII	0.57[a]	0.68[b]	0.50[a]	0.75[b]	*0.030*

[ab] within row, and within main effect, indicate significant difference; P < 0.05
$_1$GV (germinal vesicle); GVBD (germinal vesicle breakdown); MI (metaphase I); MII (metaphase II)

Table 3.3.8 The development of parthenogenetically-activated oocytes from small and medium sized ovarian follicles from 161 day old LIGHT and HEAVY gilts

	Target LW at 161 days of age		Follicle size		Pooled SEM
	LIGHT	HEAVY	Small	Medium	
Proportion cleaved[1]	0.85	0.89	0.76[a]	0.97[b]	*0.01*
Proportion ≥ 2-cell stage[2]	0.09	0.03	0.58	0.75	*0.09*
Proportion blastocysts[2]	0.36	0.36	0.26	0.47	*0.11*

[ab] within row, and within main effect, indicate significant difference; P < 0.05.
[1]Proportion of activated oocytes that cleaved. [2]As cleaved

3.3.3.4 Developmental competence of oocytes obtained from 175 day old gilts

After 44 – 46 hours of IVM, the proportion of oocytes at the GV stage tended to be higher for the LOW compared to the HIGH treatment group (0.10 versus 0.03; P = 0.061), was similar for oocytes in the LIGHT and HEAVY treatment groups (0.09 versus 0.04), and was not affected by follicle size (**Table 3.3.9**). The proportion of oocytes at the GVBD stage and MI stage was unaffected by treatment (**Table 3.3.9**). The proportion of oocytes at the MII stage following IVM was significantly (P < 0.05) lower in the LOW compared to the HIGH treatment (0.40 versus 0.54), but was unaffected by either target liveweight at 161 days of age or follicle size (**Table 3.3.9**). There was no significant effect of target liveweight gain at 161 days of age or target ADG from 161 to 175 days of age on the proportion of activated oocytes that cleaved, or the proportion of cleaved oocytes that developed beyond the two-cell stage, or to the blastocyst stage (

Table 3.3.10).

Table 3.3.9 Meiotic stage of *in vitro* matured oocytes obtained from small (1 – 2.9 mm) and medium (3 – 6 mm) follicles of LIGHT versus HEAVY and Low versus High non-cycling gilts at 175 days of age (data expressed as the proportion of oocytes in each meiotic stage following IVM to the total number of oocytes assessed).

	Meiotic Stage[1]			
	GV	GVBD	MI	MII
LIGHT-LOW small follicles	0.18	0.39	0.10	0.33
LIGHT-HIGH small follicles	0.03	0.01	0.48	0.48
HEAVY-LOW small follicles	0.06	0.07	0.50	0.36
HEAVY-HIGH small follicles	0.05	0.04	0.51	0.41
LIGHT-LOW medium follicles	0.13	0.00	0.44	0.43
LIGHT-HIGH medium follicles	0.04	0.06	0.28	0.63
HEAVY-LOW medium follicles	0.03	0.07	0.45	0.46
HEAVY-HIGH medium follicles	0.02	0.03	0.29	0.66
Pooled SEM	*0.04*	*0.12*	*0.14*	0.09
Pooled across target LW at 161 days				
LIGHT	0.09	0.12	0.33	0.47
HEAVY	0.04	0.05	0.44	0.47
Pooled SEM	*0.02*	*0.060*	*0.07*	0.09
Pooled across target ADG: 161-175 days				
LOW	0.10*	0.13	0.37	0.40[a]
HIGH	0.03*	0.03	0.39	0.54[b]
Pooled SEM	*0.02*	*0.060*	*0.07*	0.09
Follicle Diameter				
1 - 2.9 mm	0.08	0.13	0.40	0.40*
3 – 6 mm	0.05	0.04	0.39	0.54*
Pooled SEM	*0.02*	*0.060*	*0.07*	0.09

[ab] Superscripts within column, and main effect, indicate significant difference; $P < 0.05$; *$P < 0.1$. [1]GV (germinal vesicle); GVBD (germinal vesicle breakdown); MI

(metaphase I); MII (metaphase II)

Table 3.3.10 The development of parthenogenetically-activated oocytes from medium sized (3 – 6 mm) ovarian follicles from LIGHT versus HEAVY and Low versus High non-cycling gilts at 175 days of age

	Proportion oocytes:		
	Cleaved[1]	$\geq$ 2 Cell stage[2]	Blastocysts[2]
LIGHT-LOW	0.73	0.94	0.19
LIGHT-HIGH	0.81	0.90	0.10
HEAVY-LOW	0.91	0.93	0.29
HEAVY-HIGH	0.82	0.90	0.26
Pooled SEM	*0.07*	*0.02*	*0.09*
Pooled across target LW at 161 days			
LIGHT	0.77	0.92	0.14
HEAVY	0.86	0.91	0.27
Pooled SEM	*0.07*	*0.02*	*0.09*
Pooled across target ADG: 161-175 days			
LOW	0.82	0.93	0.22
HIGH	0.81	0.90	0.20
Pooled SEM	*0.07*	*0.02*	*0.09*

[1]Proportion of activated oocytes that cleaved. [2]As a proportion of those oocytes that cleaved

3.3.3.5 Follicular fluid steroid concentrations: 161 day old gilts

Gilt liveweight at 161 days of age had no effect on the concentration of progesterone (P4), androstenedione (A4), testosterone (T) and oestradiol (E2) in the follicular fluid collected from follicles of 161-day old gilts (Table 3.3.11). Compared to follicular fluid from small follicles, concentrations of progesterone,

androstenedione, testosterone, and oestradiol were significantly higher (P < 0.05) in the follicular fluid of medium sized follicles (**Table 3.3.11**). The ratio of testosterone to androstenedione was significantly (P < 0.05) higher in the follicular fluid from LIGHT compared to HEAVY gilts (2.89 versus 1.71), and from small compared to medium follicles (3.21 versus 1.39; **Table 3.3.12**). The ratios of oestradiol to progesterone, oestradiol to testosterone, and androstenedione to progesterone were significantly higher (P < 0.05) in the follicular fluid from medium compared to small follicles (**Table 3.3.12**).

Table 3.3.11 Concentration of progesterone (P4), androstenedione (A4), testosterone (Testo), and oestradiol (E2) in follicular fluid samples collected from 1 – 2.9 and 3 – 6 mm follicles from LIGHT versus HEAVY non-cycling gilts at 161 days of age

	Follicular fluid concentrations of			
	P4 (nmol/l)	A4 (nmol/l)	Testo (nmol/l)	E2 (nmol/l)
Target LW at 161 days of age				
LIGHT	84.7*	29.0	57.5	10.3
HEAVY	101.3*	49.7	65.5	13.1
Pooled SEM	*8.08*	*12.16*	*6.71*	*4.14*
Follicle diameter				
1 – 2.9 mm	79.0[a]	18.0[a]	51.2[a]	5.6[a]
3 – 6 mm	107.0[b]	60.7[b]	71.9[b]	17.9[b]
Pooled SEM	*8.08*	*12.16*	*6.71*	*4.14*

[ab] Superscripts, within main effects and columns, indicate significant difference; P < 0.05. *P < 0.1

Table 3.3.12 Ratio of steroids in the follicular fluid of 1 – 2.9 and 3 – 6 mm follicles collected from LIGHT versus HEAVY, non-cycling gilts at 161 days of age

	Steroid ratios in the follicular fluid					
	A4:P4	Testo:P4	Testo:A4	E2:A4	E2:Testo	E2:P4
Target LW at 161 days of age						
LIGHT	0.31	0.67	2.39[b]	0.29	0.13	0.09
HEAVY	0.47	0.64	1.71[a]	0.29	0.2	0.12
Pooled SEM	*0.11*	*0.64*	*0.44*	*0.07*	*0.05*	*0.04*
Follicle diameter						
1 – 2.9 mm	0.22[a]	0.65	3.21[b]	0.3	0.10[a]	0.06[a]
3 – 6 mm	0.56[b]	0.66	1.39[a]	0.29	0.23[b]	0.15[b]
Pooled SEM	*0.11*	*0.64*	*0.44*	*0.07*	*0.05*	*0.04*

[ab] Superscripts, within main effects and columns, indicate significant difference; $P < 0.05$

3.3.3.6 Follicular fluid steroid concentrations: 175 day old gilts

For follicles collected from 175 day old gilts, follicular fluid concentrations of progesterone were unaffected by feeding treatment, and were similar for small and medium follicles (**Table 3.3.13**). Concentrations of oestradiol tended to higher in the follicular fluid from HIGH compared to LOW gilts ($P = 0.066$) and from medium compared to small follicles ($P < 0.063$). Follicular fluid concentrations of androstenedione tended to be lower in the LOW compared to the HIGH treatment (41.6 ± 13.4 versus 71.1 ± 24.4 nmol/l; $P = 0.082$), and were significantly lower ($P < 0.05$) for small compared to medium follicles (33.2 ± 10.60 versus 79.5 ± 23.90 nmol/l; **Table 3.3.13**). Average daily gain from 161 to 175 days of age and follicle size had no effect on follicular fluid concentrations of testosterone; however, concentrations of testosterone were

significantly lower (P < 0.05) in samples collected from LIGHT compared to HEAVY gilts (42.8 ± 7.14 versus 78.1 ± 18.62 nmol/l; **Table 3.3.13**).

The ratio of oestradiol to progesterone was significantly higher P < 0.05) in the follicular fluid from HIGH compared to LOW gilts (0.44 versus 0.30), but was similar in the follicular fluid of LIGHT and HEAVY gilts, as well as 1 – 2.9 and 3 – 6 mm follicles. The ratio of testosterone to androstenedione was significantly higher (2.03 versus 1.14; P < 0.05), while the ratio of androstenedione to progesterone was significantly lower (0.58 versus 0.74; P < 0.05) in follicular fluid from small compared to medium follicles. Follicular fluid ratios of oestradiol to testosterone, oestradiol to androstenedione and testosterone to progesterone were unaffected by dietary treatment or follicle size.

Table 3.3.13 Concentration of progesterone (P4), androstenedione (A4), testosterone (Testo), and oestradiol (E2) in follicular fluid samples collected from 1 – 2.9 and 3 – 6 mm follicles from LIGHT versus HEAVY and LOW versus High gilts at 175 days old

	Follicular fluid concentrations of:			
	P4	A4	Testo	E2
	(nmol/l)	(nmol/l)	(nmol/l)	(nmol/l)
LIGHT-LOW small follicles	127	27.6	43.1	6.1
LIGHT-HIGH small follicles	90	47.3	49.4	13.5
HEAVY-LOW small follicles	120	26.3	43.8	6.2
HEAVY-HIGH small follicles	202	31.8	63.2	13.6
LIGHT-LOW medium follicles	176	36.6	20.8	8.3
LIGHT-HIGH medium follicles	196	79.4	57.8	38.8
HEAVY-LOW medium follicles	134	76.1	97.3	19.3
HEAVY-HIGH medium follicles	175	125.8	108	43.4
Pooled SEM	*37.20*	*20.48*	*18.35*	*13.90*
<u>Pooled across target LW at 161 days</u>				
LIGHT	147	47.7	42.8[a]	16.7
HEAVY	158	65	78.1[b]	20.6
Pooled SEM	*18.6*	*10.24*	*9.18*	*6.95*
<u>Pooled across target ADG: 161-175 days</u>				
LOW	139	41.6*	51.3	10*
HIGH	166	71.1*	69.6	27.3*
Pooled SEM	*18.6*	*10.24*	*9.18*	*6.95*
<u>Follicle Diameter</u>				
1 - 2.9 mm	135	33.2[a]	49.9	9.8*
3 – 6 mm	170	79.5[b]	71	27.4*
Pooled SEM	*18.6*	*10.24*	*9.18*	*6.95*

[ab] Superscripts, within main effects and columns, indicate significant difference; $P < 0.05$. *$P < 0.1$

3.4 Discussion

The present study was designed to test the hypothesis that reducing pre-pubertal liveweight gain through nutritional restriction would retard ovarian follicle growth and alter the intra-follicular environment such that oocyte developmental competence, or 'oocyte quality', was impaired. It was also hypothesised that long-term nutritional restriction during the pre-pubertal period would result in carry-over effects on ovarian and intra-follicular characteristics, regardless of nutritional intake during the peri-pubertal period. The current data partially support the hypotheses, demonstrating a negative influence of feed restriction during either the pre-pubertal or peri-pubertal period on ovarian development and circulating IGF-I concentrations, with lower growth rates resulting in a reduced number of 3 – 6 mm (medium sized) follicles present at 161 and 175 days of age, as well as a reduction in the proportion of oocytes that reached Metaphase II *in vitro*. However, despite demonstrating carry-over effects of long-term growth restriction on antral follicle populations, the current data indicate that acute changes in feed intake during the 14 days prior to ovary collection have the greatest effect on oocyte quality. Together, these findings support the growing body of evidence (Zak et al., 1997; Quesnel et al., 1998; Ferguson et al., 2003) that reducing nutritional intake impairs follicle growth and oocyte developmental competence. However, to my knowledge, this is the first study to report the influence of feed restriction prior to puberty on the developmental competence of oocytes obtained from non-cycling, peri-pubertal gilts. Similarly, the current data are the first to demonstrate the negative influence of moderate (i.e. above maintenance), as opposed to severe, feed restriction on antral follicle populations and intra-follicular characteristics of pre-pubertal gilts.

3.4.1 Endocrinology

Secretion of IGF-I by hepatocytes is determined by nutritional status (Cosgrove and Foxcroft, 1996), and in agreement with previous reports (Cosgrove et al., 1992; Booth et al., 1996; Simmen et al., 1998; Diskin et al., 2003), the current data demonstrate a positive association between nutrient intake and circulating IGF-I levels, with IGF-I decreasing in response to restrictive feeding and increasing following realimentation. However, a residual effect of feeding level between 70 and 161 days of age on circulating IGF-I concentrations of 175-day old gilts was also observed, regardless of whether gilts received a high or a low feed intake between 161 and 175 days of age. This is consistent with the data from previous studies demonstrating a linear, gradual decrease in plasma IGF-I from the start of dietary restriction and a gradual increase following realimentation (Booth et al., 1996; Stagg et al., 1998; Diskin et al., 2003). Equally, this finding could be explained by the higher liveweight and backfat of HEAVY compared to LIGHT gilts at 175 days of age, and studies involving heifers indicate that, regardless of feeding level, IGF-I levels are higher in heavier, fatter animals (Adamiak et al., 2005).

Consistent with previous reports that nutritional manipulation of feed intake, liveweight and fatness alters circulating steroid concentrations (Miller et al., 1999; Sangsritavong et al., 2002; Ferguson et al., 2003), the dietary regimens in my study resulted in significant differences in peripheral levels of plasma oestradiol in both 161 and 175 day old gilts. Peripheral oestradiol concentrations reflect ovarian secretion, which in turn is related to both the number and oestrogenic capacity of the follicles present on the ovary (Camous et al., 1985; Boukhliq et al., 1996), as well as the rate at which oestradiol is removed from the blood by the liver (Camous et al., 1985; Sangsritavong et al., 2002). An inverse relationship between feed intake and peripheral steroid levels has been widely demonstrated, with hepatic clearance of steroids from the

circulation increased by elevations in feed intake (Parr, 1991; Ashworth et al., 1999b; Miller et al., 1999; Sangsritavong et al., 2002; Ferguson et al., 2003). Although a positive association between acute changes in feed intake and liver weight has been demonstrated in both the current and previous studies (Ashworth et al., 1999b; Miller et al., 1999), changes in hepatic blood flow, as opposed to liver weight per se, are most likely responsible for nutritionally-induced alterations in oestradiol clearance (Miller et al., 1999). Further, it has previously been demonstrated that steroid clearance rates in both gilts and ewes are unaffected by liveweight and fatness (Bouhkliq et al., 1996; Miller et al., 1999), suggesting that the decreased plasma oestradiol concentrations of 161- and 175-day old LIGHT gilts reflect the lower number of medium follicles present on the ovaries of these animals. Interestingly, despite altering the number of medium follicles, liveweight gain between 161 and 175 days of age had little effect on peripheral oestradiol levels in 175 day old gilts; however, this may have been due to alterations in oestradiol clearance. Specifically, a negative association between the number of medium follicles and oestradiol levels is evident from the data derived from LIGHT-High and HEAVY-Low gilts, and based on the data from previous studies (Ferguson et al., 2003), it could be suggested that the acute elevation in feed intake experienced by LIGHT-High gilts increased oestradiol clearance, while the acute reduction in feed intake experienced by HEAVY-Low gilts decreased oestradiol clearance via the hepatic system.

3.4.2 Ovarian folliculogenesis

The current data demonstrate that chronic (91 days) and acute (14 days) periods of moderate feed restriction elicit a 35% and 29% reduction, respectively, in the number of medium follicles present on the ovaries of 161 and 175 day old gilts. These data provide the first evidence that moderately restricting feed intake during the pre-pubertal period suppresses ovarian follicle growth and alter the

distribution of follicles between size categories. Although previous studies have reported a suppression of follicular growth when pre-pubertal gilts are fed at maintenance as opposed to ad libitum levels (Cosgrove et al., 1992; Booth et al., 1996), the level of dietary restriction imposed in the current study provided gilts with approximately 2.2 to 2.5 times their maintenance requirement, compared to those in the high intake groups which were fed at 3.0 to 3.5 times maintenance. The present data clearly demonstrate the sensitivity of follicular growth to modest alterations in feed intake, with subtle reductions in feed intake severely impairing ovarian activity.

