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HORMONAL MANIPULATION OF ESTRUS AND OVULATION IN THE GILT

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HORMONAL MANIPULATION OF ESTRUS AND OVULATION IN THE GILT

BY

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ABSTRACT

HORMONAL MANIPULATION OF ESTRUS AND OVULATION IN THE GILT

By

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To meet weekly breeding targets, it is occasionally necessary to inject exogenous gonadotrophins to induce estrus in prepubertal gilts. However, estrus and ovulation responses to gonadotrophin treatment have often proven unpredictable. The objective of the studies outlined in this thesis was to examine possible reasons for this unpredictability. In the initial experiment, prepubertal gilts received an injection of either eCG (equine chorionic gonadotrophin) or a combination of eCG and hCG (human chorionic gonadotrophin), or were not injected controls. At the time of injection, the gilt ovaries had 1 to 2 mm follicles. The estrus and ovulation responses were consistently lower for eCG than for the combination of eCG-hCG, and the failure to express estrus in eCG-hCG treated gilts was not associated with a premature rise in progesterone. In the second experiment, gilts were selected at 150 d or 180 d of age and assigned to injection of eCG-hCG, eCG alone, pretreatment with follicular stimulating hormone (FSH) followed by eCG, or controls. The estrus and ovulatory responses of gilts were consistently higher for gilts treated with the combination eCG and hCG than for eCG groups. In the third experiment, gilts were assigned to injection of hCG alone, pretreatment with FSH in polyvinylpyrrolidinone (PVP) followed by hCG, or FSH pretreatment followed by eCG. Pretreatment with FSH increased ovulatory response to hCG compared to hCG alone. Pretreatment with FSH also increased ovulatory response to hCG compared to eCG.

DEDICATION

To the eternal care, to my parents and sister

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AI	artificial inseminations
Dpc	days post coitum
eCG	equine chorionic gonadotrophin
FSH	follicle stimulating hormone
GnRH	gonadotrophin releasing hormone
hCG	human chorionic gonadotrophin
IM	intramuscular
IU	international unit
LH	luteinizing hormone
NPD	non-productive days
PVP	polyvinylpyrrolidinone
SEM	standard error of the mean
IGF-1	insulin growth factor-1
IGF BP	insulin growth factor binding protein
PAPP-A	pregnancy-associated plasma protein-A

CHAPTER 1

LITERATURE REVIEW: SWINE REPRODUCTIVE PHYSIOLOGY

Oogenesis and follicular development in the pig

In the early swine embryo (24 to 26 d post coitum; dpc), primordial germ cells migrate from the yolk sac into the undifferentiated gonad, the genital ridge, and organize into the sex chords. The germ cells then undergo mitosis and their number increases from 5×10^3 at about 24 dpc to more than 1×10^6 at 50 dpc (Black and Erickson 1968). In the absence of testis determining factor, the sex cords fragment into cellular clusters, each enclosing a primitive germ cell. These clusters differentiate into primitive follicular cells and the bulk of the genital ridge becomes the ovary. The germ cells change into oogonia by successive mitotic divisions and then into primary oocytes through the first meiotic division. The oocytes are then arrested at the G2 and do not resume meiosis until immediately before ovulation. The time needed for transformation of oogonia to oocytes is longer in pigs than in other mammalian species (Black and Erickson 1968). The first oogonia enter meiotic prophase before day 40 of intrauterine development and their transformation is not complete until approximately 30 days post partum (Fig.1.1).

Primary oocytes are surrounded by one or two layers of flattened follicular cells forming the ovarian primordial follicles. The first primordial follicles are observed by 56 dpc and increase in number throughout prenatal life (Bielańska-Osuchowska 2006). Approximately 500,000 primordial follicles are present in both ovaries by 10 d post- --

partum in swine, and constitute the maximum number available throughout reproductive life (Black and Erickson 1968).

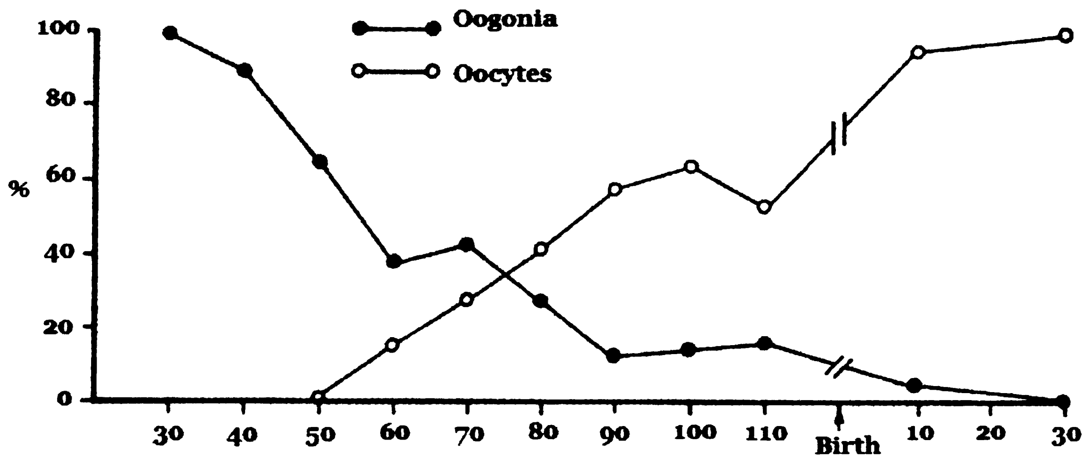

Figure 1.1 Distribution of oogonia and oocytes in developing gilts (From Black and Erickson 1968)

Within 10 d post partum some primordial follicles develop to the primary follicle stage (0.12 mm), which is an oocyte with one to three layers of granulosa cells (Knox 2005). Initiation of growth of primordial follicles involves endocrine actions and regulatory effects of local factors from the somatic cells of the follicle (Hirshfield 1991) and probably from the growing oocyte (Picton *et al.* 1998).

Follicular cells continue to proliferate to form several layers of granulosal cells, as well as the surrounding theca cells, becoming transformed into secondary follicles (Sacristan *et al.* 1996; Fig. 1.2). This follicular development from the primary to secondary follicle is independent of pituitary gonadotrophins, being observed also in hypophysectomized animals. The follicle enters the secondary stage with 3 to 20 layers of granulosa cells and a diameter of 0.14 to 0.40 mm. Growth beyond 0.4 mm is associated with the formation of an antrum. In the antral (tertiary) class, most follicles range in size from 0.4 to 1.5 mm with 10 to 30 layers of granulosa cells (Knox 2005). The follicular fluid is derived from the blood as well as substances synthesized by the theca and granulosa cells. During antrum formation granulosa cells continue to proliferate and start to develop FSH receptors. At the same time, theca cells appear completely differentiated forming two concentric layers, the internal theca (with LH receptors) and external theca. During maturation, cells of the theca and granulosa layers synthesize and secrete significant amounts of steroids that along with FSH and LH allow follicles to grow and reach their final development (Cardenas and Pope 2002).

As described by Richards (2001), once the follicle reaches a critical size, the granulosa cells attached to the basal lamina become distanced from the oocyte-cumulus complex and establish a second micro-environment in the follicle. This separation may necessitate the regulation of mural granulosa cells by factors/hormones other than those from the oocyte-cumulus complex. These include IGF-1 and FSH receptors in granulosa cells, and LH receptors in theca cells. Locally, FSH dictates specific changes in granulosa cell gene expression, enhanced proliferation, and the formation of the antrum that separates the oocyte-cumulus component from the mural layer of endocrine cells.

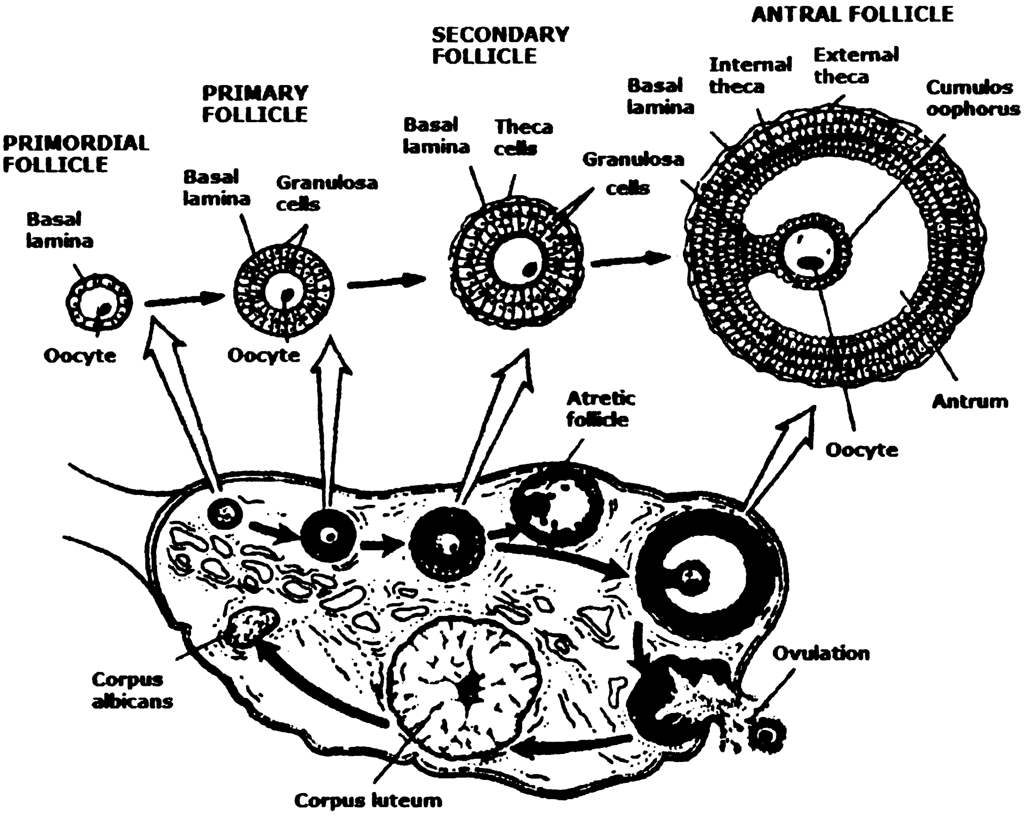

Figure 1.2 Representative scheme of the structure of the ovary and the different phases in the development of the follicle, corpus luteum and corpus albicans (From Sacristan 1996)

Ultimately, granulosa cells express genes for LH receptor, P450 aromatase, activin, and inhibin. Theca cells differentiate to produce androgens via $P450_{17\alpha}$. All these changes increase steroidogenesis that inhibits secretion of FSH, starving small follicles into atresia and leading to the positive feedback of estradiol to trigger the ovulatory LH surge.