Reproductive activity is ranked behind growth with respect to nutrient prioritisation (Cosgrove and Foxcroft, 1996), and it is interesting that a significant, positive correlation between growth rate from 70 to 161 days of age and the number of 3 – 6 mm follicles was evident for HEAVY but not LIGHT gilts. Based on these data, it could be suggested that when feed intake, and thus growth rate, are restricted, follicular growth is dependant on genetic variability in sexual maturation (Beltranena et al., 1993). Although the present data do not allow the identification of the threshold level of intake beyond which follicular growth is influenced by nutrient intake as opposed to genetic determinants, it is evident that increasing daily liveweight gain from 0.7 to 1.0 kg / day induces a corresponding increase in follicular growth.

Although a decrease in follicle number has previously been demonstrated in pre-pubertal gilts when dietary intake was reduced to maintenance levels (Cosgrove et al., 1992; Booth et al., 1996), the moderate level of feed restriction used in the present study did not induce a reduction in total follicle number. In fact, low level feeding between 161 and 175 days of age resulted in the accumulation of small (1 - 3mm) follicles and a consequential increase in the total number of surface follicles present on the ovaries of 175 days old gilts. The previously-reported stimulatory effect of realimentation on follicular growth

(Cosgrove et al., 1992; Booth et al., 1996; Hazeleger et al., 2005) is supported by the current data, as demonstrated by the increased number of medium follicles present on the ovaries of LIGHT-High 175-day old gilts compared to both 161-day old LIGHT and 175-day old LIGHT-Low gilts. However, irrespective of liveweight gain between 161 and 175 days of age, the ovaries of 175-day old LIGHT gilts contained significantly fewer medium follicles than those of HEAVY gilts. Specifically, the ovaries of 175-day-old LIGHT-High gilts contained approximately 35% fewer medium follicles than those of HEAVY-High gilts, demonstrating a negative effect of chronic feed restriction on subsequent follicular development. Approximately 20% more medium follicles were observed on the ovaries of HEAVY-Low compared to LIGHT-Low 175-day old gilts, which appears to support earlier work in rodents (Schneider and Wade, 1987) demonstrating that body reserves can act as a buffer against the suppressive effects of food deprivation on reproductive activity.

A suppressive effect of restrictive feeding on follicle growth beyond 3 mm is evident from the current data. Follicle growth up to 4 mm can occur independently of LH pulsing (Driancourt et al., 1995); however, a stimulatory effect of LH on the growth of 1 – 3 mm follicles has also been reported (Quesnel et al., 2000). Although inhibited LH pulsing has been demonstrated in severely feed-restricted prepubertal and cycling gilts (Prunier et al., 1991; Prunier et al., 1993; Booth et al., 1996; Ferguson et al., 2003), previous data indicate that moderate feed restriction does not appear to affect LH release (Beltranena et al., 1993). Importantly, previous studies involving gonadotrophin antagonist-treated gilts also demonstrate that increased follicle growth in response to high feed intake occurs independently of LH (Cosgrove et al., 1992; Quesnel et al., 2000). Consequently, although direct effects of treatment on hypothalamic-pituitary release of LH were not examined in this study, it is suggested that alterations in circulating concentrations of metabolites and hormones, as opposed to suppressed LH secretion, were responsible for

mediating the negative impact of feed restriction on follicle growth reported here.

The current data are consistent with previous studies in which lower plasma IGF-I levels were associated with reduced ovarian follicle growth (Cosgrove et al., 1992; Booth et al., 1996). IGF-I binding sites have been demonstrated in the oocytes, granulosa and thecal cells of both preantral and antral porcine follicles (Quesnel, 1999), and a direct stimulatory effect of IGF-I on antrum formation, follicle steroidogenic capacity and follicular growth has previously been established in pigs (Cosgrove and Foxcroft, 1996; Quesnel and Prunier, 2000; Cardenas and Pope, 2002; Mao et al., 2004). Studies in cattle have identified a positive correlation between follicular fluid and plasma concentrations of IGF-I (Echternkamp et al., 1990), with dietary-induced increases in circulating IGF-I stimulating steroidogenesis and follicle growth (Armstrong et al., 2001; Armstrong et al., 2002; Webb et al., 2007). A positive correlation between plasma IGF-I and IGFBP-3 has also been established in barrows fed a low energy diet (Lee et al., 2002), and there is also strong evidence that circulating IGFBP-3 levels are correlated with both dietary intake and growth rate (Vestergaard et al., 1995; Rausch et al., 2002; Katsumata et al., 2002). A stimulatory effect of IGFBP-3 on IGF-I induced somatic cell proliferation has been proposed (Howard and Ford, 1992). The IGFBP-3 present in follicular fluid is predominantly of extra-follicular origin (Wandji et al., 2003), and a causal relationship between acute increases in energy intake and both increased intra-follicular bioactivity of IGF-I and granulosa cell aromatase activity has been demonstrated in cattle (Armstrong et al., 2002; Webb et al., 2004). Based on the available evidence it is conceivable that the decreased levels of IGF-I observed in LIGHT and Low gilts reduced the sensitivity of granulosa and theca cells to gonadotrophins and impaired the proliferation and steroidogenic capacity of somatic cells, thus retarding follicular growth and altering the intra-follicular environment (see later discussion). Further, the gradual changes in

IGF-I observed in LIGHT-High and HEAVY-Low gilts in response to acute changes in feed intake could explain the observed differences in follicular populations between these gilts and those that were fed on either a low or a high plane throughout the study (i.e. LIGHT-Low and HEAVY-High gilts).

3.4.3 Intra-follicular characteristics and oocyte developmental competence

In the present study, chronic and acute restriction of feeding level resulted in an 11% and 14% reduction, respectively, in the proportion of oocytes maturing to metaphase II *in vitro.* This finding is in agreement with the data of Ferguson et al. (2003) which demonstrated that feeding cycling, 220 day old gilts at maintenance compared to 2.6 times maintenance levels for 19 days during their third oestrous cycle resulted in a 20% reduction in the proportion of peri-ovulatory oocytes that reached metaphase II *in vitro*. It is noteworthy that the high level of feeding imposed by Ferguson et al. (2003) is similar to the low level of feed intake (2.2 to 2.5 times maintenance) used in the current study. The present data therefore indicates that even moderate reductions in dietary intake impair oocyte growth and development, furthermore it appears that nutritional influences on oocyte development occur as dietary intake is increased from maintenance all the way through to 3.3 times maintenance. Clearly, these differences in oocyte maturation reflect alterations in follicular function, and it has been suggested that varying feed intake alters follicular fluid profiles of growth hormones and steroids which influence the capacity of the oocyte to become developmentally competent (Zak et al., 1997; Hunter, 2000; Grupen et al., 2003). In particular, nutritionally-induced alterations in gonadotrophin and metabolic hormone profiles influence somatic cell differentiation and steroidogenic activity (Cosgrove et al., 1992; Quesnel and Prunier, 2000; Webb et al., 2004), and strong circumstantial evidence exists to support the involvement of follicular fluid steroid concentrations in the

acquisition of oocyte competence (Ding and Foxcroft, 1994; Grupen et al., 2003; Huynh et al., 2003).

Restrictive feeding reduces the ability of follicular fluid to support meiotic progression to metaphase II (Zak et al., 1997), and positive associations between oocyte meiotic competence and follicular fluid concentrations of both progesterone and oestradiol have previously been demonstrated (Ding and Foxcroft, 1992; van de Leemput et al., 1998) In agreement with the study of Ferguson et al. (2003), in which concentrations of oestradiol were lower in the follicular fluid of maintenance-fed gilts, a numerical ($P < 0.1$) but not significant decrease in concentrations of both androstenedione (29.5 nmol/l) and oestradiol (17.3 nmol/l) was observed in the follicular fluid derived from gilts that were restrictively fed between 161 and 175 days of age. Restrictive feeding between 161 and 175 days of age reduced the ratio of oestradiol to progesterone in the follicular fluid, and together, these data can be interpreted in terms of a suppressive effect of dietary restriction on the steroidogenic capacity of somatic cells. Certainly a synergistic relationship between the IGF-I system and gonadotrophins in the regulation of progesterone biosynthesis and aromatase activity is well established (Poretsky et al., 1999; Spicer et al., 2002); however, it is not clear from the current data whether this reflects reduced conversion of progesterone to androgen substrates or aromatisation of androgens to form oestradiol. Interestingly, chronic feed restriction did not alter intra-follicular steroid concentrations; however, the ratio of testosterone to androstenedione was higher in the follicular fluid of LIGHT compared to HEAVY gilts, and an association between an increased proportion of testosterone relative to androstenedione and reduced developmental competence of pre-pubertal oocytes is evident from the data of Grupen et al. (2003).

The ability to develop to the blastocyst stage is the most accurate indicator of oocyte developmental competence or 'capacitation' (Sirard et al., 2006). Bagg

et al. (2006) concluded that parthenogenetic development is an accurate method of determining the potential of oocytes to develop to the blastocyst stage, and the blastocyst formation rates observed in this study are similar to those reported in previous studies involving parthenote embryos of pre-pubertal oocytes (Grupen et al., 2003; Bagg et al., 2006). It is, therefore, unexpected that despite impairing meiotic maturation, restrictive feeding did not significantly affect cleavage or blastocyst formation rates. However, irrespective of liveweight gain between 161 and 175 days of age, a numerical, but not significant, increase in blastocyst formation rates (27% versus 14%) was observed in oocytes derived from 175 day-old LIGHT compared to HEAVY gilts. This finding could suggest a carry-over effect of chronic feed restriction on oocyte cytoplasmic development, even though blastocyst formation rates at 161 days of age were similar for oocytes derived from LIGHT and HEAVY gilts. It is possible that the size of the follicles from which the oocytes were derived may explain the lack of an effect of dietary treatment on the development of parthenote embryos. Oocyte developmental competence is acquired gradually during the latter stage of antral follicle growth, and the acquisition of meiotic competence does not signify complete developmental competence (Moor et al., 1990). The accumulation of the transcription factors vital for the completion of the early stages of embryogenesis occurs during the latter stages of antral follicle growth (Hytell et al., 1997; Krisher, 2004). Therefore, although metabolic effects on the acquisition of meiotic competence were evident within the cohort of oocytes used in this study, nutritional effects on the accumulation of essential transcription factors may not become evident until the final stages of follicle growth. Equally, defects during cytoplasmic maturation can manifest as increased incidences of chromatin condensation and polyspermy (Guruya, 1985), which would not have been detected in the present study due to the use of parthenogenetic activation (Grupen et al., 2003).

Consistent with previous studies involving prepubertal gilts (Marchal et al., 2002), a higher proportion of oocytes obtained from medium compared to small follicles matured to metaphase II in vitro. Similarly, a significantly higher proportion of activated oocytes from the medium follicles of 161 day-old gilts cleaved following activation, and a numerical, but not significant, increase in blastocyst formation was observed for oocytes derived from medium compared to small follicles. A positive correlation between follicle size and oocyte developmental competence has previously been established, with the ability of oocytes to progress to metaphase II and develop to the blastocyst stage increasing dramatically with follicle growth beyond 3 mm (Marchal et al., 2002). Progesterone biosynthesis and androgen aromatisation also increase as the follicle grows (Ainsworth et al., 1980; Foxcroft and Hunter, 1985), and this is demonstrated by the observed differences between the ratios of steroids as well as the absolute concentrations of steroids present in the follicular fluid derived from medium compared to small follicles. Together the current data appear to provide further evidence of a relationship between oocyte meiotic maturation and follicular fluid oestradiol concentrations. However, it remains to be established whether intra-follicular steroid concentrations directly affect oocyte growth and development, or are merely indicative of the 'health' status of the follicle (van de Leemput, 1998; Dode and Graves, 2002). Equally, it should be noted that both follicular fluid samples and collected oocytes were pooled in this study, thus making it difficult to make definitive statements as to a causal relationship between intra-follicular steroid profiles and the observed differences in oocyte developmental competence.

3.4.4 Conclusion and Implications

In conclusion, the current study has demonstrated that moderately restricting the feed intake of pre-pubertal gilts impairs follicular growth beyond 3 mm and reduces the developmental competence of oocytes derived from the

proliferating antral follicle pool. These findings extend those of previous studies investigating the influence of severe feed restriction on follicular growth and function, by demonstrating the sensitivity of the ovarian follicle to more moderate reductions in food intake. In addition, the current study suggests that chronic restriction of food intake has carry-over effects on subsequent follicular growth, with the negative effects of long-term dietary restriction on follicular growth still apparent 14 days after increasing feed intake from 2.5 to 3.0 times maintenance. Alterations in the dynamics and size of the proliferating follicle pool have been identified as determinants of the timing of oestrous in weaned sows as well as ovulation rate in the pig (Hunter and Weisak, 1990; Quesnel et al., 1998). Consequently, the reduced follicle growth and altered intra-follicular steroidogenic activity observed in restrictively fed gilts could impair their ability to initiate an ovarian response to the rise in LH secretion associated with boar stimulation. In addition, previous studies demonstrate that nutritional regimes which impair oocyte meiotic competence also reduce embryo survival (Ferguson et al., 2003; Ferguson et al., 2006), suggesting that the level of dietary restriction used in the current study would reduce both the ovulation rate and embryo survival of gilts mated at their pubertal oestrus. The implications of nutritionally-induced alterations in follicular growth and oocyte developmental competence on the timing of the pubertal response to boar stimulation and potential litter size are pursued in some detail in the following chapter.

Chapter 4   Effect of pre-pubertal growth rate on gilt response to boar stimulation and fecundity

4.1 Introduction

Due to the current trend of decreasing longevity of sows within the breeding herd and resultant increase in annual replacement rates it is common for replacement gilts to constitute between 20% and 30% of the breeding herd (Hughes and Varley, 2003; Koketsu et al., 2005; Levis et al., 2005). As a consequence, gilt reproductive performance exerts a major impact on overall breeding herd productivity. Facilitating gilt entry into the breeding herd and maximising subsequent reproductive performance depends on the combination of appropriate nutritional management and effective puberty stimulation and mating strategies. However, incidences of reproductive disorders and failure, including delayed or asynchronous puberty attainment and low first litter sizes, are common within cohorts of replacement gilts (Whittemore, 1996; Evans and O'Doherty, 2001).

Nutritional status profoundly affects reproductive activity in the female pig (Cosgrove and Foxcroft, 1996; Prunier and Quesnel, 2000). Nutritional restriction of pre-pubertal growth rate can retard sexual maturation and delay puberty attainment (Beltranena et al., 1993; Prunier et al., 1993), most likely mediated through metabolic status, rather than any aspect of body size (Schneider and Wade, 1987; Rozeboom et al., 1993; Booth et al., 1994; Foster and Nagatini, 1999; Klindt et al., 2001). Alterations in the availability of certain metabolites have a considerable influence on ovarian follicle growth and development (Cosgrove and Foxcroft, 1996; Cardenas and Pope, 2002; Webb et al., 2004). Data derived from weaned sows also support a causal relationship between nutritional intake and ovarian development and the timing of the return to oestrus post-weaning and subsequent litter size (Zak et al., 1997; Quesnel et al., 2000). Studies involving rats and ewe lambs also indicate that retarded ovarian development may be responsible, at least in part, for delayed puberty

attainment in restrictively-fed animals (Ronnekliev et al., 1978; Bronson, 1986; Schillo et al., 1992). Importantly, inadequate nutritional intake during the oestrous cycle prior to ovulation decreases the number of ova shed and reduces embryo survival in gilts (Almeida et al., 2000; Prunier and Quesnel, 2000; Quesnel et al., 2000b Ferguson et al., 2003). Reducing the feed intake of growing, cycling gilts to maintenance requirements alters the dynamics of the proliferating follicle pool, thus affecting the number of follicles selected for ovulation (Almeida et al., 2000; Quesnel et al., 2000). The impact that alterations in pre-mating nutrition have on embryo survival appear to be partially mediated by changes in follicle steroidogenesis and oocyte maturation which influence the developmental competence of the oocytes released at ovulation (Zak et al., 1997; Ferguson et al., 2003). Specifically, compared to high feeding levels, maintenance feeding prior to ovulation significantly reduces the proportion of oocytes in the presumptive ovulatory pool that are able to reach metaphase II (Ferguson et al., 2003).

It was shown in Chapter Three that both chronic and acute periods of moderate dietary restriction impair ovarian follicle growth in pre-pubertal 161- and 175 day old gilts; expressed as reduced growth of 3 – 6 mm follicles and altered intrafollicular steroidogenesis. It was hypothesised that this nutritionally-induced retardation of follicle growth would impair the ability of restrictively-fed gilts to initiate an ovarian response to the rise in LH secretion associated with boar stimulation, thus negatively affecting gilt responsiveness to boar stimulation and reducing pubertal ovulation rates. The data presented in Chapter Three also demonstrated that while the proportion of oocytes capable of reaching metaphase II is reduced by both chronic and acute restrictive feeding, neither the cleavage rates nor the blastocyst formation rates of parthenogenetically-activated oocytes were affected by the imposed dietary regimes. This finding appears to contradict recent data derived from cycling

gilts which provides strong evidence that pre-mating feeding regimes that reduce the ability of oocytes to reach metaphase II also reduce embryo survival up to day 30 of gestation (Ferguson et al., 2007). It is, therefore, appropriate to investigate whether the response of pre-pubertal gilts matches the response of cycling gilts; that is, that moderately restricting the pre-and peri-pubertal feed intake of gilts prior to first mating elicits a reduction in embryo survival.