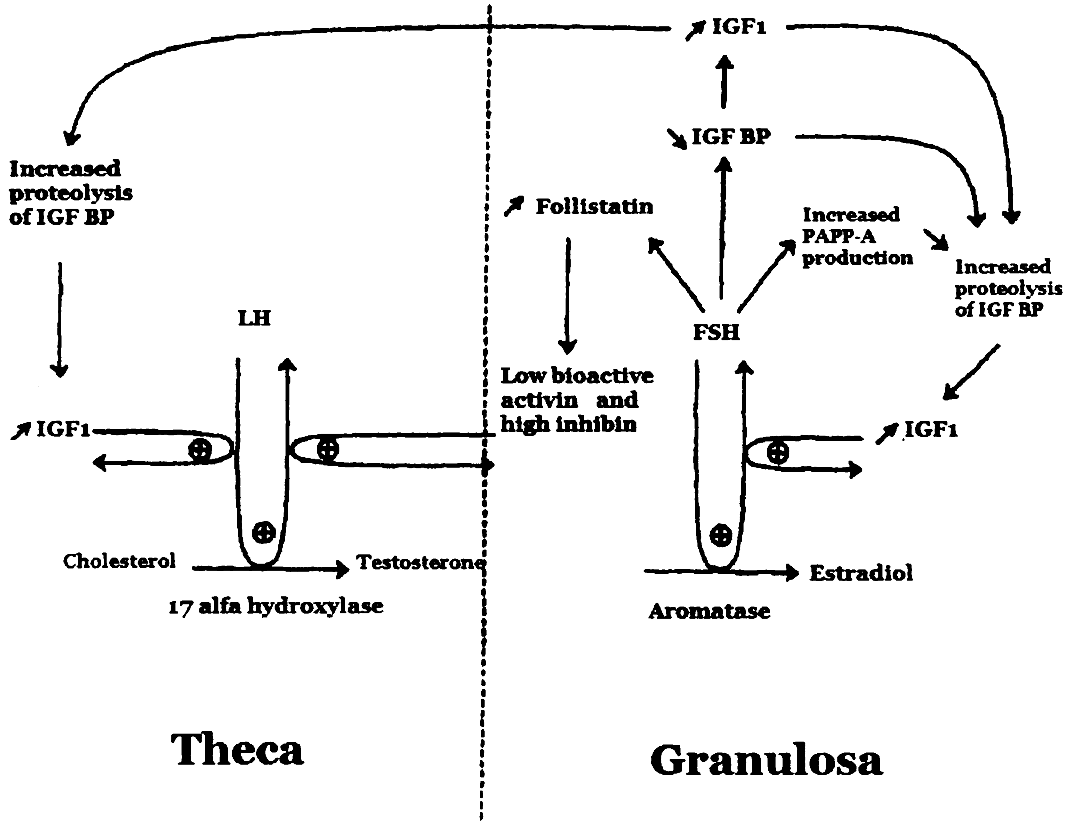

Figure 1.3 Mechanism involved in the development of an estrogen-active dominant follicle (From Driancourt 2001).

One controller of granulosa cell differentiation is FSH although IGF-1 and estradiol exert supportive roles (Driancourt 2001, Fig. 1.3). For example, although IGF-1 and estradiol cannot induce expression of sterodigenic enzymes, they enhance the effect of FSH to further increase aromatase activity. The FSH reduces amounts of IGF binding protein (IGF BP) and stimulates production of follistatin and a protease (pregnancy-associated plasma protein-A, PAPP-A) that also degrades IGF BP within the follicular fluid. The net decrease in IGF BP leads to an increase in free IGF-I that potentiates the effect of LH and FSH. Also, the increased follistatin binds follicular activin changing the inhibin/activin balance towards inhibin. A paracrine effect of inhibin produced within the granulose cell layer also potentiates the action of LH in the theca layer.

Hormonal profiles and ovarian development before puberty

LH and FSH secretion: In prepubertal gilts, LH is a key hormone that appears to control ovarian development and the age at puberty (Evans and O'Doherty 2001). Concentrations of LH in blood decrease from birth to about 40 d of age, then increase to 120 d of age, then decline to a nadir by about 180 d of age (Colenbrander *et al.* 1977; Pelletier *et al.* 1981; Diekman *et al.* 1983; Camous *et al.* 1985). From this nadir until first ovulation (puberty), LH concentrations increase (Pelletier *et al.* 1981; Prunier *et al.* 1993), as is typical in most species (Fig. 1.4). This peripubertal increase in LH secretion is characterised by an increase in mean LH concentrations and pulse frequency (Pelletier et al. 1981; Prunier *et al.* 1993), and is associated with final maturation of ovarian follicles (Beltranena *et al.* 1993) culminating in the preovulatory LH surge.

The FSH concentrations in blood are high early in life and then decrease after 70 to 125 days of age, and do not increase as puberty approaches (Diekman *et al.* 1983; Camous *et al.* 1985; Prunier *et al.* 1993). Estradiol concentrations are low throughout the majority of the prepubertal period but increase prior to puberty (Esbenshade *et al.* 1982; Lutz *et al.* 1984; Camous *et al.* 1985). Progesterone concentrations only increase after puberty with the formation of corpora lutea (Esbenshade *et al.* 1982; Prunier *et al.* 1993).

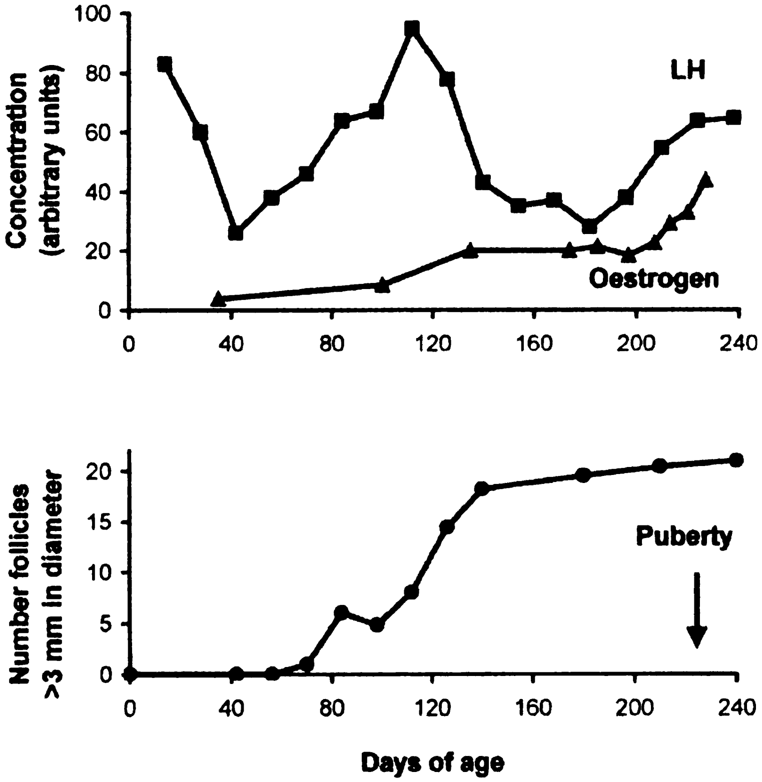

Figure 1.4 Changes in mean luteinizing hormone (LH) and oestrogen concentrations and numbers of follicles greater than 3 mm in diameter in gilts during sexual maturation (From Evans *et al.* 2001).

Regulation of gonadotrophin secretion (the gonadostat theory): Regulation of gonadotrophin secretion during the prepubertal period occurs via changes in GnRH secretion. Synthesis of GnRH in the female is in neurosecretory cells located in two separate areas in the hypothalamus, the tonic center and the surge center. Anatomically, the surge center consists of three hypothalamic nuclei known as the preoptic nucleus, the anterior hypothalamic area and the suprachiasmatic nucleus. The tonic center consists of two nuclei, the ventromedial and arcuate nuclei. Axons from cells of the surge and tonic centers extend into the pituitary stalk regions where the nerves terminate on a capillary network called the hypothalamo-hypophyseal portal system. This system allows GnRH to act immediately on the cells of the pituitary causing the release of the gonadotrophins FSH and LH.

The tonic center is responsible for basal secretion of GnRH. The neurons in this center release small pulses of GnRH over a prolonged period of time (tonic release of GnRH occurs during the entire estrous cycle). The surge center is responsible for the preovulatory release of GnRH. In contrast to the tonic release of GnRH, the preovulatory surge occurs only once during the estrous cycle. Prior to puberty, the tonic centre has a high sensitivity to negative feedback by estrogen and therefore GnRH secretion is inhibited. During the immediate peripubertal period, the prevailing theory is that the negative feedback sensitivity of the tonic centre to estradiol decreases allowing greater GnRH secretion (gonadostat hypothesis). This permits LH secretion to increase (Pelletier *et al.* 1981; Prunier *et al.* 1993) even in the face of increasing estradiol concentrations (Lutz *et al.* 1984; Camous *et al.* 1985). When the estrogen concentrations in the blood reach a high level the surge center becomes positively stimulated and releases larger quantities of GnRH. This stimulates the surge of LH causing ovulation and triggering the onset of the puberty. While there is evidence to support this theory (Berardinelli *et al.* 1984; Lutz *et al.* 1984), others have been unable to demonstrate a reduction in estradiol negative feedback prior to first ovulation (Elsaesser *et al.* 1991).

Ovarian dynamics before puberty: Antral follicles are first observed histologically at approximately 65 d of age and emerge from the ovarian surface at approximately 80 d of age (Black and Erickson 1968). The formation and increase in number of antral follicles around 100 d may be due to the early transient increase in LH secretion (Evans and O'Doherty 2001) and FSH secretion (Camous *et al.* 1985). Although these first antral follicles formed during the prepubertal period potentially have the ability to ovulate, all of them suffer atresia. The increased incidence of atresia after 120 d is coincident with a 60% decrease in mean plasma concentrations of FSH and LH (Guthrie and Garret 2000; Fig. 1.6). Only during the last steps of the prepubertal stage will these follicles complete final development, establishing puberty.

Follicle turnover has been demonstrated in swine during the pre-pubertal period between days 140 and 180 of age using laparoscopy repeated every 20 days (Grasso *et al.* 1988). Changes in the morphological aspects of the ovaries from "honeycomb type" (with large numbers of small follicles) to "grape type" (with several large follicles) were shown to occur within a few days (Bolamba *et al.* 1994). This may be taken as evidence that follicular waves occur in gilts at these ages (Driancourt 2001), as has been shown in prepubertal heifers (Evans *et al.* 1994; Melvin *et al.* 1999). However, in contrast with prepubertal heifers, these changes in prepubertal gilts appear to be regulated by local intraovarian factors other than by gonadotrophic and sex steroids hormones (Bolamba *et al.* 1994), since asynchrony is observed between left and right ovaries in ovarian morphology and changes.

Hormonal profiles and ovarian development after puberty

GnRH secretion: In the cycling female the tonic and surge centers are regulated by the level of progesterone and estradiol in blood. Although progesterone in the female exerts a negative feedback on both the tonic and the surge centers, it mostly exerts its effects on the tonic center. In contrast, the surge center responds to estradiol with a positive feedback. During the luteal phase (high circulating levels of progesterone) GnRH episodes from the tonic center occur every 4 to 8 h (Senger 2003). Such basal secretion, while allowing for some follicular growth, will not allow sufficient follicular development for the production of high levels of estradiol. Therefore, females under the influence of progesterone do not cycle during the luteal phase or gestation.

During the early follicular phase, negative feedback by progesterone on the hypothalamus is removed and GnRH is released by the tonic center at higher frequencies than during the luteal phase (every 1.5 to 2 h). This stimulates increased release of FSH and LH that in turn increases the secretion of estradiol by the follicles. During the late follicular phase when estradiol reaches a high (threshold) level, the surge center is positively stimulated and releases large quantities of GnRH causing the release of the surge of LH that induces ovulation, as described earlier for puberty.

LH secretion: In sows, cows and the ewes, LH is secreted in a pulsatile manner that changes from a low-frequency, high-amplitude mode of secretion during the luteal phase to a high-frequency, low-amplitude mode of secretion during the follicular phase (Clarke 1989). Studies on GnRH secretion in sheep show that the increase in plasma LH pulse frequency may reflect the removal of a negative feedback influence of progesterone on the hypothalamic GnRH pulse generator (Clarke *et al.* 1987). Conversely, Guthrie and Bolt (1990) and others have been unable to demonstrate an increase in LH concentrations, frequencies or amplitudes in gilts undergoing natural luteolysis.

As the follicular phase progresses the amplitude of LH pulses varies between species (Clarke 1989). In swine, the growth of ovarian follicles causes a progressive rise in the plasma concentrations of estradiol that effect a short-term pituitary 'clamp' of LH secretion during the late follicular phase of the cycle (Prunier *et al.* 1987; Clarke 1989). Indeed, from studies in experimental models it is clear that estrogen can exert a short-term negative feedback action at the pituitary level (Britt *et al.* 1991). Plasma LH levels in ovariectomized pigs given a single injection of estrogen first showed a negative feedback response and then a positive feedback response. Estrogen apparently inhibits or greatly reduces the release of GnRH for a period of approximately 54 to 60 h, and then causes a release of GnRH that induces a surge of LH proportional to the amount of GnRH released. The physiological significance of such a 'clamp' would be to allow the gonadotrophs to prepare cellular machinery for the subsequent positive feedback event.

FSH secretion and ovarian dynamics: In swine, changes in follicular fluid concentrations of steroids indicate two surges of follicle growth and increased steroidogenesis (Guthrie and Cooper 1996), although follicle waves do not develop to the same extent as in cattle probably due to ovarian progesterone suppression of LH secretion. In the pig, the LH receptor appears at a relatively immature stage of follicular development (~2 mm diameter; Liu *et al.* 1998, 2000), while in the cow LH receptors do not appear until follicles reach 9 to 10 mm (Xu *et al.* 1995). Taken together, it may explain why the cow has extended follicular waves with appearance of dominant follicles during the luteal phase, whereas in the pig follicles do not grow further than 4 mm. The first surge of follicle activity consists of the replenishment of the follicle population between days 2 and 8 of the estrous cycle, followed by an increased incidence of atresia between days 5 and 7 of small and medium follicles. The second surge in follicle activity consists of the selection and growth of ovulatory follicles, accompanied by atresia of subordinate follicles during the follicular phase of the cycle (days 15-21). The pattern of follicle growth during the mid to late luteal phase in pigs (7-15 d) has been characterized as continual growth and atresia (Guthrie and Cooper 1996).

In cows, mares and ewes, the emergence of follicle waves is preceded by a transient increase in plasma FSH secretion. In pigs, changes in follicle number and size distribution during the estrous cycle are, in part, also associated with changes in circulating concentrations of FSH (Guthrie *et al.* 1995). Increased atresia among small and medium follicles coincides with a decrease in circulating levels of FSH starting 1.5 to 2 d after the initiation of the decline in circulating progesterone (Guthrie and Bolt 1990; Knox *et al.* 2003). Secretion of FSH remains suppressed until the preovulatory LH surge. Concentrations of FSH are transiently increased on days 2 and 3 after ovulation (periovulatory FSH release), and at the same time the ovaries are replenished with small (1 - 2 mm diameter) and medium (3 - 6 mm) follicles (35 to 40 per animal) (Guthrie 2005). Increased atresia on days 6 and 7 among the small and medium follicles coincides with decreased circulating concentrations of FSH and may be a result of inhibin-induced negative feedback on secretion of FSH (Guthrie *et al.* 1995).

Estrogen and progesterone secretion: Estradiol is secreted by follicles during the entire estrous cycle, but concentrations remain low during the luteal phase due to the inhibitory effect of progesterone at the hypothalamic level. Once luteolysis occurs and preovulatory follicles are selected, circulating estradiol levels progressively increase until triggering the preovulatory release of GnRH. The mechanism involved is that, during follicular development LH binds to LH-specific membrane receptors located on the cells of the theca interna of the developing follicle. The binding of LH to its receptors activates a cascade of intracellular events ultimately resulting in conversion of cholesterol to testosterone. Testosterone then diffuses out of the cells of the theca interna and enters the granulosal cells, which have receptors for FSH. When FSH binds to its receptor, it causes the conversion of testosterone to estradiol via induced aromatase activity. During late stages of development, preovulatory follicles obtain LH receptors in granulosa cells, so allowing the LH surge to exert its full effect.

Concentrations of progesterone in circulation are low immediately after ovulation and then begin to increase by day 3 or 4 of the estrous cycle. Luteal phase circulating concentrations of progesterone are maximal by day 7 to 12 and then, with the regression of corpora lutea (luteolysis; 14-15 d), concentrations of progesterone in circulation decline rapidly and remain low throughout the follicular phase.

Follicular recruitment and selection

At recruitment, a cohort of follicles begins its final growth phase while, at selection, the preovulatory follicles are chosen and the remaining recruited follicles become atretic. The relative roles of LH and FSH shift throughout the different stages. During recruitment FSH plays a more important role than LH in antral follicle growth. As the follicles enter the selection phase, inhibin and estradiol are produced (by the follicle) and inhibit FSH secretion from the pituitary, while LH secretion increases. Finally, the large follicles produce more and more estrogen that finally prompts the preovulatory center to release a surge of LH.

Recruitment and selection are relatively short-term processes when compared to the preantral stages. The time interval from when a follicle leaves the resting pool until antrum formation is estimated to require 83 days in pigs while the time from antrum formation until the follicle reaches the ovulatory stage takes only an additional 20 days (Morbeck *et al.* 1992). From their estimate of antral follicle growth rate, Morbeck *et al.* (1992) hypothesized that the follicles undergoing antrum formation at the beginning of the cycle reach 3 mm in diameter on d 14 to 16 and constitute the population from which the ovulatory follicles are recruited.

Recruitment: The term recruitment has been used to describe two points during follicle development. The dormant primordial follicles are recruited into the growing follicle pool in a continuous manner (initial recruitment), whereas increases in circulating FSH during each estrous cycle recruit a cohort of antral follicles (cyclic recruitment) (McGee and Hsueh 2000). During initial recruitment, intraovarian and/or other unknown factors stimulate some primordial follicles to initiate growth, whereas the rest of the follicles remain quiescent for months or years. After initial recruitment, follicles grow but oocytes remain arrested in the prophase of meiosis. For those follicles not recruited, the default pathway is to remain dormant.