The objective of the current study was to test two hypotheses: (i) that dietary regimes that reduce the growth of 3 – 6 mm follicles and impair development of the follicle-oocyte complex will delay puberty attainment and reduce potential litter size; (ii) that nutritional intake during the period immediately preceding the start of boar exposure, as opposed to liveweight, will determine the timing of the pubertal response. This was achieved by studying the effects of moderately restricting feed intake during two periods within the prepubertal period (70 to 161 days, chronic, of age and 161 to 175 days, acute, of age) on the timing of gilt puberty in response to boar stimulation commencing at 175 days of age and ovulation rate and embryo survival of gilts mated at the pubertal oestrus.

4.2 Methods

The two experiments described in this chapter were conducted at the University of Adelaide, Pig and Poultry Production Institute (PPPI) at Roseworthy, South Australia. Animal trials were conducted with approval from the animal ethics committees of Primary Industries and Resources South Australia and The University of Adelaide. Experiments three and four followed the same design as for experiments one and two (reported in Chapter 3), which was a 2 x 2 factorial (reported in this chapter), incorporating two target liveweights (LW) at 161 days of age (70 kg; LIGHT versus 100 kg; HEAVY), and two target rates of average daily liveweight gain (ADG) between 161 days of age and puberty (0.5 kg / day; LOW versus 1.0 kg / day; HIGH). Gilts commenced boar exposure at 175 days of age and were artificially inseminated at the pubertal oestrus. Experiment three ran from the 2nd February 2004 until 17th June 2004 (summer/autumn); and experiment four ran from the 8th December 2004 until 6th May 2005 (summer/autumn); with boar exposure commencing on the 18th May 2004 and 23rd March 2005, respectively

The number of animals used, animal housing and feeding for both of the experiments reported in this chapter are described in the General Methods Section 4.2.1. However, there were two differences in experimental procedure. Gilts in experiment three were slaughtered, and reproductive tracts collected, 10 days after the first detection of oestrus, whereas gilts in experiment four were slaughtered, and reproductive tracts collected, 22 days after the first detection of oestrus. In experiment three the majority of embryos collected by uterine flushing were fragmented, making it impossible to count the number of embryos or measure their diameter with any degree of confidence. Consequently, slaughter time was delayed in experiment four to ensure that valid embryo data could be collected. In experiment three, gilts received boar

exposure twice daily in order to accurately identify the onset and duration of oestrus, and hence time of ovulation and embryo age. However, the delayed time of slaughter in experiment four meant that estimating time of ovulation was unnecessary, and due to labour restrictions boar exposure was only conducted once daily.

4.2.1 General methods: experiments three and four

4.2.1.1 Animals and housing

Gilts were individually identified using ear tags, and weaned according to standard practice at the Roseworthy piggery, which corresponded to a weaning age of 20.2 ± 0.2 and 19.8 ± 0.1 days and a weaning liveweight (LW) of 6.7 ± 0.1 kg and 6.6 ± 0.1 kg in blocks one and two respectively. Gilts were selected at approximately 70 days of age and 29.4 ± 0.6 kg liveweight in experiment one, and 69 days of age and 28.3 ± 0.3 kg liveweight in experiment two. At selection, 96 Large White/Landrace crossbred gilts (48 gilts/experiment) were weighed, stratified according to weight and allocated to either the LIGHT or HEAVY feeding treatment: n = 24 gilts/treatment/block. Gilts in the LIGHT treatment group were fed to attain a liveweight of 70 kg at 161 days of age, whilst those in the HEAVY treatment group were fed to attain a liveweight of 100 kg at 161 days of age. Where possible, litter sisters were allocated evenly across treatments. From selection until approximately 126 days of age, gilts were housed in grower rooms, in pens of eight, with a space allowance of 1.2 square metres per gilt. Gilts were then moved to finisher rooms where they were housed in their original groups, with a space allowance of 1.3 square metres per gilt.

At approximately 161 days of age, gilts were weighed, and within treatment (LIGHT or HEAVY) stratified according to weight, and allocated to gain liveweight at a rate of either 0.5 kg per day (LOW) or 1.0 kg per day (HIGH): n = 12 gilts/treatment/experiment. Gilts continued to be housed in finisher rooms, but were re-sorted into pens of six, with a space allowance of 1.7 square metres per gilt. The grower and finisher rooms contained no male pigs, were fitted with adjustable side blinds, and gilts were exposed to a natural lighting regime.

4.2.1.2 Gilt liveweight, backfat and maximum muscle depth measurements

Gilts were weighed at selection and at weekly intervals thereafter, as well as at the start of boar exposure, the onset of the pubertal oestrus and slaughter. P2 backfat and maximum eye muscle depth (MMD) were measured (Section 3.2.3.1) at 160 and 172 days of age.

4.2.1.3 Gilt feeding

At all times, gilts received the standard diets used by the Roseworthy piggery, with the specifications of these diets changing depending on gilt age (**Table 2.2.1**). From weaning until selection at approximately 70 days of age, gilts had ad-libitum access to feed. Following selection, gilts were fed once daily, between 8 and 9 am, with the ration broadcast on the floor of the pen. The daily feed intake required to attain a gilt liveweight of 70 kg (LIGHT) or 100 kg (HEAVY) by 161 days of age was calculated as described in Section 3.2.2.

From approximately 161 days of age until their first artificial insemination, all gilts received the female finisher diet (Table 2.2.1). The amount of feed required to attain the target average daily liveweight gains (ADG) from 161 days of age

until puberty, that is 0.5 kg per day (LOW) or 1.0 kg per day (HIGH), was calculated for gilts weighing from 60 to 150 kg as described in section 3.2.2. From their second artificial insemination through to slaughter gilts received 2.0 kg / day of a dry sow diet (**Table 2.2.1**).

4.2.1.4 Collection of blood samples and hormone assays

Pre-prandial blood samples were collected as described in Section 3.2.3.2. In experiment three, blood samples were collected at first detection of oestrus, 72 hours after first detection of oestrus, and at exsanguination, approximately 240 hours after first detection of oestrus. In experiment four, blood samples were collected at 158 and 173 days of age, as well at first detection of oestrus and 72 hours after first detection of oestrus. In experiment four, blood samples were not collected at 240 hours after first detection of oestrus in order to avoid stressing gilts during the initiation of embryo implantation. Concentrations of oestradiol and IGF-I were determined in blood plasma samples taken at 158 and 173 days of age. Oestradiol and progesterone concentrations were determined in blood plasma samples taken at oestrus, as well as 72 and 240 hours after initial oestrus detection. Plasma concentrations of oestradiol (E2) and progesterone (P4) were determined as described in Section 3.2.5. Plasma concentrations of IGF-I were determined as described in Section 3.2.5. The IGF-I assay included six replicates of two control samples containing 71.5 ng/ml and 5.6 ng/ml, which were used to estimate the intra-assay coefficients of variation of 5.7% and 9.0%.

4.2.2 Boar contact and oestrus detection

From selection into the experiment until commencement of boar exposure, gilts had no contact with male pigs. From approximately 153 days of age until the

start of boar exposure, gilts were checked daily, in their pens and without boar contact, for signs of vulval swelling and reddening as well as signs of behavioural oestrus. Boar exposure began at 174 days of age in experiment three, and at 175 days of age in experiment four. The detection mating area (DMA) was situated in a separate building approximately 100 metres from the finisher rooms in which gilts were housed, and consisted of four pens measuring 3 metres by 3.5 metres and lined on two sides by inwards facing boar pens. The attainment of puberty was defined as the first signs of a standing reflex, either in response to the manual application of pressure to the gilts back (the "backpressure test"; Lamberson et al., 1996), or mounting by the boar. The timing of puberty attainment was recorded for all gilts, and the term 'days-to-puberty' refers to the number of days from the start of boar exposure to the start of the pubertal oestrus. Gilts were artificially inseminated at the pubertal oestrus, and then moved to individual stalls where they remained until slaughter. All gilts received two artificial inseminations, once at detection of oestrus and again 24 hours later. All artificial inseminations took place in the DMA, with fence-line contact with a boar during the procedure. Inseminations were performed as described in Section 2.2.3.

4.2.2.1 Boar exposure: Experiment Three

Each group of gilts, as penned, was taken twice a day to the DMA, where they received fifteen minutes of supervised full physical contact with a vasectomized boar. Boar exposures began at 07:00 and 15:00 hours, and four vasectomized boars, greater than 10 months of age, were used in a daily rotation. After gilts had received their second artificial insemination, the presence of a standing reflex in response to boar contact was tested, at 07:00 and 15:00 hours, by running a mature boar up and down in front of the individual stalls for 15 minutes. The timing of oestrus onset was determined as the mid-point between

the previous period of boar exposure when gilts did not show a standing reflex and the time when a standing reflex was first observed. The end of oestrus was defined as the mid-point between the last exhibition of a standing reflex in response to fenceline boar contact and the time when the gilt ceased to exhibit such a response. In this way the duration of oestrus was estimated and the time of ovulation was calculated using the equation described in Almeida et al. (1991): Time of ovulation = duration of oestrus x 0.409 + 22.7 (r = 0.57). Gilts not detected in oestrus by day 28 after commencement of boar exposure were slaughtered to confirm pre-pubertal status, and ascribed a nominal days-to-puberty of 33 days (Siswadi and Hughes, 1995).

4.2.2.2 Boar exposure: Experiment Four

Each group of gilts, as penned, was taken once a day to a detection mating area (DMA), where they received twenty minutes of supervised full physical contact with a vasectomized boar. Boar exposures began at 08:00 hours, and three vasectomized boars, greater than 10 months of age, were used in a daily rotation. Gilts not detected on oestrus by day 42 after commencement of boar exposure were slaughtered to confirm pre-pubertal status, and ascribed a nominal days-to-puberty of 47 days (Siswadi and Hughes, 1995).

4.2.3 Reproductive parameters: ovulation rate and embryo measurements

Gilts were slaughtered at a commercial abattoir, and reproductive tracts were collected within 20 minutes of exsanguinations. The reproductive tracts were taken to the laboratory and processed within 3 hours of collection. The ovaries from each gilt were weighed, and the corpora lutea were dissected and weighed individually. The number of corpora lutea was taken to represent the number of oocytes ovulated at the pubertal oestrus.

4.2.3.1 Reproductive parameters: Experiment Three

Gilts were slaughtered 10 ± 0.3 days after the first detection of oestrus, where day 0 refers to the first 24 hours after oestrus was initially detected, and reproductive tracts collected. Embryos were retrieved by treating each uterine horn to three consecutive flushes of 20 ml of saline (O'Leary et al., 2004). Embryos were collected and where possible counted; however, in the majority of cases embryos were fragmented making it impossible to count with any degree of confidence. Consequently gilts were described as pregnant based on the presence of embryos and data on embryo number are not presented here. The uterus was trimmed of mesentery, separated from the cervix and weighed prior to flushing.

4.2.3.2 Reproductive parameters: Experiment Four

Gilts were slaughtered 22.2 ± 0.1 days after their first artificial insemination, and reproductive tracts recovered. The uterus was trimmed of mesentery and dissected, the number of viable and non-viable embryos recorded, embryo crown-to-rump length measured, and the distance between embryos was also measured. Embryos were described as viable or non-viable based on their gross morphology and crown-to-rump length. An embryo was classified as non-viable if its crown-rump length was more than two standard deviations less than the mean for that animal (Virolainen et al., 2004). The total number of viable embryos observed in both horns was expressed as total embryo number. Embryo survival was calculated based on the total number of viable embryos, and expressed as a percentage of the number of corpora lutea observed on both ovaries. Embryos were removed from the uterus, and the uterine horns were separated from the cervix and weighed.

4.2.4 Statistical analysis

All statistical analysis were conducted separately for Experiments Three and Four. Values in the text are described as mean $\pm$ standard error. A two-way analysis of variance (ANOVA) was used to examine the effects of "target liveweight at 161 days of age" (LIGHT or HEAVY) and "target average liveweight gain from 161 days of age to puberty" (LOW or HIGH) on all variables. Differences between treatments were examined using least significant difference. A generalised linear regression model (binomial distribution) was used to study treatment effects on the proportion of gilts attaining puberty. Unless otherwise specified, a significant interaction was not observed between liveweight at 161 days of age and daily liveweight gain from 161 days of age to puberty on the parameters measured. Correlations between variates were determined using linear regression. All analyses were performed using Genstat, 6th Edition (Committee of the Statistics Department, Rothamsted Experimental Station, Harpenden).

4.3 Results

4.3.1 Experiment Three

4.3.1.1 Gilt liveweight, growth rate, P2 backfat and MMD

As intended, ADG from selection until 161 days of age and liveweight at 161 days of age were significantly lower ($P < 0.01$) for LIGHT compared to HEAVY gilts: 0.53 ± 0.01 versus 0.78 ± 0.01 kg / day and 77.6 ± 1.13 versus 100.6 ± 1.69 kg (**Table 4.3.1**). At 160 days of age, LIGHT gilts had significantly lower ($P < 0.01$) P2 backfat and MMD compared to HEAVY gilts: 7.3 ± 0.24 versus 10.2 ± 0.38 mm and 41.2 ± 0.72 versus 48.3 ± 0.77 mm.

Table 4.3.1 Liveweight (LW), average daily gain (ADG), P2 backfat and maximum eye-muscle depth (MMD) of 175 day old gilts fed to attain either a LIGHT or HEAVY liveweight at 161 days of age and a LOW or HIGH liveweight gain from 161 days of age to puberty

	LW at 161 days of age		ADG from 161 days of age to puberty		*Pooled SEM*
	LIGHT	HEAVY	LOW	HIGH	
LW at 161 days of age (kg)	77.6[a]	100.6[b]	89.2	89.5	*2.13*
ADG: 161 to 175 days of age (kg/day)	0.76	0.74	0.48[a]	1.01[b]	*0.05*
LW: 175 days of age (kg)	88.2[a]	111.0[b]	96.4[a]	102.9[b]	*2.21*
P2 backfat: 172 days of age (mm)	7.9[a]	11.0[b]	9.1	9.8	*0.46*
MMD at 172 days of age (mm)	45.5[a]	50.4[b]	47.7	48.2	*1.19*

[a,b] Superscripts, within row and main effect, indicate significant difference; $P < 0.01$

At 175 days of age, LIGHT gilts had significantly lower (P < 0.01) liveweights than HEAVY gilts (88.2 ± 1.45 versus 111.0 ± 1.87 kg), and LOW gilts were significantly lighter (P < 0.01) than HIGH gilts (96.4 ± 2.61 versus 102.9 ± 3.03 kg: **Table 4.3.1**). Liveweights at 175 days of age were 85.7 ± 1.64, 90.8 ± 2.22, 107.1 ± 2.20, and 114.9 ± 2.670 kg for the LIGHT-LOW, LIGHT-HIGH, HEAVY-LOW and HEAVY-HIGH gilts respectively. Average daily liveweight gain from 161 to 175 days of age was significantly lower (P < 0.01) for LOW compared to HIGH gilts: 0.48 ± 0.03 versus 1.01 ± 0.03 kg / day (**Table 4.3.1**). At 172 days of age, P2 backfat and MMD were unaffected by ADG from 161 days of age to puberty; however, LIGHT gilts had significantly lower (P < 0.01) P2 backfat (7.9 ± 0.29 versus 11.0 ± 0.36 mm) and MMD compared to HEAVY gilts (45.5 ± 0.82 versus 50.4 ± 0.84 mm; **Table 4.3.1**).

4.3.1.2 Puberty attainment

Mean days to puberty were significantly higher (P < 0.05) for LIGHT compared to HEAVY gilts, with LIGHT gilts attaining puberty approximately 8 days later than HEAVY gilts: 24.1 ± 2.38 versus 16.4 ± 2.10 days (**Table 4.3.2**). Average daily liveweight gain from 161 days of age to puberty did not significantly affect mean days to puberty or gilt age at puberty (**Table 4.3.2**). At puberty attainment, LIGHT gilts were approximately 22 kg lighter than HEAVY gilts (96.6 ± 2.56 versus 118.6 ± 2.79), and LOW gilts weighed approximately 11 kg less than HIGH gilts (**Table 4.3.2**). Average daily liveweight gain from 161 days of age to puberty was significantly lower for LOW compared to HIGH gilts (0.45 ± 0.03 versus 1.02 ± 0.04 kg / day), but was similar for LIGHT and HEAVY gilts (0.76 ± 0.09 and 0.71 ± 0.09 kg / day; **Table 4.3.2**). No relationship between age at puberty and liveweight at puberty was detected using a linear regression approach (P > 0.05; **Figure 4.3.1**).

The effect of treatment on the timing and synchrony of puberty attainment is shown in **Figure 4.3.2**. The pattern of puberty onset and the proportion of gilts attaining puberty was unaffected by ADG from 161 days of age to puberty, with 63% and 54% of HIGH and LOW gilts attaining puberty. Although a similar proportion of LIGHT and HEAVY gilts attained puberty within 7 days of the start of boar exposure (17% and 21%), a significantly smaller (P < 0.05) proportion of LIGHT gilts reached puberty by day 14 (29% versus 63%), day 21 (33% versus 75%) and day 28 (42% versus 75%) of boar exposure. At slaughter, none of the gilts that failed to exhibit visible signs of oestrus during the experimental period had CLs on their ovaries.
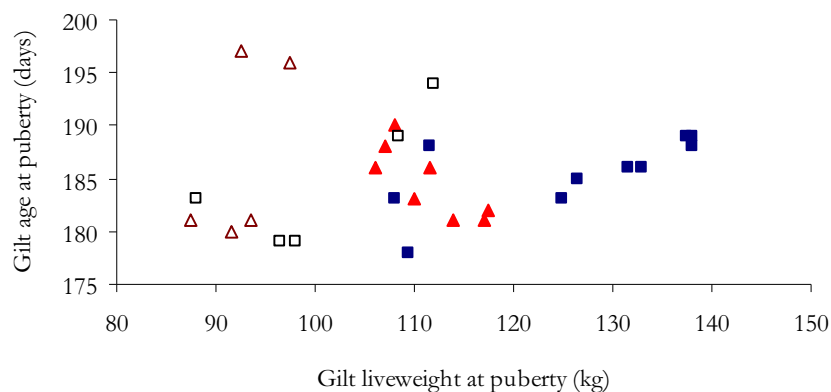
Figure 4.3.1 Relationship between age and liveweight at puberty for Experiment three. Closed squares (■) represent HEAVY-HIGH gilts, closed triangles (▲) represent HEAVY-LOW gilts, open squares (□) represent LIGHT-HIGH gilts and open triangles (△) represent LIGHT-LOW Gilts
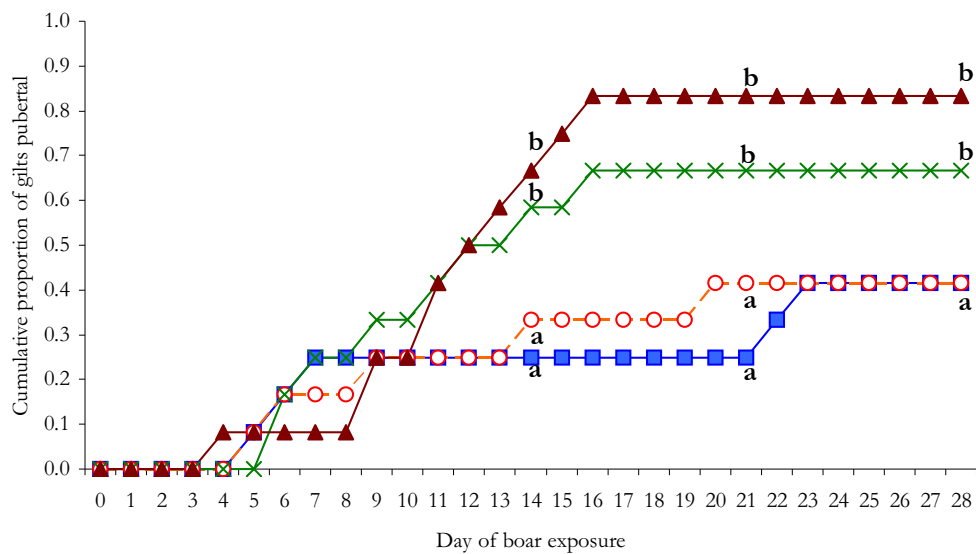
Figure 4.3.2 Cumulative proportion of gilts, in experiment three, attaining puberty in response to twice daily contact boar contact. Closed squares (■) represent LIGHT-LOW gilts, open circles (○) represent LIGHT-HIGH gilts, crosses (x) represent HEAVY-LOW gilts, and closed triangles (▲) represent HEAVY-HIGH gilts. [a,b] Significant difference between LIGHT and HEAVY gilts (P < 0.05).

Table 4.3.2 Mean days to puberty, age and liveweight at puberty for LIGHT versus HEAVY and LOW versus HIGH gilts in experiment three

Target LW at 161 days of age	Mean days-to-puberty[1]			Age at puberty (days)[1]			LW at puberty (kg)		
	Target ADG from 161 days to puberty		Pooled across target ADG	Target ADG from 161 days to puberty		Pooled across target ADG	Target ADG from 161 days to puberty		Pooled across target ADG
	LOW	HIGH		LOW	HIGH		LOW	HIGH	
LIGHT	24.5	23.8	24.1[b]	198.7	198.0	198.3[b]	92.5	100.6	96.6[a]
HEAVY	17.8	15.0	16.4[a]	192.0	189.2	190.6[a]	111.4	125.8	118.6[b]
Pooled across target LW	21.1	19.4		195.3	193.6		102.0[c]	113.2[d]	
Pooled S.E.M			*3.23*			*3.21*			*2.55*

[ab] Superscripts within column indicate significant difference; $P < 0.05$. [cd] Superscripts within row indicate significant difference; $P < 0.05$

[1] Gilts not pubertal by day 28 of boar exposure were ascribed a nominal days-to-puberty of 33 days, so $n = 12$ gilts / treatment

4.3.1.3 Reproductive parameters

Corpora lutea (CL) were observed in all of the gilts that exhibited oestrus, and on average the ovaries of LOW gilts possessed three fewer CLs than those of HIGH gilts: 15.0 ± 0.76 versus 11.6 ± 0.71; P < 0.01 (**Table 4.3.3**). Ovarian weight and total luteal weight were significantly lower (P < 0.01) for LOW compared to HIGH gilts: 14.7 ± 0.87 versus 18.2 ± 1.13 g, and 6.0 ± 0.32 versus 8.7 ± 0.61 g, respectively (**Table 4.3.3**). Liveweight at 161 days of age did not significantly affect ovulation rate, ovarian weight or luteal weight (**Table 4.3.3**). Mean CL weight was similar for LIGHT and HEAVY gilts (0.56 ± 0.04 and 0.58 ± 0.03 g) as well as for LOW and HIGH gilts (0.55 ± 0.03 and 0.59 ± 0.03 g), The uteri of LIGHT gilts were 84 g lighter than those of HEAVY gilts (428 ± 22.1 versus 512 ± 22.3 g; P < 0.01), whereas the uteri of LOW and HIGH gilts were similar in weight (491 ± 26.6 and 481 ± 31.4). The number of CL was positively correlated with ADG from 161 days of age to puberty (R^2 = 0.25; P < 0.01); but there was no relationship between days to puberty and CL number. There were no treatment effects on the proportion of mated gilts that possessed embryos following uterine flushing at 10 ± 0.2 days post mating. Embryos were recovered from 79% and 81% of LIGHT and HEAVY gilts, and 70% and 85% of LOW and HIGH gilts, respectively.

Table 4.3.3 Ovulation rate, ovarian weight and luteal weight at day 10 ± 0.3 post mating for gilts starting boar exposure at 175 days of age and mated at the pubertal oestrus in experiment three

Target LW at 161 days of age	Ovulation rate			Ovarian Weight (g)			Luteal Weight (g)		
	Target ADG from 161 days to puberty		Pooled across target ADG	Target ADG from 161 days to puberty		Pooled across target ADG	Target ADG from 161 days to puberty		Pooled across target ADG
	LOW	HIGH		LOW	HIGH		LOW	HIGH	
LIGHT	11.0	15.0	13.0	13.8	17.3	15.6	5.7	8.3	7.0
HEAVY	12.1	15.0	13.6	15.5	19.0	17.3	6.3	9.0	7.7
Pooled across target LW	11.6[a]	15.0[b]		14.7[a]	18.2[b]		6.0[a]	8.7[b]	
Pooled S.E.M			*0.89*			*1.13*			*0.56*

[ab] Superscripts within row indicate significant difference: P < 0.05

4.3.1.4 Plasma hormone concentrations

With the exception of two gilts, one each in the HEAVY-HIGH and HEAVY-LOW treatment groups (progesterone = 0.69 and 0.35 ng/ml, respectively), plasma concentrations of progesterone at first detection of oestrus were below the minimum detection limit. Concentrations of progesterone were significantly lower in plasma samples collected from LOW compared to HIGH gilts at 72 hours (3.6 ± 0.52 versus 6.5 ± 0.94 ng/ml; $P < 0.01$) and 240 hours (24.3 ± 1.87 versus 30 ± 2.77 ng/ml; $P < 0.05$) after first detection of oestrus. There was a positive correlation between progesterone concentrations at both 72 and 240 hours after oestrus and luteal weight ($R^2 = 0.15$, $P < 0.05$; and $R^2 = 0.35$, $P<0.01$), as well as between progesterone concentrations at 240 hours after oestrus and the number of CLs ($R^2=0.37$; $P < 0.01$). There was no relationship between plasma progesterone and embryo number or embryo survival.

4.3.2 Experiment Four

4.3.2.1 Gilt liveweight, growth rate, P2 backfat and MMD

As intended, gilts with a LIGHT target liveweight at 161 days of age had a significantly lower (P < 0.01) average daily liveweight gain (ADG) between 70 and 161 days of age compared to gilts with a HEAVY target liveweight: 0.51 ± 0.01 versus 0.81 ± 0.01 kg per day. At 161 days of age, LIGHT gilts were significantly lighter (P < 0.01) than their HEAVY counterparts (**Table 4.3.4**), and had significantly lower (P < 0.01) P2 backfat (7.7 ± 0.28 versus 10.9 ± 0.37 mm) and MMD (44.1 ± 0.83 versus 50.7 ± 0.70 mm).

Table 4.3.4 LW, ADG, P2 backfat and MMD of 175 day old gilts fed to attain either a LIGHT or HEAVY liveweight at 161 days of age and a Low or High liveweight gain from 161 days of age to puberty in experiment four

	LW at 161 days of age		ADG from 161 days of age to puberty		_Pooled SEM_
	LIGHT	HEAVY	LOW	HIGH	
LW at 161 days of age (kg)	77.8[a]	101.8[b]	89.2	89.5	_1.42_
ADG: 161 to 175 days of age (kg/day)	0.76	0.71	0.48[a]	1.09[b]	_0.04_
LW at 175 days of age (kg)	89.5[a]	112.6[b]	96.0[a]	106.1[b]	_2.24_
P2 backfat: 172 days of age (mm)	7.8[a]	11.9[b]	9.7	10.0	_0.48_
MMD: 172 days of age (mm)	46.1[a]	52.4[b]	50.4	48.7	_1.19_

[a,b] Superscripts, within row and main effect, indicate significant difference; P < 0.01

As a result of the imposed dietary treatments, ADG from 161 to 175 days of age was significantly higher (P < 0.01) for HIGH compared to LOW gilts (1.09 ± 0.06 versus 0.48 ± 0.04 kg / day). At 175 days of age, LIGHT gilts had

significantly lower (P < 0.01) liveweights compared to HEAVY gilts, and LOW gilts were significantly lighter than HIGH gilts (**Table 4.3.4**). P2 backfat and MMD at 172 days of age was unaffected by target ADG from 161 to 175 days of age; however, LIGHT gilts had significantly lower (P < 0.01) P2 backfat and MMD compared to HEAVY gilts: 7.8 ± 0.29 versus 11.9 ± 0.44 mm, and 46.1 ± 0.55 versus 52.4 ± 0.69 mm, respectively (**Table 4.3.4**).

4.3.2.2 Puberty attainment

Mean days to puberty were significantly higher (P < 0.05) for LOW compared to HIGH gilts (25 ± 3.0 versus 17 ± 2.03 days), and LOW gilts were approximately 8 days older at first oestrus than HIGH gilts: 200 ± 3.00 versus 192 ± 2.12 days of age; P < 0.05 (**Table 4.3.5**). LIGHT gilts reached puberty approximately 6 days later than HEAVY gilts (P > 0.05). There was a significant (P < 0.01) interaction between liveweight at 161 days of age and ADG from 161 days of age to puberty on mean days to puberty and gilt age at puberty. LIGHT-LOW gilts were significantly older at first oestrus, and mean days to puberty were significantly longer compared to LIGHT-HIGH, HEAVY-LOW and HEAVY-HIGH gilts: 207.6 ± 4.60 days of age versus 189.6 ± 2.82, 192.5 ± 2.72 and 194.0 ± 3.16 days of age (P < 0.01); and 32.8 ± 4.44 days versus 14.9 ± 2.71, 18.1 ± 2.67 and 18.6 ± 3.04 days (P < 0.01). At slaughter, none of the gilts which failed to exhibit visible signs of oestrus during the experimental period had CLs on their ovaries.

Gilt liveweight at puberty was significantly lower (P < 0.01) for LIGHT compared to HEAVY gilts (104.4 ± 2.61 versus 125.7 ± 2.23 kg), and LOW gilts were 11.5 kg lighter at first oestrus compared to HIGH gilts (**Table 4.3.5**). Between 161 days of age and puberty, ADG was significantly higher (P < 0.01) for LIGHT compared to HEAVY gilts (0.88 ± 0.09 kg/day versus 0.71 ± 0.05), and was

significantly lower (P < 0.01) for LOW compared to HIGH gilts (0.56 ± 0.02 versus 1.03 ± 0.04 kg/day). No relationship between age at puberty and liveweight at puberty was detected using a linear regression approach (P > 0.05; **Figure 4.3.3**).

Table 4.3.5 Mean days to puberty, age and liveweight at puberty for LIGHT versus HEAVY and Low versus High gilts in experiment four

Target LW at 161 days of age	Mean days-to-puberty[1]			Age at puberty (days)[1]			LW at puberty (kg)		
	Target ADG from 161 days to puberty		Pooled across target ADG	Target ADG from 161 days to puberty		Pooled across target ADG	Target ADG from 161 days to puberty		Pooled across target ADG
	LOW	HIGH		LOW	HIGH		LOW	HIGH	
LIGHT	32.8[f]	14.9[e]	23.8	207.6[f]	189.6[e]	198.6	98.8	110.0	104.4[a]
HEAVY	18.1[e]	18.6[e]	18.4	192.5[e]	194.0[e]	193.2	119.8	131.6	125.7[b]
Pooled across target LW	25.4[c]	16.8[a]		200.0[c]	191.8[d]		109.3[c]	120.8[d]	
Pooled S.E.M			*3.26*			*3.36*			*2.80*

[ab] Superscripts within column indicate significant difference; P < 0.05. [cd] Superscripts within row indicate significant difference; P < 0.05

[ef] Indicate significant interaction between Target LW at 161 days of age and Target AD from 161 days of age to puberty; P < 0.05

[1] Gilts not pubertal by day 42 of boar exposure were ascribed a nominal days-to-puberty of 47 days

Treatment affects on the timing and synchrony of puberty onset in response to boar exposure are described in **Figure 4.3.4.** The proportion of gilts that reached puberty within 42 days of the start of boar exposure was 87% and 100% for LIGHT and HEAVY gilts, and 88% and 100% for LOW and HIGH gilts (P > 0.05). However, there was a significant (P < 0.05) interaction between liveweight at 161 days of age and ADG from 161 days of age to puberty on the pattern and timing of puberty attainment. The proportion of gilts that were pubertal by day 7, 14, 35 and 42 of boar exposure was similar for LIGHT–LOW, LIGHT-HIGH, HEAVY-LOW, and HEAVY-HIGH gilts. However, a significantly lower (P < 0.05) proportion of LIGHT-LOW gilts exhibited first oestrus within 21 and 28 days of the start of boar exposure compared to LIGHT–HIGH, HEAVY–LOW and HEAVY-HIGH gilts: 25% versus 73%, 75% and 55%; and 25% versus 91%, 92% and 82%, respectively (**Figure 4.3.4**).
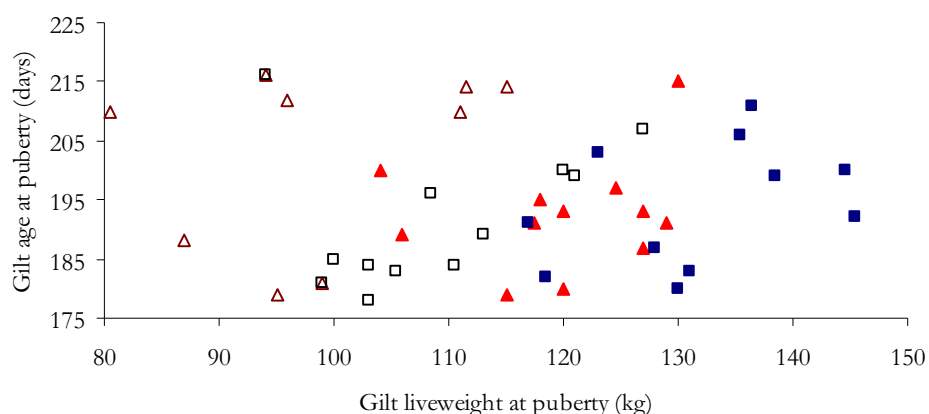
Figure 4.3.3 Relationship between age and liveweight at puberty in experiment four. Closed squares (■) represent HEAVY-HIGH gilts, closed triangles (▲) represent HEAVY-LOW gilts, open squares (□) represent LIGHT-HIGH gilts and open triangles (△) represent LIGHT-LOW gilts
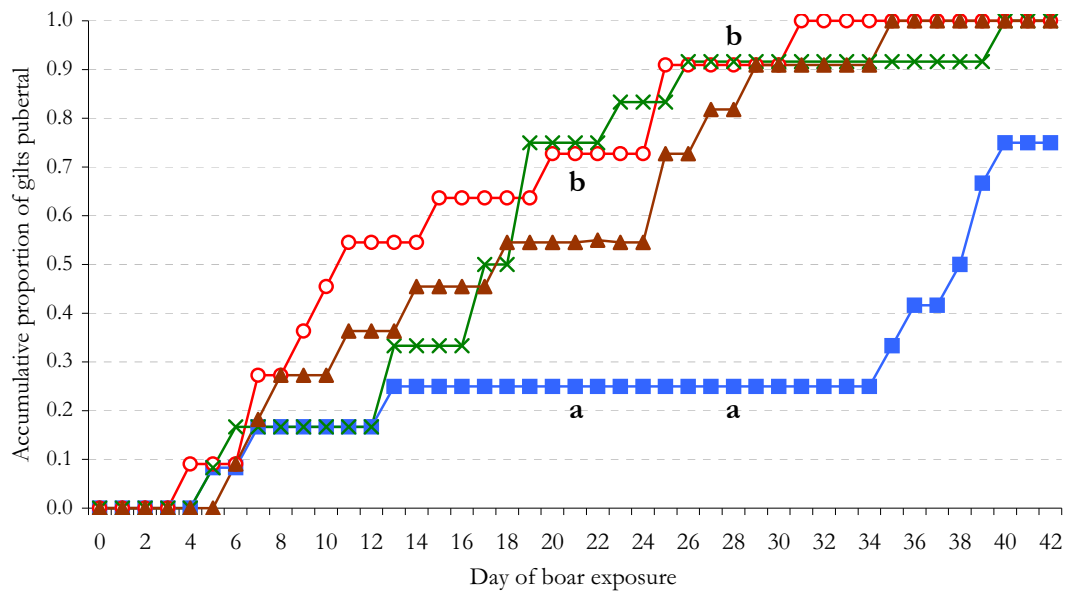
Figure 4.3.4 Cumulative proportion of gilts, in experiment four, attaining puberty in response to twice daily contact boar contact. Closed squares (■) represent LIGHT-LOW gilts, open circles (○) represent LIGHT-HIGH gilts, crosses (x) represent HEAVY-LOW gilts, and closed triangles (▲) represent HEAVY-HIGH gilts. [a,b] A significantly lower (P < 0.05) proportion of LIGHT-LOW gilts were pubertal.