Cyclic recruitment starts after puberty onset and results from cyclic increases in circulating FSH forming cohorts of antral follicles, which begin final growth after entering the gonadotrophin-dependent stage of follicle growth. During cyclic recruitment, only a limited number of follicles survive, and the default pathway is to undergo atresia. Oocytes in these follicles have already completed their growth, acquired a zona pellucida, and are competent to resume meiosis (McGee and Hsueh 2000). The number of recruited follicles growing in the cohort appears to be highly variable between species. It ranges from over 50 in pigs, to 5 to 10 in cattle and 1 to 4 in horses (Driancourt 2001). In pigs, follicular recruitment occurs between days 14 and 16 of the estrous cycle (Clark *et al.* 1982; Foxcroft and Hunter 1985) or shortly after weaning in sows. On day 16, approximately 40 to 50 follicles, 3 to 6 mm in diameter, are present in both ovaries and represent the population of recruited follicles (Grant *et al.* 1989).

In cattle, the emergence of follicular waves is preceded by a transient increase in FSH that stimulates the development of antral follicles. However, in pigs, a clear increase in FSH concentrations coincident with the period of follicular recruitment has not been detected (Flowers *et al.* 1989; Guthrie and Bolt 1990; Cardenas and Pope 2002). Nevertheless, plasma FSH concentrations are consistently elevated at the time of follicular recruitment and Guthrie and Bolt (1990) suggest that alterations in the FSH:LH ratio may be critical in the recruitment process.

Selection: Only a small proportion of recruited follicles (about 30 to 40%) are selected to complete final maturation and ovulate. The remaining follicles and the oocytes they contain will degenerate and disappear from the ovaries through atresia. Therefore, the final number of ovulatory follicles is determined by how many follicles are recruited and by the ability of recruited follicles to continue to grow and avoid atresia (Cardenas and Pope 2002). The intensity of selection (measured by the proportion of the cohort follicles surviving) is highly variable among species. It can be very low in some horses, which have a small cohort size, while it is very high for cattle (one selected follicle out of 5 cohort follicles) and swine (12 selected follicles out of a cohort size of 50) (Driancourt 2001). Selection mechanisms seem to differ between cattle and pigs, due to the different follicular receptors present at the time of selection (Liu *et al.* 1998, 2000; Xu *et al.* 1995; Fig 1.5).

Lucy (2007) proposed two different models for follicular selection in cattle (Missouri and Cornell, Fig. 1.6). Both models suggest that one follicle has a developmental advantage over others at the time of follicular selection. This developmental advantage may be conferred by individual aspects of the follicle at the beginning of the follicular wave, including its vascular bed or the number or health of the granulosa or theca cells.

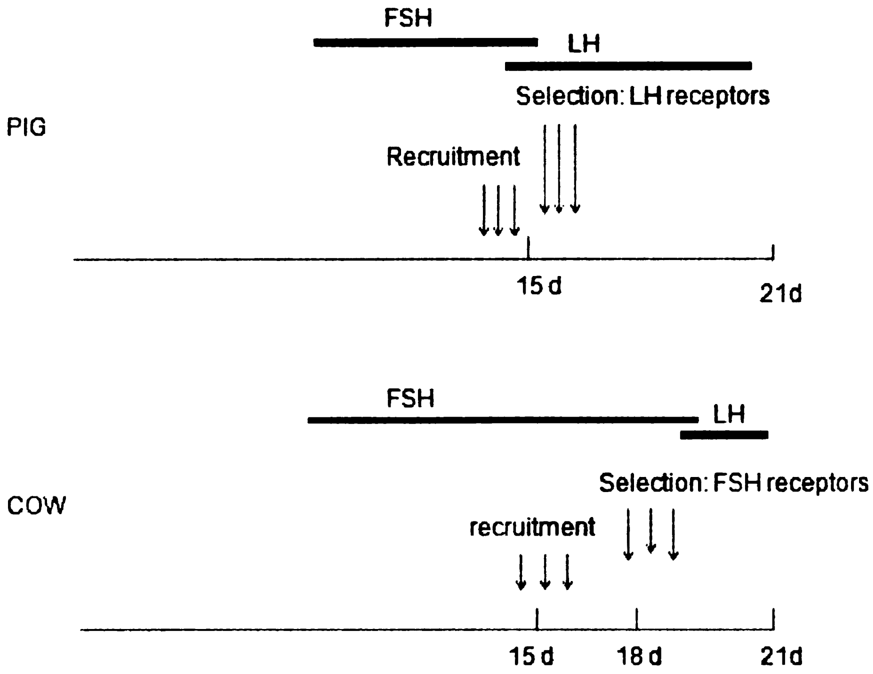

Figure 1.5 Different selection mechanisms between cattle and pigs (based on data from Lucy 2007 and Driancourt 2001).

According to the Missouri model (Xu *et al.* 1995), follicles that acquired LH receptors before others could theoretically starve the other follicles of gonadotrophins by selectively inhibiting FSH while not inhibiting LH. This model could explain selection in pigs because the earlier development of the LH receptor in granulosa cells (Liu *et al.* 1998, 2000) may enable a cohort of follicles to respond to LH when blood FSH concentrations are suppressed and so escape atresia. However, in cows the acquisition of LH receptors by granulosa cells is not a key component of follicular selection, since it appears to occur after, rather than before, selection has occurred (Fortune *et al.* 2001).

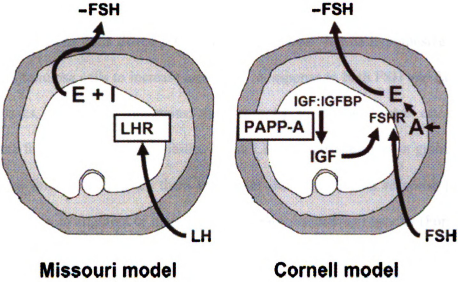

Figure 1.6 Models for events during follicular selection. According to the Missouri model, the initiating event is an increase in the LH receptor (LHR) in the granulosa cell layer. According to the Cornell model, the initiating event is the increase in pregnancy-associated plasma protein-A (PAPP-A). In each case, there is an increase in estradiol (E) and inhibin (I) that feeds back negatively on FSH secretion at the hypothalamo-pituitary axis. A = androgen; FSHR = FSH receptor (From Lucy 2001).

According to the Cornell model (Fortune *et al.* 2004), an increase in FSH enhances the IGF BP protease (pregnancy-associated plasma protein-A) that degrades IGF BP within the follicular fluid, leading to an increase in free IGF-I within the follicular fluid. The IGF-I is synergistic with FSH for estradiol synthesis within the follicle, and therefore the dominant follicle grows faster and also produces more estradiol. The increase in estradiol decreases FSH and starves the remaining follicles into atresia. This model can explain selection in cows because the FSH receptor continues to be expressed in the granulose cell layer throughout development (Xu *et al.* 1995). It is not clear why granulosa cells develop LH receptors in cows. It was hypothesized that this allows the granulosa cells to increase aromatase in response to both FSH and LH and that this increases, or at least maintains, their capacity to produce more estradiol than subordinate follicles. Another possibility is that LH receptors develop on granulosa cells of dominant follicles to prepare them for further differentiation in response to the LH surge, rather than to augment or maintain their estradiol-secreting capacity (Fortune *et al.* 2004).

Stimulating pubertal estrus

Weaned pig output per week is mostly dependant on breeding of a sufficient number of females each week (Dial *et al.* 1996). In order to have sufficient females available for breeding, a predictable supply of service-ready gilts (ie. gilts in estrus when needed) is necessary. This is most easily achieved by having gilts show an early puberty and then maintain regular cyclic activity. The only effective methods for stimulating an earlier puberty are exposure to a boar and the injection of gonadotrophic hormones.

Boar exposure: Boar exposure is the most common practice for stimulating an earlier puberty. Adequate estrus stimulation requires direct physical contact between the boar and the gilts, while estrus detection may only need fence-line contact. To ensure efficacy of boar exposure, it is important to follow the rules of boar contact, as reviewed previously (Kirkwood and Thacker 1992; Hughes 1997). These rules are:

1. Gilts must be old enough; the usual advice is at least 160 days of age although recent evidence suggests that 180 days may be better. This is because the gilts need to be mature enough to respond to the boar stimulus.

2. Boars must be old enough (ie. at least 10 months of age). A major part of the boar stimulus is pheromone production from the submaxillary salivary gland, and this gland is not fully developed until 9 or 10 months of age.

3. Gilts should be in physical contact with the boar for at least 15 min per day. As stated above, fence-line contact is not as effective as full physical contact.

4. Twice daily exposure will improve the response.

5. Provide adequate space (eg. >1.5 m^2 per gilt) since crowding may delay puberty and will make estrus detection more difficult.

6. House gilts at least 1 m away from boars to prevent gilts becoming refractory to boar stimuli (a detection problem).

7. If gilts do not respond as expected, use a different stimulus boar.

If gilts are not bred at their first estrus, boar exposure should continue (eg. at least 5 min every 2 to 4 d) in order to promote regular estrous cycles. In the absence of continued boar exposure, many gilts exhibit irregular inter-estrus intervals making estrus detection difficult.

Exogenous hormones: If boar exposure does not appear to be effective, for example due to a seasonal effect, then hormonal induction of estrus should be considered. Commercially available gonadotrophin preparations contain equine chorionic gonadotophin (eCG), which can be used alone or combined with human chorionic gonadotrophin (hCG). A common combination is 400 IU eCG with 200 IU hCG (PG600®; Intervet). The injection of PG600 is effective for the induction of fertile estrus in prepubertal gilts but when administered to prepubertal gilts, up to 30% may not exhibit behavioral estrus and about 30% of those exhibiting behavioral estrus may fail to cycle regularly (Table 1.1).

Table 1.1. Estrus responses of gilts to PG600* injection

Percent estrous	Percent ovulating	Percent cycling	Source
88	100	87	Paterson (1982)
--	97	60	Paterson (1982)
70	99	--	Tilton *et al.* (1995)
78	--	67	Kirkwood (1999)

*PG600®: combination of 400IU eCG + 200 IU hCG (Intervet Intenational)

The etiology of unpredictable responses is unknown, but possibly due to the hCG component of PG600 inducing an immediate ovulation or luteinization of medium or large ovarian follicles, and the subsequent progesterone production then suppressing behavioral estrus.

Table 1.2 Long-term performance of gilts bred at a PG600*-induced or natural first estrus

	Control	PG600-bred	PG600-skipped
Number of gilts bred	132	140	60
First farrowing rate	89.4	70.0	89.8
First litter size	9.9	9.6	10.7
P1 bred <7 days	68.3	65.4	67.3
Litters per sow	3.5	3.4	3.2
Pigs per sow	45.0	45.8	45.1

From Kirkwood *et al.* (2000)

*PG600®: combination of 400IU eCG + 200 IU hCG (Intervet)

Typical results from treating gilts with PG600 were documented by Kirkwood (1999). Estrus detection rates during a 3-week period were 78% and 37.5% for PG600-treated and untreated gilts, respectively. Further, for non-bred (skipped) and non-pregnant PG600-treated gilts, a 2:1 ratio of regular to irregular returns was observed, confirming the 70% incidence of normal estrous cycles in hormone-stimulated gilts. The farrowing rate of hormone-induced gilts was lower but there was no adverse effect on long-term sow performance (Kirkwood *et al.* 2000; Table. 1.2). This suggests that if the use of hormones is deemed necessary, it need not adversely affect long-term sow performance. However, an improvement in estrus response of pre-pubertal gilts to PG600 is desirable. However, in order to achieve this objective, it will be necessary to determine why some gilts do not appear to respond to PG600 injection.

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CHAPTER 2

EFFECT OF ECG OR ECG PLUS HCG ON ESTRUS EXPRESSION AND OVULATION IN PREPUBERTAL GILTS.

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Abstract

To meet weekly breeding targets, it is occasionally necessary to inject exogenous gonadotrophins to induce estrus in prepubertal gilts. However, the gilt estrus response to equine chorionic gonadotrophin (eCG) either alone or in combination with human chorionic gonadotrophin (hCG) can be unpredictable. The objective of the present study was to examine possible reasons for this unpredictability. Prepubertal gilts (90 kg and 153 d of age, n=109) received an injection of either 600 IU eCG or a combination of 400 IU eCG and 200 IU hCG (PG600), or were not-injected (controls). The gilts were then exposed to a mature boar for 15 min daily for 7 d for estrus detection. At the time of injection, real-time ultrasound revealed that the gilt ovaries had primarily 1 to 2 mm follicles. Blood samples were obtained at time of hormone injection (day 0) and at d 3, 7, and 10 for assay of serum progesterone concentrations. The estrus responses by 7-d were 15.5%, 73.3%, and 0%, for eCG, PG600, and control gilts, respectively ($P<0.001$). The estrus response improved ($P<0.05$) with increasing body weight. Based on circulating progesterone levels, all estrous gilts ovulated except for 4 of the PG600 gilts. Failure to express estrus in PG600 treated gilts was not associated with a premature rise in progesterone.

Keywords: swine, gilts, gonadotrophins, estrus, ovulation

Introduction

The largest single component of herd non-productive days is often the gilt entry-to-service interval. To minimize this interval, gilts should reach puberty as soon as possible either after arrival on the farm or entry into the breeding herd. The ability to meet the weekly breeding target requires a predictable supply of service-ready gilts (ie. gilts in estrus when required), which is most easily achieved by having gilts show an early puberty and then maintain predictable cyclic activity. When gilt availability is limiting, a common method used to stimulate the onset of estrus is the injection of gonadotrophins, such as the combination of 400 IU eCG and 200 IU hCG (PG600®, Intervet). While this product is effective for the induction of a fertile estrus in prepuberal gilts, in practice up to 30% of treated gilts do not show a behavioural estrus response within 7 d (Kirkwood 1999). However, based on blood progesterone determinations at 10 d after treatment, most non-responding gilts do appear to have ovulated (Tilton *et al.* 1995).

Taking the above findings into consideration, we hypothesized that the 30% non-estrous response is due to the hCG component of PG600, the hCG being a LH analogue causing some gilts to either ovulate or suffer luteinization of medium or large follicles. In either event, the premature production of progesterone would block behavioral estrus and the gilts will be deemed to have failed to respond to treatment. We further suggest that if our hypothesis were true, then injection of eCG alone (ie. no hCG component) would result in an improved estrus response because used alone, this molecule would provide a much lower LH activity (Combarnous *et al.* 1984; Guthrie *et al.* 1990).

Materials and Methods

These studies were performed during October and November 2006 at Michigan State University Swine Facility with the approval of the institutional Animal Care Committee. To examine responses to different gonadotrophin preparations, 109 prepubertal Yorkshire x Landrace gilts (90 kg, 153 d) were employed. Gilts were housed at 8 to 12 animals per pen and fed ad libitum a corn-soybean meal finisher diet formulated to provide 13.7 MJ ME/kg, 9.8% crude protein, 0.6% lysine. At selection, gilts were assigned by weight and age to receive an intramuscular (IM) injection of 600 IU eCG (Pregnecol®; Bioniche Animal Health, Bellville, Ontario, n=45) or 400 IU eCG plus 200 IU hCG (PG600®; Intervet, Millsboro, Delaware, n=45). A third group of gilts served as uninjected controls (n=19). All treatments were represented in each pen. The dose of eCG (600 IU) was chosen on the basis of it being a label dose for sows at weaning and that it is the 'total' gonadotrophin content of PG600.

From 2 d until 7 d after the time of hormone injection, all gilts were subject to direct exposure to a mature boar for 15 min daily to facilitate detection of estrus. Estrus was defined as the expression of a rigid standing reflex in the presence of the boar. Fewer control gilts were employed because we anticipated few, if any, would exhibit estrus during the 7 d study period. To further characterize the estrus and ovulation responses to hormone treatment, blood samples were obtained from all gilts via jugular venipuncture at the time of hormone injection (0 d) and at 3, 7, and 10 d. Serum samples were assayed for progesterone (P4) concentrations in a single assay using a commercial kit (Diagnostic Product Corp., Los Angeles, California). Assay sensitivity and intra-assay coefficient of variation were 0.1 ng per mL and 2.7%, respectively. An elevation in P4 to >1 ng per mL on 3 d was considered indicative of premature ovulation (or luteinization), while low levels on 3 d followed by elevations on 7 d and/or 10 d was taken to indicate a normal ovulatory response. Immediately prior to hormone injection, the first 25 gilts assigned to each hormone treatment were subject to transrectal B-mode ultrasonography of their ovaries using an Aloka SSD 500 (Aloka Inc, Wallingford, Connecticut) with a 7.5-MHz linear array transducer as described by Knox and Althouse (1999). The diameters of the largest 3 follicles were recorded. Data were analysed using the Number Cruncher Statistical System (NJ Hintze, Kayeville, Utah). Treatment differences on the estrus response rates were examined by Chi square and differences in age, weight, and follicle diameter tested by GLM analysis of variance.

Results

There were no differences between treatments for gilt age or weight at the start of the study (Table 2.1). Similarly, there was no difference in ovarian follicular diameter prior to hormone injection (Table 2.1). Most gilts had a maximum follicular size of 2 mm although 3 mm follicles were measured in 7 of the eCG-treated and 5 of the PG600-treated gilts, and 2 of the PG600-treated gilts had 4 mm follicles. The PG600-treated gilts with the largest follicles ovulated normally.

All gilts had non-detectable P4 concentrations on 0 d. As anticipated, no control gilts exhibited estrus during the study period, and none had elevated serum P4 concentrations. Contrary to expectation, more ($P<0.001$) PG600-treated gilts than eCG-treated gilts exhibited estrus by 7 d after injection (Table 2.1). All eCG-treated gilts exhiting estrus had serum P4 concentrations indicative of normal ovulation. Elevated P4 concentrations were not detected in any anestrus eCG-treated gilt. Of the 33 (73%) PG600-treated gilts exhibiting estrus, 4 failed to ovulate. Of the 12 anestrus PG600-treated gilts, none had elevated P4 on d 3 after injection and only 1 had serum P4 concentrations indicating a normal ovulation.

There was an effect of body weight at treatment on the incidence of estrus, with more of the gilts weighing >90 kg exhibiting estrus than their lighter counterparts ($P<0.05$). However, because too few eCG-treated gilts exhibited estrus, the effect was only significant for PG600-treated gilts (Table 2.2). The two weight classes did not differ in age (154.9±5.0 vs. 157.5±3.8 days for the lighter and heavier gilts, respectively, $P=0.8$).