4.3.2.3 Reproductive parameters

Ovulation rate at the pubertal oestrus was significantly lower for LOW compared to HIGH (13.1 ± 0.45 versus 15.3 ± 0.57; P < 0.01); but was similar for LIGHT and HEAVY gilts: 13.8 ± 0.54 and 14.4 ± 0.61; P = 0.05 (

Table 4.3.6). The number of viable embryos, embryo survival, mean CL weight, total weight of the luteal tissue and uterine weight was not significantly affected by treatments (P > 0.05). LIGHT-LOW, HEAVY-LOW, LIGHT-HIGH and HEAVY-HIGH gilts had 12.6 ± 0.69, 13.6 ± 0.59, 15.1 ± 0.63 and 15.3 ± 0.99 corpora lutea, and 9.7 ± 1.0, 10.4 ± 0.81, 11.6 ± 0.89 and 10.6 ± 0.87 viable embryos on day 22 of gestation, respectively. There was no relationship between CL number and the number of viable embryos present on day 22 of gestation; however, there was a significant (P < 0.05) negative correlation between CL number and embryo survival (R^2 = 0.13).

The ovaries of LIGHT and HEAVY gilts were of similar weight (12.0 ± 0.66 and 12.8 ± 0.38 g), while there a tendency (P = 0.07) for LOW gilts to have lighter ovaries compared to HIGH gilts (11.7 ± 0.36 versus 13.0 ± 0.55 g). Luteal weight was similar for LIGHT and HEAVY gilts (5.7 ± 0.35 and 5.5 ± 0.19 g) and for LOW and HIGH gilts (5.4 ± 0.30 and 5.8 ± 0.24 g). Mean CL weight was also similar for LIGHT and HEAVY gilts (0.41 ± 0.02 and 0.39 ± 0.02 g) and for LOW and HIGH gilts (0.41 ± 0.2 and 0.40 ± 0.02 g). Uterine weight was unaffected by treatment; the uteri of LIGHT, HEAVY, LOW and HIGH gilts weighed 350 ± 9.4, 342 ± 17.2, 362 ± 17.7 and 330 ± 9.6 g, respectively. Following post-mortem, all of the gilts that exhibited oestrus were found to have ovulated. There was no significant affect of treatment on the proportion of gilts pregnant on day 22 post-mating: 78% and 75% of LIGHT and HEAVY gilts, and 78% and 75% of LOW and HIGH gilts were pregnant (P > 0.05).

Table 4.3.6 Ovulation rate, number of embryos and embryo survival at approximately day 22 post mating for pregnant LIGHT versus HEAVY and LOW versus HIGH gilts that started boar exposure at 175 days of age and were mated at the pubertal oestrus in experiment four

Target LW at 161 days of age	Ovulation Rate			Number of embryos			Embryo Survival (%)		
	Target ADG from 161 days to puberty		Pooled across target ADG	Target ADG from 161 days to puberty		Pooled across target ADG	Target ADG from 161 days to puberty		Pooled across target ADG
	LOW	HIGH		LOW	HIGH		LOW	HIGH	
LIGHT	12.6	15.1	13.8	9.7	11.6	10.7	77.8	78.6	78.2
HEAVY	13.6	16.0	14.4	10.4	10.6	10.5	78.1	72.4	75.3
Pooled across target LW	13.1[c]	15.2[d]		10.1	11.4		78.0	75.5	
Pooled S.E.M	0.58			0.81			6.07		

[ab] Superscripts within column indicate significant difference; P < 0.05. [cd] Superscripts within row indicate significant difference; P < 0.05

4.3.3 Plasma hormone concentrations

At 158 days of age, plasma concentrations of oestradiol were significantly lower (P < 0.01) for LIGHT compared to HEAVY gilts (4.1 ± 0.30 versus 7.5 ± 0.44 pg / ml), and plasma IGF-I concentrations were significantly lower (P < 0.01) for LIGHT compared to HEAVY gilts: 37.4 ± 2.25 versus 61.9 ± 2.70 ng / ml ( ). At 173 days of age, concentrations of oestradiol tended to be lower (P = 0.053) in plasma samples collected from LIGHT compared to HEAVY gilts (6.0 ± 0.30 versus 7.8 ± 0.85 pg / ml), but were similar for LOW and HIGH gilts (**Table 4.3.8**). At 173 days of age, plasma concentrations of IGF-I were similar for LIGHT and HEAVY gilts, but were significantly lower (P < 0.01) for LOW compared to HIGH gilts (42.5 ± 2.61 versus 55.1 ± 2.04 ng / ml; **Table 4.3.8**).

Table 4.3.7 Concentrations of E2 and IGF-I in plasma collected LIGHT versus HEAVY 158 day old gilts in experiment four

	LW at 161 days of age		
	LIGHT	HEAVY	*SEM*
Plasma Oestradiol (pg / ml)	4.1[a]	7.5[b]	*0.37*
Plasma IGF-1 (ng / ml)	37.4[a]	61.9[b]	*2.42*

[ab] Superscripts within row indicate significant difference; P < 0.05

With the exception of one HEAVY-HIGH gilt (progesterone = 0.54 ng/ml), plasma concentrations of progesterone at first detection of oestrus were below the minimum detection limit. Progesterone concentrations in samples collected 72 hours after oestrus were significantly higher for HIGH compared to LOW gilts (7.1 ± 0.48 versus 4.6 ± 0.50 ng/ml; P < 0.01). Plasma concentrations of progesterone increased more rapidly between oestrus and 72 hours post oestrus in HIGH compared to LOW gilts (0.095 ± 0.007 versus 0.06 ± 0.007 ng/ml/hour; P < 0.01). There was no relationship between plasma progesterone 72 hours after oestrus and embryo number or survival. Plasma concentrations

of oestradiol at oestrus were similar for LIGHT and HEAVY gilts (10.9 ± 1.59 and 11.6 ± 1.63 pg/ml), but were significantly lower (P < 0.05) for LOW compared to HIGH gilts: 9.1 ± 1.22 versus 13.4 ± 1.87 pg/ml. There was no correlation between plasma oestradiol concentrations at oestrus and either ovulation rate or luteal weight.

Table 4.3.8 Concentrations of IGF-I and E2 in plasma collected from 173 day-old LIGHT versus HEAVY and LOW versus HIGH gilts in experiment four

Target LW at 161 days of age	Plasma IGF-1 (ng/ml)			Plasma Oestradiol (pg/ml)		
	Target ADG from 161-175 days of age		Pooled across target ADG	Target ADG from 161-175 days of age		Pooled across target ADG
	LOW	HIGH		LOW	HIGH	
LIGHT	38.2	55.0	46.6	6.0	6.0	6.0*
HEAVY	46.8	55.0	51.0	7.5	8.1	7.8*
Pooled across target LW	42.5^a	55.1^b		6.7	7.0	
Pooled SEM			*3.23*			*0.90*

[ab] Superscripts within row indicate significant difference; P < 0.01.
[*] Within column P = 0.053

4.4 Discussion

It was established in chapter three that both chronic (91 days) and acute (14 days) periods of dietary restriction reduce the growth of ovarian follicles beyond 3 mm, and impair the meiotic competence of oocytes derived from 175-day-old, pre-pubertal gilts. The studies reported in this chapter were designed to test two hypotheses: one, that nutritional intake during the period immediately preceding the start of boar exposure, as opposed to liveweight, determines the timing of the pubertal response; and two, that the negative effects of dietary restriction on ovarian follicle growth and oocyte meiotic competence would result in delayed puberty attainment and a reduction in potential litter size. These hypotheses were tested by studying the effects of the dietary regimes used in chapter three on the responsiveness of 175-day old gilts to daily boar stimulation and their potential first litter size when mated at the pubertal oestrus.

4.4.1 Puberty attainment

The frequency and duration of boar contact are important factors determining the timing of gilt response (Paterson et al., 1989); however, due to logistical limitations the frequency of boar exposure was reduced from two periods of 15 minutes each day in experiment three to one period of 20 minutes each day in experiment four. It is generally accepted that conducting boar stimulation daily for at least 15 – 20 minutes until the onset of first oestrus optimises the effectiveness of boar stimuli (Paterson et al., 1989; Hughes et al., 1990; Hughes et al., 1993; Hughes, 1994). Although more recent evidence does suggest an improvement in gilt response when boar contact is conducted thrice daily (Philip and Hughes, 1995; Hughes and Thorogood, 1999), contradiction exists within the available literature as to whether the timing of puberty attainment remains unaffected (Hughes, 1994; Philip and Hughes, 1995) or is enhanced (Hughes and Thorogood, 1999) when the frequency of boar contact is increased

from once to twice daily. In the current studies, no adverse effects of the reduced frequency of boar stimulation on the timing of puberty attainment were detected. Although the reduced frequency of boar contact utilised in experiment four was associated with an improved pubertal response of LIGHT-HIGH gilts, the timing of the pubertal response of gilts in the LIGHT-LOW, HEAVY-HIGH and HEAVY-LOW groups was similar in both experiments. It is, therefore, unlikely that the observed difference in the timing of the pubertal response of the LIGHT-HIGH gilts between the two studies was caused by the change in the application of boar contact, and as a consequence the pubertal data from both studies will be considered together in the following discussion.

Overall, the current data demonstrate that while puberty attainment in response to boar stimulation is delayed, but not prevented, following chronic, moderate restriction of feed intake, acute nutritional restriction during the 14 days prior to commencement of boar contact has little affect on the timing of the pubertal response. These data, therefore, extend previous reports of delayed puberty attainment following long-term restriction of feed intake during the pre-pubertal period (Beltranena et al., 1993; Prunier et al., 1993), but also provide the first evidence in gilts that increased liveweight and body reserves can act as a buffer against the inhibitory effects of feed deprivation on reproductive activity and puberty attainment.

The current data support previous reports (Beltranena et al., 1991) of a positive relationship between liveweight at commencement of boar stimulation and pubertal weight, with HEAVY gilts approximately 20 kg heavier at puberty onset than their LIGHT counterparts. However, as with the previous data of Rozeboom et al. (1995) and Paterson et al. (2002), it is clear from the current studies that enormous variation exists in the liveweight and growth rate at which gilts attain puberty. Therefore, in contradiction to the suggestion that puberty onset is associated with the attainment of a critical liveweight and body fatness (Gaughan et al., 1997), the current findings indicate that puberty onset

is neither triggered by, nor dependant on, the achievement of a critical threshold for liveweight (Rozeboom et al., 1995). Equally, it is possible that all gilts used in this study had passed this threshold prior to commencing boar exposure. Under conditions of maximal protein accretion rate, Beltranena et al. (1993) failed to identify a relationship between reproductive development and the level or rate of fat deposition, and the data of Burnett et al. (1988) demonstrates a decreasing influence of gilt liveweight and body tissue reserves on sexual maturity with increasing age. Together, these data support the view that minimum threshold levels of liveweight and body tissue reserves act in a permissive manner to allow, rather than actively trigger, the onset of first oestrus (Kirkwood and Aherne, 1985; Patterson et al., 2002). It has been suggested that puberty occurs long after these permissive levels have been reached (Foxcroft et al., 1996), with the timing of activation of the neuroendocrine changes responsible for puberty attainment determined by an inherent, genetically programmed pubertal clock (Plant, 2002; Ojeda et al., 2005). However, activity of the reproductive neuroendocrine axis is permitted by the availability of sufficient metabolic fuels (Schneider, 2004), and the attainment of a specific metabolic status has been proposed as a likely trigger for the onset of first oestrus in gilts (Rozeboom et al., 1995). As the animal grows, alterations in the partitioning of ingested nutrients and a reduction in basal metabolic rate increase the availability of energy for use in reproductive activity (Foster and Nagatini, 1999). This developmental change in metabolism, mediated by altered levels of metabolites and metabolic hormones, may be responsible, at least in part, for permitting the pre-pubertal increase in the frequency of GnRH release (Hall et al., 1995; Foster and Nagatini, 1999; Messer and l'Anson, 2000).

Although innate variation in the rate of reproductive development may be an important factor determining gilt age at puberty (Beltranena et al., 1993), higher rates of growth accelerate reproductive development and increase gilt responsiveness to boar stimuli (Gaughan at el., 1997). Equally, the delayed

pubertal response of LIGHT-LOW gilts, demonstrates that long-term nutritional restriction of daily liveweight gain decreases gilt sensitivity to boar stimuli. Early puberty attainment in the gilt probably depends on the ability of male-induced alterations in GnRH release to override the negative feedback effects of oestrogen (Martin et al., 1988). The exhibition of a pubertal response to boar stimulation, and indeed the timing of this response, will therefore be determined by the sensitivity of the GnRH pulse generator to negative oestrogen feedback. Advancing sexual maturity is associated with a reduction in the sensitivity of the GnRH pulse generator to negative oestrogen feedback and increased activity of the GnRH neuronal network (Barb et al., 1998; Elsaesser et al., 1998; Plant, 2002; Ojeda et al., 2005). Therefore, the efficacy with which boar contact accelerates puberty attainment ultimately depends on the sexual maturity, or physiological age, of the gilt (Eastham et al., 1986). Accepting that a slower response to boar stimulation is indicative of a less developed hypothalamic-pituitary-ovarian axis (Kirkwood and Hughes, 1979), it is suggested that LIGHT-LOW gilts were less sexually mature at 175 days of age compared to their HEAVY-HIGH and HEAVY-LOW counterparts.

When nutritionally restricted growth occurs during the later stages of development, it becomes difficult to differentiate between growth-induced and nutrition-induced changes in metabolic signals and reproductive activity (Foster and Nagatini, 1999). Specifically, reproductive activity is ranked behind growth with respect to nutrient prioritisation, and during periods of nutritional restriction, the limited nutrients available are redirected to the more important function of maintenance and growth (Cosgrove and Foxcroft, 1996; Gaughan et al, 1997; Wade and Jones, 2004). The inclusion of the LIGHT-HIGH and HEAVY-LOW treatment groups in the current studies was designed to differentiate between growth-induced and nutrition-induced changes in metabolic signals and thus responsiveness to boar stimulation. Both groups were to have a similar liveweight at the start of boar exposure, but different metabolic status due to the effects of the different acute nutritional treatments. However, the

dramatic differences between experiments in the response of LIGHT-HIGH gilts make it difficult to draw any firm conclusions as to the influence of re-alimentation, or flush-feeding, on gilt responsiveness to boar stimuli. Whereas in experiment three the timing of the pubertal response was similar for both LIGHT-LOW and LIGHT-HIGH gilts, in experiment four flush feeding reduced the interval to puberty and increased the proportion of pubertal gilts. The reasons for this difference between the two studies are unclear; and support for both sets of data can be found in the previous literature. In the absence of any significant differences in liveweight gain, Kirkwood et al. (1988) demonstrated that 10 days of flush feeding following 20 days of restricting feed intake to 60% of *ad libitum* did not reduce the interval to puberty compared to restrictively fed gilts. Similarly, re-alimentation failed to reduce age at puberty relative to long-term restrictively-fed ewe lambs (Boulanouar et al., 1995). In apparent contradiction, the more recent studies of Klindt et al. (1999) and (2001) indicate that restricting feed intake to 74% of ad libitum between 91 and 175 days of age has little effect on gilt age at puberty as long as gilts are fed *ad libitum* during the period of boar stimulation and insemination. These studies suggest a normalising effect of flush-feeding on the interval to puberty in response to boar stimulation, and Klindt et al. (2001) concluded the improved metabolic status of flush-fed gilts was responsible for their ability to attain puberty at the same time as their continuously *ad libitum* fed counterparts. However, the lack of a negative control make it impossible to determine whether the level of dietary restriction used in the studies of Klindt et al. (1999 and 2001) would have elicited a delay in puberty. Based both on the current and previous data, it remains unclear as to whether flush feeding has no effect, or a stimulatory effect, on the timing of the pubertal response to boar stimulation.