Table 2.1. Influence of eCG (equine chorionic gonadotrophin) or eCG plus hCG (human chorionic gonadotrophin) on incidence of estrus by 7 d post-treatment and ovulation as indicated by elevated progesterone (+P4) or non-ovulation (-P4).

	eCG+hCG	eCG	Control
No. of gilts	45	45	19
Age, d[1]	154.1±2.6	154.2±2.7	150.6±3.8
Weight, kg[1]	90.1±1.3	91.4±1.3	88.5±2.0
Follicle diameter, mm[1]	2.4±0.1	2.3±0.1	--
Gilts estrus by 7 d (%)[2]	73.3	15.6	0
Gilts estrus + P4 (%)	64.4	15.6	--
Gilts estrus – P4 (%)	8.9	0	--
Gilts anestrus + P4 (%)	2.2	0	--
Gilts anestrus – P4 (%)	24.4	84.4	--

[1] Means ± SE

[2] Effect of hormone preparation, $P<0.001$ by Chi square test

Table 2.2. Influence of gilt weight on the estrus response to eCG or eCG plus hCG

	eCG+hCG	eCG	Control
No. of gilts	45	45	19
75-90 kg	13/22 (59.1%)a	2/23 (8.6%)	0
91-110 kg	20/23 (86.9%)b	5/22 (22.7%)	0

ab, Effect of weight, $P<0.05$ by ANOVA

Discussion

The results of this study confirmed an approximate 70% estrus response to PG600 but did not support the hypothesis that injection of hCG concurrent with eCG results in follicular luteinization or ovulation and premature elevation of circulating progesterone. Indeed, the poor estrus response to eCG compared to the eCG/hCG combination suggests an important role for the hCG in follicular development and subsequent estrus expression. However, a follicular ovulatory or luteinizing effect may depend on the follicular status of the ovaries at the time of stimulation (ie. presence or not of medium to large follicles). In the present study, few gilts had follicles >2 mm in diameter.

Of the PG600-treated gilts exhibiting estrus, 4 of them did not have elevated circulating progesterone levels. It is possible that these gilts underwent sufficient follicular development to cause circulating estrogen levels adequate to induce behavioural estrus, but then failed to ovulate. A failure to ovulate may be due to the PG600 activity being inadequate to complete follicular development to the point of inducing the phasic LH release. However, in the absence of measurement of circulating LH concentrations and ongoing follicular ultrasound examinations, these suggestions remain speculative.

It is probable that the response to eCG treatment, with or without concurrent hCG treatment, will depend on the gilt's level of physiological maturity, which is supported by the positive association between gilt weight and their estrus response in the present study, although gilt age was not a significant factor. Most of the gilts in the present study had only small (1 to 2 mm) follicles on their ovaries, which should have been responsive to

FSH-like stimulation (Guthrie *et al.* 1990), although the possibility that they were too immature to respond to gonadotrophic stimulation cannot be discounted. However, it was shown previously that the presence of many or only a few large (>6 mm) follicles on the ovary did not affect the estrus or ovulatory response of prepubertal gilts to eCG treatment. This suggests that eCG can stimulate development of small follicles to the point of ovulation (Bolamba *et al.* 1992).

It is possible that a period of FSH stimulation would advance follicular development (Guthrie *et al.* 1988; Bolamba *et al.* 1996) such that they would then be more responsive to eCG stimulation. Alternatively, it is possible that some minimum level of LH-like stimulation is necessary to complete follicular development (Driancourt *et al.* 1995). Previous studies examining estrus and/or ovulatory responses to eCG have yielded variable results. Response rates of 70% to 100% have been noted when 725 to 1,000 IU eCG was injected (Guthrie 1977; Dial *et al.* 1984; Britt *et al.* 1986; Esbenshade 1987; Flowers *et al.* 1989; Bolamba 1992) although estrus rates of only 25% to 52% were noted following injection of 363 to 600 IU (Britt *et al.* 1985; do Lago *et al.* 2005; Gama *et al.* 2005). The difference in responses may be due to the degree of physiological development and genetics of the gilts and/or the source of the eCG, although it is likely that the greater LH-like activity associated with higher doses improved the likelihood of ongoing follicular development.

We conclude from the present data that the gilt estrus responses to eCG will be improved with concurrent use of hCG and that, depending on their degree of physiological development, a failure of gilts to exhibit estrus in response to a combination of eCG and hCG need not involve premature ovulation or luteinization of follicles and an associated premature elevation in circulating progesterone.

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CHAPTER 3

EFFECT OF PRIOR FSH TREATMENT ON THE ESTRUS AND OVULATION RESPONSES TO ECG IN PREPUBERTAL GILTS

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Abstract

The objective of this study was to determine the effect of pre-treatment of prepubertal gilts with follicular stimulating hormone (FSH) on the estrus and ovulatory responses to equine chorionic gonadotrophin (eCG) injection at two ages. A total of 149 prepubertal Hypor gilts were selected at 150 d (n=76) or 180 d (n=73) of age and assigned to injection of 400 IU eCG plus 200 IU human chorionic gonadotrophin (hCG; PG600®, Intervet International), 600 IU eCG alone (Folligon®, Intervet), pretreatment with 72 mg FSH (Folltropin®; Bioniche Animal Health) administered as 6 x 12 mg injections at 12 h intervals with 600 IU Folligon 12 h after last FSH injection, or non-injected controls. To facilitate estrus detection, gilts were exposed to a mature boar for 15 min daily for 7 d. To determine ovulatory responses, blood samples were obtained on the day of injection and 10 d later and assayed for progesterone content. Following treatment at 150 d, 1 control gilt (5.3%) was deemed estrous but did not ovulate. Compared to treatment with Folligon alone, PG600 injection tended ($P=0.1$) to increase the estrus response (52.6% vs. 26.3%) and increased ($P<0.01$) the ovulatory response (89.5% vs. 47.4%). The estrus response in gilts pretreated with Folltropin was intermediate (42.1%) but the ovulatory response (47.4%) was the same as for Folligon alone. Following treatment at 180 d, 2 control gilts (10.5%) were deemed estrous and they also ovulated. There was no difference between hormone-treated groups for estrus or ovulatory responses, although the ovulatory response of PG600-treated gilts tended ($P=0.1$) to be higher than for the Folligon-treated group (89.5% vs. 66.7%), with Folltropin-pretreated gilts being intermediate (76.5%). These data demonstrate that the estrus and ovulatory responses of gilts were higher for PG600 than for Folligon and that while responses to PG600 were not affected by gilt age, for the combined Folligon groups, estrus response ($P<0.02$) and ovulatory response ($P<0.05$) improved with increased gilt age.

Keywords: gilts, FSH, eCG, estrus, ovulation

Introduction

To minimize the gilt entry-to-service interval, or where availability of service-ready gilts is limiting, gilts are often induced into estrus by injection of gonadotrophic hormones. A common preparation used for estrus induction is a combination of 400 IU equine chorionic gonadotrophin (eCG) and 200 IU of human chorionic gonadotrophin (hCG; PG600®, Intervet International). Subsequent to injection of PG600, it is not unusual to have only 70% of gilts show a behavioural estrus response within 7 days (Kirkwood, 1999). It was hypothesized that the 30% failure to respond to PG600 was due to its hCG component luteinizing or ovulating medium or large follicles, causing a premature rise in circulating progesterone and blockade of behavioural estrus (Manjarin, unpublished data). However, in this earlier work we did not observe any indication of ovulation or follicular luteinization subsequent to PG600 injection into 150-day old prepubertal gilts. Interestingly, when we injected 600 IU eCG alone, it resulted in only a 15% estrus response in prepubertal gilts (Manjarin, unpublished data). The poor response to this relatively low dose of eCG may have been due to the immaturity of ovarian follicles (90% <3 mm), indicating a need for the concurrent biological activity of hCG in order to adequately stimulate growth of small follicles. This is supported by the improved estrus response observed with higher doses of eCG (Dial *et al.*, 1984; Britt *et al.*, 1985), which will also have a higher LH-like activity (Dial *et al.* 1984).

Interestingly, while injection of eCG into prepubertal gilts caused atresia in the small and medium follicle populations, injections of FSH promoted their growth (Guthrie *et al.*, 1990; Bolamba *et al.*, 1996; Guthrie, 2005). Also, changes in follicle number and size during prepubertal development were temporally associated with changes in circulating FSH concentrations (Camous *et al.*, 1985; Guthrie and Garret, 2000). Further, suppression of FSH during the luteal phase of cyclic gilts decreased the small and medium follicle populations (Knox and Zimmerman, 1993; Guthrie *et al.*, 1987), which were restored by FSH replacement (Guthrie *et al.*, 1988). Taking all of the above into account, we hypothesize that administration of FSH to prepubertal gilts will increase the populations of medium sized ovarian follicles, which will enhance the gilt′s ovarian response to eCG injection.

Materials and Methods

These studies were performed on a 1,000-sow farrow-to-wean facility near Leon, Spain during July and August 2006. A total of 149 Hypor gilts were employed to examine responses to different gonadotrophin preparations. Gilts were housed at 6 to 8 animals per pen and fed ad libitum a standard finisher ration. Gilts were assigned by age (150 d or 180 d) to receive an intramuscular (IM) injection of 400 IU eCG plus 200 IU hCG (PG600®, Intervet International, Boxmeer, NL; n=19 and 19, at 150 and 180 d, respectively), 600 IU eCG (Folligon®, Intervet; n=19 and 18, respectively), or pre-treatment with 72 mg FSH (Folltropin®; Bioniche Animal Health, Bellville, Ontario, n=19 and 17, respectively) followed by 600 IU eCG. The FSH was administered as 6 x 12 mg injections at 12 h interval with the eCG being administered 12 h after final FSH injection. A fourth group of gilts served as uninjected controls (n=19 and 19, respectively).

From 2 d to 7 d after hormone injection, gilts were subject to direct exposure to a mature boar for 15 min daily to facilitate detection of estrus. To determine the ovulation responses to hormone treatment, blood samples were obtained from all gilts via jugular venipuncture at the time of hormone injection and 10 d later. Serum was harvested and frozen (-20°C) until assayed for progesterone concentrations. Serum samples were assayed for progesterone (P4) concentrations in a single assay using a commercial ELISA kit (Immulite®, Siemens Medical Solutions Diagnostics, Tarrytown, NY). Assay sensitivity and intra- assay coefficient of variation were 0.2 ng/ml and 8.1%, respectively. All gilts employed in this study had non-detectable P4 concentrations on the day of injection. An elevation of progesterone concentrations on day 10 to a minimum of 4 ng/ml was deemed to indicate an ovulatory response to treatment. The effects of treatment and gilt age on the proportional estrus and ovulation responses were examined by Chi square. For the determination of age effects, the eCG and FSH followed by eCG groups were combined.