In contrast to the variable response of LIGHT-HIGH gilts, the data from both studies clearly demonstrate little effect of acute, moderate dietary restriction during the 14 days prior to, and coincident with, boar exposure on the ability of HEAVY gilts to initiate a pubertal response. Klindt et al. (2001) concluded that *ad*

libitum feeding during the pre-pubertal period produces gilts with sufficient body reserves to commence reproductive activity, demonstrating that naturally occurring reductions in feed intake, and even loss of backfat, during the boar stimulation and breeding period had little effect on the timing of the pubertal response. Together, these findings support the suggestion that body reserves can act as a buffer against the suppressive effects of feed deprivation on reproductive activity (Schneider and Wade, 1987), with the capacity of heavier animals to mobilise fatty acids from their adipose tissue essentially protecting them from the negative effects of feed deprivation. In addition, HEAVY gilts would have been physiologically more mature than their LIGHT counterparts when boar exposure commenced. The rate of protein deposition peaks between 60 and 100 kg liveweight, decreasing as the animal approaches maturity (Gaughan et al., 1997). Gilt liveweight at the start of boar contact was approximately 89 kg and 112 kg for LIGHT and HEAVY gilts, and the reduced drive for protein deposition in the HEAVY gilts may have reduced the diversion of nutrients away from reproduction. This could have been beneficial when nutrient intake was limited.

Although a suppressive effect of chronic dietary restriction on gilt response to boar stimulation is evident from the current data, it is probable, as suggested by Plant et al. (2002), that nutritional factors modulate the timing of puberty, as opposed to acting as the ultimate cue for pubertal development. Activation of the neuroendocrine changes required for the onset of puberty is likely to be genetically determined (Plant, 2002; Ojeda et al., 2005), and the data of Beltranena et al. (1993) suggest that, in the absence of boar stimulation, innate genetic variation in the pattern of LH secretion is the most important determinant of the rate of reproductive maturation. Beltranena et al. (1993) classified gilts according to whether they exhibited a high or a low frequency of episodic LH release, and a number of authors (Paterson et al., 1989; Philip and Hughes, 1995) have proposed that gilts can be divided into three categories based on whether they exhibit a fast-, slow-, or non- response to boar

originating stimuli. Just as alterations in the frequency and/or duration of boar stimulation influence the number of gilts which fall into each category, the current data suggest that chronic nutritional restriction has the greatest effect on those gilts which would otherwise have fallen into the slow or non-responders category. Specifically, whereas a significantly higher proportion of LIGHT-LOW gilts exhibited a slow (greater than 21 days) response, or failed to respond at all within the experimental period, the proportion of gilts attaining puberty within 10 days of the start of boar exposure, thus exhibiting a fast response to boar stimulation, was similar across all four dietary treatments.

The current studies also tested the hypothesis that dietary regimes which reduce the growth of 3 – 6 mm follicles would delay puberty attainment. Overall, the current data fail to support this hypothesis. Although 14 days of restricting the feed intake of HEAVY gilts significantly reduced follicle growth beyond 3 mm in chapter three, no adverse effects on puberty attainment were observed in the current studies. Equally, although dietary repletion of LIGHT gilts consistently increased the number of 3 – 6 mm follicles present at 175 days of age in chapter three, considerable differences in the timing of the pubertal response of LIGHT-high gilts were observed in the two current studies. Factors other than the number of 3 – 6 mm follicles present at the commencement of boar stimulation appear to determine the timing of the pubertal response.

The final stages of follicle development, and subsequent ovulation, are stimulated by an increased frequency of LH pulsing (Foxcroft and Hunter, 1985; Hunter and Weisak, 1990; Knox, 2005), and there is strong evidence from a variety of species, including pigs, to suggest that inadequate tonic LH secretion is the primary inhibitor of ovulation in pre-pubertal gilts (Elsaesser et al., 1974; Day et al., 1984; Foxcroft et al., 1984; Huffman et al., 1987; Elsaesser et al., 1988; Ryan et al., 1991; Dyer et al., 1990; Elsaesser et al., 1991; Plant, 2002). Certainly a suppressive effect of severe dietary restriction on LH pulsing has

been demonstrated in both pre-pubertal and cycling gilts (Prunier et al., 1991; Prunier et al., 1993; Booth et al., 1996; Ferguson et al., 2003), with delayed puberty in severely restrictively fed animals attributed, at least in part, to their suppressed pattern of tonic LH release (Bronson, 1986; Prunier et al., 1991; Schillo et al., 1992; Prunier et al., 1993; Booth et al., 1996).

The frequency of LH pulsing is controlled by the inhibitory and stimulatory effects of ovarian steroids and peptides (Levine, 1997), as well as the stimulatory effects of metabolic hormones and metabolites, in particular glucose, insulin and IGF-I (Booth et al., 1996; Foster and Nagatini, 1999). As suggested in the sheep (Martin et al, 1988), early puberty attainment in the gilt probably depends on the ability of male-induced alterations in GnRH release to override the negative feedback effects of oestrogen. Ewes with low frequency LH pulsing coincident with ram introduction fail to ovulate (Martin et al., 1980). Although moderate restriction of feed intake appears to have little effect on LH release in pre pubertal gilts (Beltranena et al., 1993) it is likely that the chronic nutritional stress experienced by LIGHT-LOW gilts in the current study would have reduced the availability of metabolic hormones to exert a stimulatory effect on GnRH, and thus LH, release. Interestingly, recent evidence also suggests the negative feedback potential of oestradiol on LH release is enhanced during periods of nutrient restriction (Renquist et al., 2008), potentially inhibiting the effectiveness with which boar-component stimuli elicit the change in LH pulsing required to induce follicle growth and ovulation, thus explaining the reduced proportion of LIGHT-LOW gilts exhibiting a pubertal response. It is also likely the increased body reserves, or greater physiological maturity, of HEAVY-LOW gilts, would have reduced the extent to which the availability of metabolic fuels and metabolites suppressed tonic LH release coincident with the start of boar exposure and inhibited the ability of male-induced alterations in LH pulsing to induce an ovulatory response. Interestingly, studies in rodent species (Bronson, 1986; Bronson and Heidemann, 1990) and gilts (Booth et al., 1996), demonstrate a rapid increase in episodic LH release

following dietary repletion of previously feed restricted pre-pubertal animals. It is, therefore, suggested that the pattern of LH pulsing would have been restored by the 14 days of high level feeding experienced by LIGHT-HIGH gilts prior to the commencement of boar contact.

4.4.2 Endocrinology

In agreement with the findings of the previous chapter, as well as other reports (Cosgrove et al., 1992; Booth et al., 1996; Simmen et al., 1998; Diskin et al., 2003), the current data demonstrate a positive association between nutrient intake and circulating IGF-I levels. Specifically, chronic dietary restriction resulted in a 40% reduction in IGF-I levels at 158 days of age, while the plasma samples collected at 173 days of age demonstrate that IGF-I decreases in response to restrictive feeding from day 161 and increases following realimentation. However, in contrast to experiment three, the current data demonstrated no residual effect of the feeding level between 70 and 161 days of age on circulating IGF-I concentrations of 173-day old gilts. Regardless of whether gilts received a high or a low feed intake between 161 and 175 days of age, IGF-I concentrations were similar for 173-day old LIGHT and HEAVY gilts in the current study. However, plasma samples were collected 13 days after the change in feeding level as opposed to 10 days in chapter three. It is probable that this delay in the timing of plasma collection explains the failure to detect a residual effect of previous feeding level, with previous studies reporting a linear, gradual decrease in plasma IGF-I from the start of dietary restriction and a gradual increase following realimentation (Booth et al., 1996; Stagg et al., 1998; Diskin et al., 2003).

Using the same dietary regimes as the study described in chapter three, the current data also demonstrate a considerable influence of feeding level on peripheral concentrations of plasma oestradiol in both 158- and 173- day old gilts. As previously discussed in chapter three, changes in peripheral oestradiol

concentrations reflect alterations in the level of ovarian secretion and / or the extent to which oestradiol is removed from the blood via the hepatic system (Camous et al., 1985; Sangsritavong et al., 2002). Possible relationships between the imposed dietary regimes and both changes in peripheral oestradiol levels and oestradiol clearance by the liver have been discussed in chapter three, and it is suggested that these also apply to the current data.

A direct association between increasing peripheral IGF-I concentrations and the onset of puberty is well established in rodent and ruminant species (Hiney et al., 1996; Pine et al., 2006). Although IGF-I has been proposed as one of the metabolic signals linking energy balance with reproductive activity and growth processes in the female pig (Booth et al., 1996; Barb et al., 2001), a relationship between peripheral IGF-I concentrations coincident with the start of boar stimulation and the timing of the pubertal response is not evident from the current data. Specifically, despite experiencing a significant reduction in peripheral IGF-I concentrations, the pubertal response of HEAVY-LOW gilts was unaffected. However, the modulating effect of IGF-I on pituitary LH release has been demonstrated in the pig (Whitley et al., 1995), and exogenous IGF-I restores age at puberty in IGF-1 deficient rats (Pine et al., 2006). It is, therefore, suggested that IGF-I levels in the current study were in excess of any permissive threshold level required to initiate the increased release of GnRH and LH required for puberty attainment.

4.4.3 Potential litter size

The second objective of the current studies was to compare the effects of the imposed dietary regimes on ovulation rate and embryo survival when gilts were mated at their pubertal oestrus. In chapter three, the level of dietary restriction used in the current studies reduced the number of 3 – 6 mm follicles present on the ovaries of 175-day old gilts and impaired the meiotic competence of the oocytes derived from these follicles. It was, therefore, hypothesised that both

chronic and acute restriction of dietary intake would reduce the potential first litter size of gilts mated at their pubertal oestrus. Partial support for this hypothesis is provided by the present data which demonstrate that both chronic and acute periods of dietary restriction significantly reduce the pubertal ovulation rate. To my knowledge, the current study is the first to investigate the effects of flush feeding on pubertal ovulation rates, and to do so using both negative (LIGHT-LOW gilts) and positive (HEAVY-HIGH gilts) controls. Therefore, although the current data extends previous reports of increased ovulation rates in flush-fed, cycling gilts (Moore et al., 1973; Beltranena et al., 1991), they are the first to demonstrate that whilst flush feeding increases the number of ova shed at the pubertal oestrus, dietary repletion merely restored the pubertal ovulation rate of LIGHT-HIGH gilts to match those of gilts on a high feeding plane throughout the study (HEAVY-HIGH gilts). These data, therefore, confirm the unsubstantiated suggestion of Kirkwood et al. (1988) that flush feeding previously restrictively-fed gilts merely serves to normalise ovulation rates.

In the present studies, moderate restriction of feed intake for at least 14 days prior to, as well as during, boar exposure resulted in an average of 2.8 fewer ova being shed at the pubertal oestrus. Previous studies involving cycling gilts demonstrate a suppressive effect of reducing the pre-mating feed intake to maintenance levels or below on ovulation rate (Flowers et al., 1989; Ashworth et al., 1999). However, in contrast to the current findings, reducing the pre-mating feed intake of cycling gilts from 2.8 to 2.1 (Almeida et al., 2000; Almeida et al., 2001; Novak et al., 2003) or from 2.6 to 1.8 (Ferguson et al., 2006) times maintenance had no affect on ovulation rate. All the gilts used in these studies had completed at least two oestrous cycles (Ferguson et al., 2006), or one oestrous cycle and an undetermined period of oral progestagen 'altrenogest' treatment (Almeida et al., 2000; Almeida et al., 2001), and were, therefore, both physiologically and sexually more mature than the gilts used in the current study. Increased physiological maturity is associated with a reduced genetic imperative for protein deposition, and as suggested in chapter two, it is likely

that the reproductive performance of younger, lighter, and less sexually mature gilts is more responsive to differences in nutritional intake.

By demonstrating the sensitivity of the final, or pre-and peri-ovulatory, stages of follicle growth to modest alterations in dietary intake, the current findings extend those of chapter three which established the suppressive effects of moderate dietary restriction on the early, non-ovulatory, stages of follicular growth. Selection of ovarian follicles for ovulation occurs continuously during the 5 – 7 day follicular phase that precedes ovulation, and the size, and morphological dynamics, of the proliferating antral follicle pool coincident with follicle selection has been identified as a probable determinant of ovulation rate in the pig (Hunter and Weisak, 1990; Cardenas and Pope; 2002; Hunter et al., 2004; Knox, 2005). In cycling gilts, alterations in feed intake affect the growth of 1 – 6 mm follicles and influence the dynamics of the proliferating pool in cycling gilts (Quesnel et al., 2000), and using the same dietary treatments as the current study, it was demonstrated in chapter three that feed restriction during the 14 days prior to boar exposure elicits a 29% reduction in the number of medium (3 – 6 mm) follicles present on the ovaries of 175 day old gilts. Although current understanding is limited, it is likely that the pubertal ovulation is triggered by a cascade of endocrine events equivalent to the follicular phase of the oestrous cycle (Esbenshade et al., 1982; Paterson, 1982; Deligeorgis et al., 1984). Boar-induced increases in LH pulse frequency likely provide the stimulus for the commencement of follicle selection (Grant et al., 1989; Hunter and Weisak, 1990; Cardenas and Pope, 2002; Knox et al., 2005), with the avoidance of atresia and progression to the final stages of follicle growth and ovulation restricted to those follicles able to make the switch from FSH to LH dependence (Knox et al., 2005). The present data suggest that nutritional effects on pubertal ovulation rate reflect alterations in the morphological development of the follicles present at the start of the first follicular phase. Fourteen days of flush feeding of cycling gilts has been associated with considerable alterations in follicular phase profiles of FSH and LH release, with

Flowers et al. (1988) reporting both an increased frequency of LH pulsing and a premature decline in FSH concentrations prior to ovulation in restrictively-fed gilts. Therefore, although not examined in this study, it is plausible that alterations in the pattern of gonadotrophin release may have contributed to the observed differences in ovulation rate.

In the pig, it takes approximately 84 days from the re-activation of primordial follicle growth to the formation of the antral cavity (McGee and Hseuh, 2000; Findley et al., 2002), with a further 20 days required for an antral follicle to reach its ovulatory size of 8 to 10 mm (Morbeck et al., 1992). Therefore, in the current study, the follicles selected for ovulation would have exited the primordial pool and commenced growing when the gilts were approximately 90 to 105 days of age. Using Merino ewes, Nottle et al. (1997) demonstrated that an eight-week period of nutritional restriction six months prior to ovulation significantly reduced the number of ova shed, and suggested that nutritional stress coincident with the re-activation of follicle growth and the early stages of the growth of follicles may have negatively affected the subsequent ability of those follicles to avoid atresia and complete folliculogenesis. However, the current data demonstrate no residual negative effects of feed restriction between 70 and 161 days of age on the ovulation rate of LIGHT-HIGH gilts. Therefore, as with studies involving post-pubertal gilts in which dietary intake during follicle selection appears to be a critical determinant of ovulation rate (Prunier and Quesnel, 2000), the current findings suggest that nutritional effects on pubertal ovulation rate reflect alterations in the follicle's final stages of growth.

Importantly, despite impairing oocyte developmental competence in chapter three, the current data demonstrate that chronic and acute periods of moderate dietary restriction do not reduce either the absolute number of embryos or the number of corpora lutea represented by viable embryos on day 22 of gestation. These data provide the first evidence that despite altering the number of ova shed at the pubertal oestrus, moderately restricting feed intake during the pre-

pubertal period has no effect on the number of embryos surviving to day 22 of gestation. Equally, the failure of flush feeding to increase embryo number appears to contradict the previous report of larger first litter sizes when flush-fed gilts are mated at their pubertal oestrus (Rhodes et al., 1991). Although previous studies suggest that embryo survival will be reduced when cycling gilts are fed at maintenance as opposed to three times maintenance prior to mating (Ashworth et al., 1999a; Ferguson et al., 2003), the level of dietary restriction imposed in the current study provided gilts with approximately 2.2 to 2.5 times their maintenance requirement, compared to those in the high intake groups which were fed at 3.0 to 3.5 times maintenance. Previous reports of the effect of similarly modest alterations of pre-mating feed intake during the oestrous cycle prior to ovulation have produced conflicting results. Specifically, while Almeida et al. (2000) demonstrated that reducing the feed intake of cycling gilts from 2.8 to 2.1 times maintenance immediately prior to oestrus reduces the number of embryos surviving to day 28 of gestation, in the more recent study of Ferguson et al. (2006), feeding gilts at maintenance, 1.8 times maintenance or 2.6 times maintenance had no effect on the number or proportion of embryos surviving to day 27 of gestation. Interestingly, the imposed dietary treatments of Almeida et al. (2000) and Ferguson et al. (2006) elicited no changes in ovulation rate. In contrast, the present data clearly demonstrates that while pubertal ovulation rates are sensitive to modest alterations in pre-pubertal feed intake, the number of embryos surviving the pre- and peri-implantation period appear to be unaffected by subtle changes in pre-ovulatory feed intake or indeed ovulation rate

The effect of pre-mating nutrition on embryo survival reflects alterations in the developmental competence of the oocytes released at ovulation (Zak et al., 1997; Ferguson et al., 2003). A large portion of pre-implantation embryo losses have been attributed to the ovulation of meiotically-immature oocytes (Geisert and Schmitt, 2002), and Ferguson et al. (2003) demonstrated that the nutritional regimes which reduced embryo survival in the study of Ashworth et al. (1999a)

caused a significant reduction in the meiotic competence of oocytes derived from the presumptive ovulatory follicle pool. Using the same dietary regimes used in the current study, the findings presented in chapter three demonstrate that restrictive feeding for at least 14 days significantly reduced the proportion of oocytes in the proliferating follicle pool of 175 day old gilts that were able to reach metaphase II (MII) *in vitro* compared to oocytes derived from gilts on a high feeding plane. It was, therefore, expected that this would be associated with a reduction in embryo survival, but this was not found. This may be because nutritional restriction affected neither cleavage nor blastocyst formation rates following parthenogenetic activation in chapter three. However, accumulation of the transcription factors vital for the completion of the early stages of embryogenesis occurs during the latter stages of antral follicle growth (Hytell et al., 1997; Krisher, 2004), and it was suggested that nutritional effects on the accumulation of these transcription factors may not become evident until the final, or peri-ovulatory, stages of follicle growth. It is, therefore, unexpected that restrictive feeding did not significantly reduce either the number or proportion of embryos surviving the implantation period in experiment four.

Differences in oocyte maturation reflect alterations in follicular function, and nutritional effects on meiotic competence appear to be mediated by metabolic influences on somatic cell differentiation and steroidogenesis (Cosgrove et al., 1992; Quesnel and Prunier, 2000; Webb et al., 2004). In particular, the presence of a meiotically-immature oocyte likely results from altered expression, or sensitivity, of somatic cell gonadotrophin receptors. It is, therefore, suggested that the proportion of follicles containing a meiotically-immature oocyte would possess insufficient LH receptors to avoid atresia and be selected for ovulation, thus explaining the unexpectedly low embryo mortality rates of LIGHT-LOW and HEAVY-LOW gilts. Equally, the extent of embryo mortality that occurs during the first 25 days of gestation may also be determined by the number of foetuses the uterus is capable of supporting. Irrespective of pre-mating feed

intake, the current data demonstrate an inverse relationship between ovulation rate and embryo survival. Brooks and Cole (1974) proposed the existence of a maternal limit to litter size, suggesting that when the number of embryos exceeds this limit, otherwise viable embryos would fail to successfully complete implantation and would die. The existence of such a maternal limit could explain the unexpectedly low number of embryos present in the uteri of HEAVY-HIGH and LIGHT-LOW gilts, as well as previous reports (Brooks and Cole, 1974) of an apparent dissociation between nutritionally-induced increases in ovulation rate and the number of embryos surviving the implantation period. There is growing evidence that uterine capacity is in fact limited despite its elastic nature, with a number of studies involving sows demonstrating impairments to foetal development when the number of foetuses approaches the maximum uterine capacity (Vallet et al., 2002). Studies conducted primarily in sows also suggest the limiting effects of uterine capacity do not begin to affect litter size until after day 30 of gestation (Ford et al., 2002); however, it is conceivable the smaller size and immature status of the pubertal uteri result in competition for uterine space and nutrients between littermates occurring earlier in gestation.

4.4.4 Post-ovulatory progesterone profiles

The influence of post-mating nutritional intake on embryo survival appears to be mediated by alterations in peri-ovulatory progesterone concentrations (Jindal et al., 1996 and Jindal et al., 1997). Nutritional effects on the physiological development of recruited and pre-ovulatory follicles may also alter production and/or secretion of progesterone by the corpora lutea (Hunter and Weisak, 1990; Mao and Foxcroft, 1998; Almeida et al., 2000). Consistent with the data of Almeida et al. (2000), in which high pre-mating feed intakes increased peri-ovulatory progesterone without affecting ovulation rate, in my study progesterone concentrations 72 hours after first detection of oestrus were 44% and 35% lower for LOW gilts in experiments three and four, respectively. The

data from experiment three demonstrate a positive relationship between progesterone concentrations and both the number of corpora lutea and total weight of the luteal tissue, suggesting the higher progesterone concentrations of LIGHT-HIGH and HEAVY-HIGH gilts reflect the increased number of corpora lutea of these animals. Further, using the same experimental gilt model as Almeida et al. (2000), Novak et al (2003) demonstrated no affect of pre-mating feed intake on progesterone concentrations in the ovarian, oviductal, or jugular veins during the 72 hours after ovulation.

Peripheral progesterone concentrations reflect the balance between synthesis and secretion of progesterone by the luteal cells and metabolic clearance rate (Jindal et al., 1996; Mburu egt al., 1998). Although acute changes in feed intake affect clearance of steroids (Parr, 1991; Ashworth et al., 1999; Miller et al., 1999; Sangsritavong et al., 2002), differences in clearance rate between the treatment groups are unlikely. However, the rate of follicle growth is affected by nutritional intake, suggesting that timing of ovulation relative to the start of behavioural oestrus may have been different for HIGH and LOW gilts. Specifically, the more rapid increase in progesterone concentrations observed for HIGH gilts during the first 72 after first detection of behavioural oestrus could reflect an earlier ovulation in these gilts. Although changes in peri-ovulatory progesterone concentrations, induced by feeding level or exogenous supplementation, appear to have a profound affect on embryo survival (Jindal et al., 1996; Jindal et al., 1997; Vallet et al., 1998; Vallet and Christenson, 2004), the current findings demonstrate no relationship between plasma progesterone 72 hours after oestrus and embryo number or survival. Consequently, it is suggested that further study is required not only to increase understanding of the mechanisms mediating the influence of pre-ovulatory feed intake on luteal progesterone production, but also to investigate the implications of these changes on embryo development.

4.4.5 Conclusion and Implications

In conclusion, the findings from these studies suggest that the influence of nutritional restriction on sexual maturation, as expressed by gilt response to boar stimulation, depends on the timing and duration of nutritional manipulation. Chronic, but not acute, restriction of dietary intake was found to decrease the proportion of gilts capable of initiating a pubertal response to boar stimulation. These findings extend those of previous studies demonstrating that chronic dietary restriction delays, but does not prevent, puberty attainment, by indicating that increased body reserves and physiological maturity essentially buffer the gilt against the suppressive effects of nutritional stress on reproductive activity.

In contrast to previous studies investigating the effects of moderate dietary restriction on potential litter size, the current data demonstrated that despite a significant reduction in pubertal ovulation rate, moderate reductions in pre-mating feed intake do not reduce the number, or proportion, of embryos surviving the implantation period compared to high level feeding. Although the negative impacts of maintenance feeding on oocyte quality are convincing, it is likely in the group housing systems common within the pig industry that naturally-occurring variations in feed intake will only result in moderate reductions in dietary intake. Further studies are recommended to establish the effect of moderate dietary restriction on litter size in gilts as the current data suggest possible differences in the mechanisms controlling the litter size of gilts mated at the pubertal oestrus compared to gilts mated at subsequent heats.

Chapter 5   General discussion and Conclusion

5.1 Introduction

Due to decreases in the longevity of breeding sows, replacement gilts and early parity sows constitute a large, and increasing, proportion of modern breeding herds (Hughes and Varley, 2003; Levis, 2005). Breeding herd profitability has, therefore, become increasingly dependant on the efficiency of gilt management strategies as well as litter size at first farrowing. In particular, the ability to reduce the age at which gilts attain puberty, and therefore reduce the age at first mating, is extremely beneficial to the productivity of the breeding herd (Brooks and Smith, 1980; Evans and O'Doherty, 2001). However, the failure to reach puberty, delayed or unpredictable timing of the first oestrus, and small first litter sizes are common within cohorts of replacement gilts (Whittemore, 1996; Evans and O'Doherty, 2001). Towards addressing these problems and improving gilt reproductive performance this thesis had two primary aims; one, to identify whether the puberty stimulation and mating strategies developed for the genotypes of 20 to 30 years ago are suitable for today's heavier yet leaner genotypes; and two, to better understand the influence of pre-pubertal growth rate and metabolic status on reproductive maturation, puberty attainment and potential litter size. In total, four experiments are described in this thesis and, in the present chapter the results from all experiments will be integrated in an attempt to better understand the mechanisms controlling fertility and reproductive performance of the replacement gilt. Finally, areas requiring further study will also be identified.

5.2 Puberty attainment and potential litter size: effects of age and oestrus number

Exposure to a mature boar is the most effective and widely used method of stimulating and controlling precocious puberty in gilts. The efficacy with which boar contact accelerates puberty attainment ultimately depends on the sexual maturity, or physiological age, of the gilt (Eastham et al., 1986; Hughes et al.,

1990). The present data demonstrate that delaying the start of boar exposure until 182 or 203 days of age resulted in a more rapid pubertal response, with greater synchrony between contemporary females. This contradicts the findings of studies conducted in the 1970s and 80s that optimal timing and synchrony of puberty attainment occurs when gilts are 160 days old at commencement of boar contact, with no improvement in gilt response evident when boar stimulation occurred after 160 days of age (Hughes and Cole, 1976; Hughes and Varley, 1980; Hughes, 1982; Kirkwood and Hughes, 1979; Eastham et al., 1986).

Accepting that a faster gilt response to boar stimulation is indicative of a more developed hypothalamic-pituitary-ovarian axis (Kirkwood and Hughes, 1979; Deligeorgis et al., 1984), the present results indicate that physiological maturation occurs later than with previous genotypes. Therefore, despite being approximately 40% heavier at any given age compared to the gilts used in the studies conducted 20 to 30 year ago (eg Kirkwood and Hughes, 1979; Eastham et al, 1986), the gilts used in the present study were later maturing, supporting the suggestion that selection for increased lean growth has, inadvertently, also been a selection against early sexual maturation. Therefore, I propose that improving, or at least maintaining, the efficacy of boar stimulation requires the modification of current recommendations to suit today's later maturing genotypes.

The present study found that potential litter size was unaffected by the age of gilts at the start of boar exposure, puberty attainment or mating, contradicting previous reports (Paterson and Lindsay, 1980) of poor reproductive performance when puberty is stimulated at a young age. Considering the chronological delay in sexual maturation that is evident from the timing of the pubertal response, it is surprising that ovulation rates were not lower in gilts mated at a younger age. However, current genotypes have also undergone selection for increased litter size, and hence ovulation rate. In fact the present

results suggest that modern gilts have higher ovulation rates than their counterparts of 20 – 30 years ago (Brooks and Smith, 1980; Paterson and Lindsey, 1980; Archibong et al., 1992), shedding approximately 3 more ova at the pubertal oestrus. The size of the proliferating follicle pool coincident with follicle selection was recognised as a key determinant of ovulation rate in the pig by Hunter and Weisak in 1990, suggesting that selection for an increased ovulation rate may also have been a selection for an increased antral follicle pool. Although puberty attainment in response to boar contact can only occur following final maturation of the hypothalamic-pituitary-ovarian axis, Camous et al., (1985) observed little change in the size and dynamics of the antral follicle pool during the 40 days prior to puberty attainment. I, therefore, suggest that the number of ova shed by young, and theoretically physiologically less mature, gilts is no longer limited by the size or maturity of the antral follicle pool.

However, mating gilts at their second oestrus did tend to increase potential litter size, with a numerical, but not significant, increase in both ovulation rate (0.6 ova) and embryo number (1.0 embryo) evident when first mating was delayed until the second oestrus. This finding is consistent with previous reports of marginal, but not significant, improvements in first litter size when first mating occurs at the second as opposed to first oestrus (Young et al., 1990b). Equally, other published data would suggest that further improvements in embryo number would have been observed in the present study if mating had been delayed until the third oestrus (Archibong et al., 1992; Koenig and Stormshak, 1993). The physiological basis for the reported increase in ovulation rate and embryo survival when mating is delayed beyond the pubertal oestrus is not known. Although a beneficial effect on embryo survival of priming the uterus with progesterone has been demonstrated in ewes (Moore et al., 1985), a causative effect of differences in the intra-uterine environment between gilts mated at their pubertal or third oestrus on embryo survival is not supported by the study of Archibong et al. (1992). However, delaying first mating until the third oestrus has been associated with an increase in the quality of the ova

shed, as well as reduced incidences of abnormal embryo development (Archibong et al., 1992; Koenig and Stormshak, 1993). Further, at comparable times relative to oestrus expression, Smith et al. (1992) reported differences in the growth of the pre-ovulatory follicle pool between the pubertal and third oestrus. Specifically, follicle growth appeared to be slower, or delayed in pubertal gilts, possibly resulting in alterations in the quality of the ova shed, differences in follicle luteinisation, as well as alterations in the timing of ovulation relative to the start of oestrus, and thus mating (Hunter and Weisak, 1990). Bagg et al. (2004) observed no improvements in oocyte maturation in vitro following the completion of the first oestrus. Taken together, these data suggest that altered follicular growth or alterations in the timing and / or synchrony of ovulation are responsible for improvements in embryo number when first mating is delayed beyond the pubertal oestrus.

Differences in follicle growth between the pubertal and subsequent oestruses may also explain the demonstrated increase in ovulation rate in both the current, and previous, studies (Archibong et al., 1987; Archibong et al., 1992). Although the size of the proliferating follicle pool has been identified as one determinant of ovulation rate in the pig (Hunter and Wiesak, 1990), it might be more accurate to suggest that the heterogeneity of the antral follicle pool determines ovulation rate. Specifically, the oestrogenic capacity of the largest follicles is believed to stimulate the initiation of the pre-ovulatory LH surge, with the size of the ovulatory pool determined by the number of smaller follicles capable of responding with increased growth to the LH surge (Hunter and Weisak, 1990; Hunter et al., 2004; Knox, 2005). It is plausible that heterogeneity of development within the proliferating follicle pool is decreased as a result of the synchronising effects of the first (pubertal) luteal phase on follicle growth, with the increased synchrony in development responsible for the increased ovulation rate observed when mating is delayed beyond the pubertal oestrus.

Although both the current, and previous data (Hughes and Cole, 1976; Kirkwood and Hughes, 1979; Eastham et al., 1986) suggest that gilt age at start of boar exposure is a critical determinant of the timing and synchrony of the pubertal response, nutritional restriction of pre-pubertal growth rate can delay the attainment of puberty (Beltranena et al., 1993; Prunier et al., 1993). The negative effect of severe dietary restriction on ovulation rate and embryo survival is well established (Prunier and Quesnel, 2000), and I found that the potential litter size of gilts commencing boar exposure at 161 days of age was positively related to their liveweight at 18 weeks of age as well as at mating. This finding suggests the reproductive performance of young, and therefore less sexually mature, gilts might be more responsive to differences in nutritional intake than their older counterparts. Based on this finding, I suggest that if management practices continue to promote early-breeding strategies then improving gilt reproductive performance requires increased understanding of the influence of pre-pubertal nutrition and growth characteristics on sexual development and maturation.

5.3 Ovarian maturity, puberty attainment and potential litter size: relative effects of chronic versus acute dietary restriction

The influence of different periods and durations of nutritional restriction during the pre-and peri- pubertal period on the responsiveness of gilts to boar stimulation is poorly understood. The influence on ovarian follicle growth, oocyte competence and potential litter size of moderate restrictions of feed intake which still permit animal growth to occur but are less than *ad libitum* intake have not been established. Towards addressing the effects of these issues, this component of the thesis had three primary aims: one, to compare the effects of long- (chronic) or short- (acute) term nutritional changes on metabolic signals and the timing of the pubertal response to boar stimulation; two, to investigate the relationship between the dynamics of the proliferating antral follicle pool at boar stimulation and the timing of the pubertal response,

as well as ovulation rate and embryo survival following mating at the pubertal oestrus; and three, to determine the effects of moderate, as opposed to severe, nutritional restriction during different periods of follicular growth on oocyte-developmental competence, the number of ova shed and embryo survival at the pubertal oestrus. These aims were addressed in two parts, both using the same experimental model. Part one, addressed in Chapter Three, determined the effects of long- (chronic) and short- (acute) term moderate restriction on ovarian development and oocyte developmental competence in 161- and 175-day old, pre-pubertal gilts. Part two, described in Chapter Four, determined the effects of the same dietary treatments on the timing of puberty attainment and potential litter size.

Overall, the data presented in this thesis demonstrate that chronic, moderate dietary restriction during the pre- and peri-pubertal period severely impairs ovarian development, decreases the number of follicles growing beyond 3 mm, and reduces the proportion of follicles reaching metaphase II *in vitro*. Previous studies have demonstrated impaired follicle growth when the feeding level of pre-pubertal gilts is restricted to maintenance levels for 14 days (Booth et al., 1996); however, the current results demonstrate that even subtle reductions in feed intake severely impair growth of the follicle-oocyte complex. It is also noteworthy that the high level of feeding (2.6 times maintenance) used by Ferguson et al. (2003) to establish the negative effects of maintenance feeding on the ability of oocytes to reach metaphase II *in vitro* is similar to the low feeding level used in the current study. This suggests that nutritional influences on oocyte developmental competence occur as dietary intake is increased from maintenance all the way through to 3.3 times maintenance.

Whether marginal reductions in feed intake would impair ovarian function of more mature, cycling gilts or multiparous sows was not investigated in the current study. It is possible the impact of moderate dietary restriction is reduced with increasing physiological maturity as the imperative for protein

deposition and growth decreases. In contrast to previous studies focusing on cycling gilts (Almeida et al., 2000; Almeida et al., 2001; Novak et al., 2003; Ferguson et al., 2006), the present results demonstrate that dietary restriction, both chronic and acute, resulted in 2.8 less ova being shed at the pubertal oestrus. Reduced ovulation rates following restrictive feeding have been associated with a reduced frequency of LH pulsing (Flowers et al., 1988). GnRH release is controlled primarily by the inhibitory and stimulatory effects of ovarian steroids, peptides and metabolic fuels (Levine, 1997). Activity of the neuronal subpopulations responsible for GnRH, and thus indirectly for LH, release are sensitive to changes in the availability of metabolic fuels (Wade and Jones, 2004; Gamba and Prolong, 2006). In addition to developmentally induced alterations in the prioritisation of ingested nutrients and metabolic status (Plant, 2002), the sensitivity of LH secretion to oestrogen inhibition also appears to decrease with increasing gilt age (Berardinelli et al., 1984). It is possible that a higher sensitivity of the hypothalamus to oestrogen inhibition in younger gilts may increase their sensitivity to reductions in nutritional intake compared with older animals.