Results

As shown in Table 3.1, a single 150 d control gilt (5.3%) was detected as being estrous but she did not ovulate. When compared to the gilts treated with eCG alone, PG600 injection tended ($P=0.1$) to increase the estrus response (52.6% vs. 26.3%) and increased ($P<0.01$) the ovulatory response (89.5% vs. 47.4%). The estrus response in eCG-treated gilts that were pretreated with FSH was intermediate (42.1%) but their ovulatory response (47.4%) was the same as for eCG alone. Following treatment at 180 d, 2 control gilts (10.5%) were deemed estrous and they also ovulated. There was no difference between any hormone-treated group for estrus or ovulatory responses, although the ovulatory response of PG600-treated gilts tended ($P=0.1$) to be higher than for the gilts receiving eCG alone (89.5% vs. 66.7%), with the FSH-pretreated gilts being intermediate (76.5%). There was no effect of age on estrus or ovulatory responses of PG600-treated gilts but the estrus and ovulatory responses to eCG were higher in older gilts ($P<0.05$ for both).

Table 3.1. Influence of gilt age (150 vs. 180 d) on the estrus and ovulation responses to eCG plus hCG combination, eCG alone, FSH pre-treatment then eCG, or no treatment

	eCG+hCG	eCG	FSH+eCG	Control
150 days				
No. of gilts	19	19	19	19
Gilts estrus by 7 d	10 (52.6%)	5 (26.3%)	8 (42.1%)	1 (5.3%)
Gilts ovulating	17 (89.5%)	9 (47.4%)	9 (47.4%)	0
180 d				
No. of gilts	19	18	17	19
Gilts estrus by 7 d[1]	13 (68.4%)	12 (66.7%)	10 (58.8%)	2 (10.5%)
Gilts ovulating	17 (89.5%)	12 (66.7%)	13 (76.5%)	2 (10.5%)

[1] One estrous eCG+hCG treated gilt failed to ovulate

Discussion

The primary objective of the present study was to test the hypothesis that gilts pre-treated with FSH would have higher estrus and ovulation responses to eCG injection, presumably due to enhanced follicular development. The hypothesis was not supported since we observed no difference in proportions of gilts ovulating between the two eCG-treated groups. Although we did not assess ovarian follicular dynamics in this study, our FSH treatment protocol was similar to others where an effect on follicle growth was evident (Guthrie *et al.*, 1990; Bolamba *et al.*, 1996). This suggests that even if FSH was able to stimulate follicular growth to the medium follicle size, these follicles were not more responsive to eCG at either 150 or 180 days of age. It is probable that the ovarian response to eCG stimulation will depend on the gilt's level of physiological maturity, which will include the entire hypothalamo-pituitary-ovarian axis, not just at the ovarian level. This is supported by a positive association between gilt weight and their estrus response noted in our earlier study (Majarin, unpublished data) and the effect of age in the present study.

The absence of a gilt age effect in the ovarian response to PG600 indicates that during the prepubertal phase, concurrent LH activity is necessary for continued follicular development. With PG600, this LH activity is supplied by the hCG component of the product. The results of the present study, where no effect of FSH pretreatment on gilt estrus or ovulatory responses to eCG were evident also supports, albeit indirectly, the suggestion of an indispensable role for LH activity in follicular development. Indeed, development of follicles beyond 4 mm in diameter was not FSH dependent but seemed to be controlled primarily by LH pulses (Driancourt *et al.*, 1995) and is coupled with a change in FSH and LH follicular receptors (Nakano *et al.*, 1983; Liu *et al.*, 1998; Liu *et al.*, 2000). The improved estrus induction response of prepubertal gilts to higher doses of eCG (Dial *et al.*, 1984; Britt *et al.*, 1985) also argues for an indispensable role for LH activity in development of follicles to the preovulatory stage. The eCG molecule does have some LH activity (Combarnous *et al.* 1984; Guthrie *et al.* 1990) and at higher doses it is possible that the LH activity of eCG becomes sufficient to allow development of large follicles. Indeed, it has been shown that LH (Huff and Esbenshade, 1992; Guthrie *et al.*, 1990) and hCG (Bolamba *et al.*, 1991; Driancourt et. al., 1992) can stimulate follicular development, estrus and ovulation in prepubertal gilts if given in sufficient quantities, whereas treatment with FSH alone did not have any effect on the number of large follicles (Guthrie *et al.*, 1988, 1990). In contrast, however, Bolamba and Sirard (2000) did note growth of large follicles when FSH was administered for three days instead of two (93-187 ug/kg BW 24 h interval), although this could be attributable to LH contamination of the FSH used. Additional evidence in support of the role of LH in follicular development was provided in the review of Evans and O'Doherty (2001).

In conclusion, the lower ovulatory response to a relatively low dose of eCG compared to the combination of eCG and hCG, likely reflects an inability to stimulate growth of follicles, particularly at younger gilt ages. The absence of an effect of FSH pre-treatment on the response to eCG indicates that either our protocol did not affect follicular growth or that there is an indispensable role for LH activity in final follicular development.

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CHAPTER 4

EFFECT OF HCG TREATMENT ON THE ESTROUS AND OVULATION RESPONSES TO FSH IN PREPUBERTAL GILTS

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Abstract

To ensure sufficient numbers of pregnant females, particularly at hotter times of the year, hormonal induction of gilt estrus may be necessary. However, the gilt estrus and ovulation responses to gonadotrophin treatment have often proven unpredictable. The objective of the present study was to examine possible reasons for this unpredictability. Prepubertal gilts (approximately 150 d of age, n=63) were assigned to one of three treatments: injection of 300 IU hCG (n=15); pretreatment with 100 mg FSH in polyvinylpyrrolidinone administered as 2 x 50 mg injections 24 h apart, followed by 600 IU eCG at 24 h after the second FSH injection (n=23); or FSH pretreatment as above followed by 300 IU hCG at 24 h after the second FSH injection (n=25). To facilitate estrus detection, gilts were exposed to a mature boar for 15 min daily for 7 d. Blood samples were obtained on the day of eCG or hCG injection and again 10 d later and gilt ovulation responses determined on the basis of elevated progesterone concentrations. The estrus responses by 7 d were 6.7%, 17.5%, and 64.0%, for gilts treated with hCG, FSH+eCG, and FSH+hCG, respectively ($P<0.001$). The estrous gilt receiving hCG alone and one estrous FSH+hCG gilt did not ovulate, all other estrous gilts ovulated. A further 2 anestrus FSH+eCG-treated gilts ovulated. These data suggest that FSH pre-treatment facilitated the development of ovarian follicles to the point where they became responsive to hCG, but had little effect on the response to eCG.

Keywords: swine, gilts, gonadotrophins, estrus, ovulation

Introduction

The output of the breeding herd is weaner pigs, and it has been suggested that the factor most affecting the predictability of weaner pig output is the meeting of breeding targets (Dial *et al.* 1996). The ability to meet the weekly breeding target requires a predictable supply of service-ready gilts, which may necessitate the hormonal induction of estrus to ensure the availability of a sufficient number of breeding females. Gonadotrophic preparations used to stimulate estrus in gilts are equine chorionic gonadotrophin (eCG) and combinations of eCG with human chorionic gonadotrophin (hCG). We have previously confirmed that, compared to treatment with 600 IU eCG alone, estrus and ovulation responses of gilts were improved when injected with 400 IU eCG combined with 200 IU hCG (PG600®) (Manjarin *et al.* 2008a). However, responses to PG600 remained unpredictable, with 73% exhibiting estrus, but only 64% both exhibiting estrus and ovulating. We now speculate that the failure of overtly estrous gilts to ovulate may be due to the 200 IU hCG in PG600 providing an inadequate duration of LH-like activity to complete follicular development. Interestingly, the serum half-life of hCG in humans was longer after subcutaneous than after intramuscular injection (Saal *et al.* 1991) and on one occasion an improved estrus response of gilts to PG600 was noted after subcutaneous injection compared to intramuscular injection (Knox *et al.* 2000). This suggests a longer duration of gonadotrophic biological activity may be necessary for some gilts to both express estrus and ovulate. However, prolonging the duration of gonadotrophin activity by simply increasing the initial dose of PG600 did not prove useful. The administration of 1.5 or 2.0 times the label dose of PG600 to gilts did increase the number of corpora lutea but also increased the numbers of gilts with follicular cysts as well as the numbers of cysts per gilt (Breen *et al.* 2006).

Several earlier studies have shown that LH (Huff and Esbenshade, 1992) and hCG (Guthrie *et al.* 1990; Bolamba *et al.* 1991; Driancourt *et al.* 1992), in addition to their ovulatory and steroidogenic actions, can also stimulate follicular development. However, gilts with different ovarian status (number and size of follicles) at the time of hCG injection had different rates of ovulation (Bolamba *et al.* 1991), suggesting that hCG is unable to initiate growth of small follicles. In contrast, FSH seems to play a key role during initial follicle growth (Guthrie and Bolt, 1990; Knox *et al.* 2003). Taking all together, we now hypothesize that pretreatment of gilts for 2 days with FSH may advance follicular development to the point where the follicles will respond to hCG injection with onset of estrus and ovulation.

Material and methods

These studies were performed during October to December 2007 at a commercial 1,000-sow farrow-to-wean facility near Leon, Spain. Animals were cared for humanely in accordance with institutional animal care guidelines. To examine responses to different gonadotrophin preparations, 63 prepubertal Hypor gilts (approximately 150 d of age) were employed. Actual gilt ages were between 140 and 160 d but the farm recorded all gilts within this range as being 150 d. Gilts were housed at 6 to 8 animals per pen and fed ad libitum, a wheat/soybean meal finisher diet formulated to provide 14 MJ DE/kg and 0.8% lysine.