In addition to impairing ovarian development and reducing pubertal ovulation rate, chronic dietary restriction during the pre-pubertal period delayed puberty attainment. This finding is consistent with previous studies (Beltranena et al., 1993; Prunier et al., 1993), and demonstrates that reproductive activity is reduced, or postponed, during periods of moderate nutritional restriction, with the limited nutrients available most likely redirected to the more important functions of maintenance and growth (Cosgrove and Foxcroft, 1996; Gaughan et al., 1997; Wade and Jones, 2004). The efficacy with which boar contact accelerates puberty attainment reflects the maturity of the hypothalamic-pituitary-ovarian axis (Kirkwood and Hughes, 1979), suggesting that chronic dietary restriction delays sexual maturation.

The present data confirm previous reports (Cosgrove et al., 1992; Booth et al., 1996; Hazeleger et al., 2005) of a stimulatory effect of dietary repletion on follicle growth. However, the ovaries of LIGHT-HIGH gilts contained approximately 35% fewer medium follicles than those of HEAVY-HIGH gilts, indicating a negative effect of long-term dietary restriction on subsequent follicular development. Nottle et al. (1997) suggested that nutritional stress coincident with the reactivation of follicle growth and the early stages of growth may negatively affect the ability of follicles to avoid atresia and successfully complete folliculogenesis, that is to ovulate. However, it is more likely that differences in metabolic status between LIGHT-HIGH and HEAVY-HIGH gilts, as demonstrated by differences in peripheral IGF-I, are responsible for the reduced follicular growth in LIGHT-HIGH gilts. A positive relationship between the proportion of oocytes maturing to metaphase II *in vitro* and dietary intake during the period immediately prior to collection is evident from the current data. Specifically, the high level feeding, regardless of the duration, increased the proportion of oocytes maturing to metaphase II, supporting the suggesting that exposure to moderate nutritional stress earlier in follicular development has no residual negative effects on development of the follicle-oocyte complex.

Acute, moderate dietary restriction of previously well-fed gilts during the period immediately prior to, and coincident with, boar exposure did not retard the pubertal response. This finding suggests that increasing gilt liveweight and body reserves provides a buffer against the inhibitory effects of feed deprivation on puberty attainment. Klindt et al. (2001) concluded that naturally occurring reductions in feed intake during the boar stimulation and breeding period had little effect on the pubertal response of gilts, provided they possessed sufficient body reserves. Increased liveweight is also associated with an increase in physiological maturity, with the rate of protein deposition decreasing beyond 100 kg liveweight (Gaughan et al., 1997). The resulting reduction in the diversion of nutrients towards protein deposition and away from reproductive processes will be beneficial when nutrient intake is limited.

The effect of flush feeding lighter, and thus physiologically less mature, gilts on responsiveness to boar stimulation was also investigated in the two experiments described in Chapter Four. However, due to differences between the results obtained from these experiments, as well as contradiction within the available literature, it is not possible to confirm categorically whether flush-feeding affects the timing of the pubertal response. Therefore, the influence of re-alimentation of previously feed-restricted gilts is an issue that requires further investigation.

In the experiments described in Chapters Three and Four, the principal plasma indicator measured to determine metabolic state was IGF-I and, consistent with previous reports (Cosgrove et al., 1992; Booth et al., 1996), the current results demonstrate a positive association between nutrient intake and circulating IGF-I concentrations. Across all three experiments, IGF-I decreased in response to chronic dietary restriction and increased following realimentation. An association between lower plasma IGF-I concentrations and reduced ovarian follicle growth is evident both from the current and previous studies (Cosgrove et al., 1992; Booth et al., 1996). A stimulatory effect of IGF-I on antrum formation, steroidogenesis and follicle growth is evident in pigs (Mao et al., 2004), with changes in the bioavailability of intrafollicular IGF-I, and associated binding proteins, regulating the responsiveness of follicles to gonadotrophins (Guidice et al., 2000; Webb et al., 2004). It is therefore suggested that the decreased levels of IGF-I observed in restrictively fed gilts reduced the sensitivity of granulosa and theca cells to gonadotrophins, impairing the proliferation and steroidogenic capacity of the follicle wall, thus suppressing follicular growth and impairing oocyte developmental competence. A direct association between increasing IGF-I concentrations and puberty onset in rodent and ruminant species is also well established (Hiney et al., 1996; Pine et al., 2006). Exogenous IGF-I restores a normal age at puberty attainment in IGF-I deficient rats (Pine et al., 2006), and IGF-I is known to modulate pituitary LH release in pigs (Whitley et al., 1995). However, the timing of the pubertal

response in the current studies was not related to IGF-I concentrations coincident with the commencement of boar contact, suggesting that IGF-I levels were in excess of any threshold level required to permit the elevation in GnRH and LH release necessary for puberty to occur.

In order to obtain an indication of ovarian activity, oestradiol levels were measured in plasma samples collected in experiments two, three and four. Consistent with previous reports (Miller et al., 1999; Sangsritavong et al., 2002), the dietary regimens used in my studies resulted in significant differences in oestradiol in the plasma collected from 161- and 175-day old gilts. Peripheral oestradiol concentrations reflect ovarian secretion, which in turn is related to both the number and oestrogenic capacity of the follicles present on the ovary, as well as the rate at which oestradiol is cleared from the blood by the liver (Camous et al., 1985; Boukhliq et al., 1996; Sangsritavong et al., 2002). Although comparisons between studies should be treated with caution, treatment effects on peripheral oestradiol concentrations were similar across all three experiments described in Chapters Three and Four, suggesting the ovarian follicle populations of the 161- and 175- day old gilts used in experiments three and four would have been similar to their counterparts used in experiment two. It is probable that in the current studies both chronic and acute periods of moderate feed restriction elicited a significant reduction in the number of medium follicles present on the ovaries. Equally, the stimulatory effect of re-alimentation on follicular growth is evident from the data presented in Chapter Three, and is likely to have occurred in the experiments detailed in Chapter Four. Therefore, the timing of the pubertal response to boar contact does not appear to be affected by the number of follicles greater than 3 mm present on the ovary coincident with boar exposure. These findings suggest that differences in the sensitivity of the hypothalamic-pituitary ovarian axis to the negative feedback effects of oestrogen, rather than ovarian responsiveness to LH, determine the ability of the gilt to attain puberty in response to boar contact.

Pubertal ovulation rates decreased or increased in response to acute restriction or repletion, respectively, of dietary intake, demonstrating the sensitivity of the final, or pre- and peri- ovulatory, stages of follicle growth to modest, acute alterations in feeding level. An average of 2.8 fewer ova were shed at the pubertal oestrus when dietary intake was reduced during the period prior to, as well as during, boar exposure. Conversely, an acute increase in dietary intake, or flush feeding, restored pubertal ovulation rates to match those of gilts on a high-feeding plane throughout the study. To my knowledge, this is the first study to investigate the effects of flush feeding on pubertal ovulations rates using both negative and positive controls, and therefore, to confirm the suggestion (Kirkwood et al., 1988) that flush feeding merely serves to normalise ovulation rate. The present results also demonstrate no residual negative effects of feed restriction during the early stages of follicle growth on ovulation rate. This suggests that nutritional effects on pubertal ovulation rate reflect alterations in the follicle's final stages of growth. Pubertal ovulation rate also appears to be positively related to the number of medium follicles present on the ovary coincident with the start of boar exposure. Despite limited understanding, it can be assumed that the pubertal ovulation is triggered by a cascade of endocrine events equivalent to the follicular phase of the oestrous cycle. The size of the proliferating follicle pool at the time of follicle selection has previously been identified as one probable determinant of ovulation rate in the pig (Hunter and Weisak, 1990). In support of this, the current data indicate that nutritional effects on pubertal ovulation rate reflect alterations in the morphological development of the follicles present at the start of the first follicular phase.

Although acute dietary restriction reduced the proportion of oocytes derived from medium follicles that reached metaphase II *in vitro* and decreased the total number of ova shed at the pubertal oestrus, it unexpectedly did not reduce the number of viable embryos present on day 22 or embryo survival. The present results contrast with those of previous studies demonstrating that

nutritional regimes which cause a significant reduction in the proportion of presumed ovulatory oocytes that reach metaphase II *in vitro* also reduce embryo survival (Ashworth et al., 1999; Ferguson et al., 2003; Ferguson et al., 2006). However, the acquisition of meiotic competence does not signify complete developmental competence (Moor et al., 1990), and it has been suggested that the ability to develop to the blastocyst stage is the most accurate indicator of oocyte quality (Sirard et al., 2006). Accumulation of the specific transcription factors required for fertilisation and early embryo development occurs during the final stages of follicle growth, namely during the peri-ovulatory period and following luteolysis (Hytell et al., 1997; Krisher, 2004). Consequently, the failure of nutritional restriction to reduce the cleavage or blastocyst formation rates of oocytes in Chapter Three could be attributed to the early growth stage of the follicles from which they were derived. Therefore, the lack of an effect of acute dietary restriction on the number and proportion of embryos surviving the pre- and peri-implantation period was unexpected.

Nutritional effects on oocyte quality are mediated, at least in part, by the influence exerted by metabolic factors on somatic cell differentiation and steroidogenesis (Cosgrove et al., 1992; Quesnel and Prunier, 2000; Webb et al., 2004). In particular, synergies exist between the insulin-related regulatory system and the expression, or sensitivity, of somatic cell gonadotrophin receptors (Giudice, 1992; Poretsky et al., 1999; Hunter et al., 2004). Selection of follicles into the ovulatory pool is restricted to those smaller follicles with sufficiently high somatic cell expression of LH receptors to respond to the preovulatory LH surge (Foxcroft and Hunter, 1985; Knox, 2005). It is suggested that those follicles containing meiotically-incompetent oocytes would possess insufficient LH receptors to make the switch from FSH to LH dependence, thus avoiding atresia and progressing to the ovulatory stage. This could explain the unexpectedly low embryo mortality rates observed following moderate restriction of pre-mating feed intake, with only follicles containing meiotically competent oocytes being selected for ovulation.

Increased developmental heterogeneity within cohorts of shed ova could also explain the reduced embryo survival observed in gilts on a high feeding level prior to mating. Flowers et al. (1989) reported a premature decline in FSH concentrations prior to ovulation in restrictively fed gilts. Consequently, it is possible that restrictive feeding reduced the duration of follicle selection, which in addition to reducing the number of follicles selected may also have reduced the morphological heterogeneity of the ovulatory follicle pool. It is well established that the ovulatory follicle pool consists of a morphologically and biochemically heterogenous population (Hunter and Weisak, 1990; Hunter et al., 2004), characterised by asynchronies in follicle diameter, granulosa cell number and oocyte development (Grant et al., 1989; Pope et al., 1990; Xie et al., 1990). If high level feeding increased the duration of follicle selection, as is suggested by the increased ovulation rate, then the heterogeneity of the ovulatory follicle pool would also be expected to increase. Disparities in follicle development and oocyte maturity at ovulation have been identified as the principal cause of asynchronous development between litter-mates during the first 12 days of gestation, with later ovulated, or less mature, oocytes becoming the least developed embryos (Hunter and Wiesak, 1990; Pope et al., 1990; Xie et al., 1990). These less developed blastocysts may not be protected against the embryo-toxic effects of the growth factors that are released into the uterine lumen in response to oestrogen secreted by their more developed litter mates (Pope et al., 1990; Pusateri et al., 1990; Vallet et al., 1996; Geisert and Yelich, 1997).

It is also conceivable the smaller size and immature status of the pre-pubertal uteri increases competition between littermates for uterine space and nutrients. Consequently, although studies in sows suggest the limiting effects of uterine capacity on embryo survival do not come into effect until after day 30 (Ford et al., 2002), an apparent dissociation between nutritionally induced increases in ovulation rate and the number of embryo surviving the implantation period is evident from early studies involving gilts (Brooks and Cole, 1974). Support for

the existence of a maternal limit to first litter size (Brooks and Cole, 1974) is provided by the present finding that irrespective of pre-mating feed intake embryo survival is inversely related to ovulation rate.

5.4   Conclusion

In summary, the current findings strongly suggest that sexual maturity, as measured by responsiveness to boar exposure, occurs later in modern lean genotypes, and that current gilt recommendations (eg Hughes et al., 1996) need to be modified accordingly. Puberty is attained more rapidly, and with greater synchrony, within cohorts of gilts, when first boar exposure commences at either 182 or 203 days of age, whereas the pubertal response is delayed and asynchronous when boar exposure commences at 161 days.

The present results suggest that ovulation rate at the pubertal oestrus is unlikely to limit first litter size. However, both ovulation rate and embryo number tend to increase when first mating is delayed until the second oestrus, most likely reflecting reduced developmental heterogeneity between the follicles and oocytes forming the ovulatory pool. Further studies are required to establish whether further improvements in ovulation rate and embryo number would occur if first mating was delayed until the third oestrus. However, the choice of when the gilt is to be mated/inseminated should be determined by the body condition status required to maximise lifetime performance and longevity within the herd. Equally, the extra cost associated with feeding the gilt and maintaining cyclicity should be taken into account to determine the cost-effectiveness of delaying first mating beyond the pubertal oestrus.

Collectively, the experiments reported in this thesis also provide evidence to support the hypothesis that moderately restricting dietary intake during the pre- and peri- pubertal period exerts a significant influence on the reproductive success of the replacement gilt. Although puberty is not dependent on the

attainment of a specific threshold level of liveweight or body tissue status, chronic reductions in nutrient supply significantly delay sexual maturation and reduce potential litter size. Conversely, there are clear indications that growing gilts to attain a high liveweight coincident with boar exposure provides the animal with a metabolic buffer against the negative effects of moderate dietary restriction on responsiveness to boar stimulation. However, it is also evident that, regardless of liveweight, subtle changes in nutrition prior to, and during, boar stimulation will profoundly affect the size and morphology of the proliferating follicle pool as well as pubertal ovulation rates. Surprisingly, embryo number and survival were unaffected by pre-mating feed intake. The mechanisms for this remain unknown, but it is possible that high level feeding prolongs the duration of follicle selection, thus increasing heterogeneity within the pool of ovulatory oocytes and as a result increasing early embryo mortality, and that this effectively counteracts any beneficial effects on follicular growth and oocyte meiotic competence.

5.5 Future Research

Based on the data described in this thesis, a number of areas require further study. The results of both the present and previous studies demonstrate the need to determine whether delaying first mating until the third oestrus will increase first litter size. Although an increase in first litter size appears likely if first mating is delayed until the third oestrus, the additional costs associated with such a strategy demand firm evidence of a beneficial effect before its adoption should be recommended. Equally, further research is required to establish whether any monetary gains associated with any increase in first litter size outweigh the additional non-productive days and production costs associated with a delayed mating strategy.

More information is also required to elucidate the influence of nutritional manipulation prior to first mating on gilt responsiveness to boar contact and

first litter size. In particular, additional studies are required to establish whether flush feeding does in fact affect the timing of the pubertal response. A next step in research is also to identify the mechanisms regulating the effects of moderate, compared to severe, nutritional restriction of potential litter size. Specifically, previous studies provide convincing evidence of a decrease in ovulation rate, oocyte quality and embryo survival in response to maintenance feeding; however, it is likely that in the group housing systems commonly used to rear replacement gilts that naturally-occurring variations in feed intake will only result in moderate reductions in dietary intake. Comparisons between the current data and previous reports suggest possible differences in the mechanisms controlling the litter size of gilts mated at the pubertal oestrus compared to gilts mated at subsequent oestruses. It would be useful to identify whether litter size following mating at the pubertal oestrus is limited by the maturity or synchrony of the ova that are shed or by the uterine space and nutrients available to implanting embryos. Understanding of these mechanisms would enable the development of management strategies to increase first litter size following a pubertal mating. Such strategies might include the use of exogenous hormone treatments to increase synchrony within the proliferating follicle pool prior to puberty. Equally, this knowledge would allow the development of more effective selection strategies to improve litter size in gilts, and previous reports suggest that selection for increased uterine capacity as opposed to ovulation rate may be a more effective approach to increasing litter size.

5.6   Practical implications

The implications listed below serve to indicate the possible application of the data detailed in this thesis to modern pig production systems. Optimal response to boar stimulation, in terms of the timing and synchrony of the pubertal response, is significantly improved when boar exposure commences at 182 days of age or later. This finding confirms that modern genotypes are

later maturing than those of 20 – 30 years ago, supporting suggestions that selection for improved production traits is a selection for delayed sexual maturation, and demonstrates the need to modify gilt management strategies accordingly.

Based on the current data, it is clear that first litter size in no longer limited by the number of ova shed, suggesting that selection for improved uterine capacity may provide the key to increasing first litter size.

The reproductive performance of today's gilts appears to be sensitive to moderate changes in nutritional management during the pre- and peri- pubertal period. Higher liveweights and body tissue reserves provide a buffer against the negative effects of moderate nutritional stress on puberty attainment and first litter size. Therefore, I suggest that gilts should be fed to maximise liveweight and body tissue reserves at the start of boar stimulation.

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Chapter 7   Appendix