At selection, gilts were assigned to receive an intramuscular (IM) injection of 300 IU hCG (Veterin Corion®; Divasa, Spain) (n=15), or 100 mg FSH (Folltropin®; Bioniche Animal Health, Bellville, Ontario) mixed with polyvinylpyrrolidinone K-30 (PVP; Plasdone C-30, average mol wt. 58,000, ISP Technologies Inc., Wayne, NJ, USA), administered as 2 x 50 mg injections 24 h apart, followed by 600 IU eCG (Folligon®; Intervet International, Boxmeer, NL) at 24 h after the second FSH injection (n=23), or 100 mg FSH as above, and then 300 IU hCG at 24 h after the second FSH injection (n=25). The dose of eCG was chosen because of it's known efficacy in weaned sows and it also reflects the total gonadotrophin content of PG600 (400 IU eCG plus 200 IU of hCG), a product of known efficacy in gilts. The dose of hCG was chosen to reflect the LH-like activity of PG600, ie. 200 IU of hCG with the 400 IU eCG likely having 20 to 40% LH-like activity (Combarnous *et al.* 1984; Guthrie *et al.* 1990). The objective of administering FSH in PVP solution was to prolong the period of biological activity (Jackson *et al.* 2006). The PVP solution (30%) was prepared by dissolving 15 mg of PVP in 40 ml of distilled and deionized water and was stored at 4°C until use. For each injection, 3.125 ml PVP solution was mixed with 0.875 ml (50 mg) of FSH.

To facilitate detection of estrus, gilts were subject to direct exposure to a mature boar for 15 min daily from 1 d to 7 d after time of hormone injection. Gilts were deemed to be estrous if they adopted a rigid stance in the presence of the boar. To determine the ovulation responses to hormone treatment, blood samples were obtained from all gilts via jugular venipuncture at the time of hCG or eCG injection (day 0) and 10 days later. Serum was harvested and frozen (-20°C) until assayed for progesterone (P4) concentrations. Serum samples were assayed for P4 in a single assay using a commercial ELISA kit (Immulite®, Siemens Medical Solutions Diagnostics, Tarrytown, NY). Assay sensitivity and intra-assay coefficient of variation were 0.2 ng/ml and 8.1%, respectively. All gilts employed in this study had non-detectable P4 concentrations on the day of injection. An elevation of P4 concentrations on day 10 to a minimum of 5 ng/ml was deemed to indicate an ovulation response to treatment (Althouse and Hixon 1999). Treatment differences for estrus and ovulation response rates were examined by Chi square.

Results

As shown in Table 4.1, of the 25 gilts receiving FSH plus hCG, 16 (64%) exhibited estrus by 7 days and all but one of these estrous gilts had serum P4 concentrations indicative of ovulation. None of the 9 anestrus FSH plus hCG-treated gilts ovulated. One gilt receiving hCG alone exhibited estrus, but neither she nor any other gilt in this group ovulated. Of the 23 FSH plus eCG-treated gilts, 4 (17.4%) exhibited estrus but only 3 of these ovulated. Of the 19 FSH plus eCG-treated gilts that remained anestrous, 3 had elevated serum P4 concentrations indicating ovulation.

Table 4.1. Effect of injecting hCG, or FSH followed by eCG, or FSH followed by hCG, into 150-day gilts on the incidence of estrus by 7 days and ovulation as indicated by elevated progesterone (+P4), or non-ovulation (-P4).

	hCG[1]	FSH + eCG[2]	FSH + hCG[3]
No. of gilts	15	23	25
Gilts estrus by 7 d (%)[4]	1 (6.66%)	4 (17.4)	16 (64.0)
Gilts ovulating (%)	0	6 (26.1)	15 (60.0)

[1] 300 IU hCG

[2] 100 mg FSH in PVP as 2 injections 24 h apart then 600 IU eCG 24h after last FSH

[3] 100 mg FSH in PVP as 2 injections 24 h apart then 300 IU hCG 24h after last FSH

[4] Effect of hormone preparation, $P<0.001$ by Chi square test

Discussion

The results of this study support our hypothesis that pre-treating gilts with FSH will enhance the estrus and ovulation responses to injection of hCG. Further, although we did not monitor ovarian follicular dynamics, we suggest that the better responses were due to an FSH-associated advancement of follicle maturity, presumably to the 4 mm stage that becomes responsive to LH-like activity. That prior FSH activity was necessary for expression of the hCG effect on estrus and ovulation responses is also indicated by the lack of effect of hCG when not pre-treated with FSH.

The present results also support our previous findings (Manjarin *et al.* 2008a,b) and literature evidence (do Lago *et al.* 2005; Gama *et al.* 2005) that, in the absence of additional LH-like activity, 600 IU eCG, is relatively non-efficacious for induction of estrus and ovulation in prepubertal gilts. The eCG molecule does have some LH-like activity (Combarnous *et al.* 1984; Guthrie *et al.* 1990) but apparently not enough to drive follicular development when only 600 IU is administered. Estrus responses to eCG are increased when higher doses are administered (eg. Bolamba *et al.* 1992; Dial *et al.* 1984), presumably due to the associated increase in LH-like activity. In a previous study, we noted no effect of FSH pre-treatment on the efficacy of eCG for estrus induction, although in that study only 72 mg of FSH was injected and it was administered as 6 serial injections (Manjarin *et al.* 2008b). The present study employed PVP to extend the biological activity of the 100 mg FSH but, again, FSH failed to affect the ovarian response to eCG. The FSH pretreatment was effective for priming the ovary to respond to hCG, which does suggest an effect on follicle development. However, based on the present data, the magnitude of the effect was not sufficient to allow the follicles to respond to eCG stimulation.

Driancourt *et al.* (1995) observed in gilts that follicle development to about the 4 mm stage was dependant only on FSH while growth beyond this diameter appeared to be controlled by LH pulses. Indeed, granulosa cells of immature follicles contained exclusively FSH receptors, but these declined by the 4 mm stage and were absent in larger follicles (Liu *et al.* 1998; 2000). Interestingly, treatment in vitro with FSH promoted LHr mRNA expression (LaBarbera, 1981), which likely explains how the FSH pre-treatment was able to prime the ovary to respond to the hCG injection.

In conclusion, pretreatment with FSH increased the ovulation response to hCG compared to hCG alone, supporting an indispensable role for FSH during early follicle maturation but a transition to LH dependence. A better understanding of this relationship may facilitate the development of improved protocols for hormonal induction of estrus and ovulation.

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CHAPTER 5
SUMMARY AND CONCLUSIONS

Summary

Three studies were performed to examine the reasons for unpredictability of estrus and ovulatory response to equine chorionic gonadotrophin (eCG) either alone or in combination with human chorionic gonadotrophin (hCG) in prepubertal gilts. Estrus responses were determined on the basis of expression of a standing heat reaction in the presence of a boar while ovulation responses were assessed on the basis of elevations of circulating progesterone concentrations 7 to 10 days after treatment.

In the first study, prepubertal gilts (90 kg and 153 d) received an injection of either 600 IU eCG or a combination of 400 IU eCG and 200 IU hCG (PG600), or were non-injected controls, and were then exposed to a mature boar for 15 min daily for 7 days for estrus detection. Also at the time of injection, gilt′s ovaries were subjected to real-time ultrasound examination. Blood samples were obtained at time of hormone injection (d 0 and at d 3, 7, and 10) for assay for serum progesterone. Both the estrus and ovulation responses were better following PG600 injection than following eCG injection, with responses improving with increasing gilt weight. There was no evident association between reproductive responses and size of ovarian follicles.

In the second study, prepubertal gilts were selected at 150 d or 180 d of age and assigned to injection of PG600, 600 IU eCG alone, pretreatment with 72 mg FSH administered as 6 injections at 12 h intervals with 600 IU eCG injected 12 h after last

FSH injection, or to be non-injected controls. Gilts were exposed to a mature boar for 15 min daily for 7 d and blood samples obtained on the day of injection and 10 d later and assayed for progesterone content. Again, responses to PG600 were superior to those following eCG injection with no evident effect of FSH pre-treatment. Responses were improved for older gilt's age.

In the third study, prepuberal gilts (150 d of age) were assigned to treatments involving injection of 300 IU hCG alone, pretreatment with 100 mg FSH administered as 2 injections 24 h apart, followed by 300 IU hCG at 24 h after the second FSH injection or FSH pretreatment as above followed by 600 IU eCG at 24 h after the second FSH injection. Gilts were exposed to a mature boar for 15 min daily for 7 d and blood samples obtained on the day of eCG or hCG injection and again 10 d later. Estrus and ovulation responses were minimal to hCG treatment alone but were improved by FSH pretreatment prior to eCG injection. When FSH pretreatment was followed by hCG, estrus and ovulation responses rivaled those following PG600 injection.

Conclusions

The results of experiment 1 indicate that when ovaries contain only small (<3 mm) follicles, the failure of 30% of gilts to exhibit estrus in response to a combination of eCG and hCG does not involve premature ovulation or luteinization of follicles caused by the hCG component. Data from experiment 2 demonstrate that the lower ovulatory response to a relatively low dose (600 IU) of eCG compared to the combination of eCG and hCG likely reflects an inability to stimulate follicle growth, particularly at younger gilt ages. However, the absence of an effect of FSH pre-treatment on the response to eCG, compared to eCG alone, indicates that the failure to stimulate growth is likely not due to an inability to initiate development of small follicles. Interestingly, pre-treatment with FSH increased ovulation responses to hCG compared to hCG alone, suggesting that FSH may play a crucial role during first steps of follicular maturation while the greater ovulation response obtained to hCG compared to eCG after FSH pretreatment suggests an indispensable role for LH-like activity once follicles have reached certain development.