

## Ovarian follicular growth in sows\*

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The resumption of ovarian follicular development during lactation and after weaning in sows is a complex process that ultimately determines rebreeding efficiency of sows. Ovarian follicular development before weaning is heterogeneous because multiple patterns of development are observed when individual sows are compared. Sows can have relatively inactive ovaries before weaning with follicles of < 2 mm in diameter. Other sows have non-ovulatory follicular waves in which follicles grow to approximately 5 mm and subsequently regress before weaning. Sows may also have preovulatory follicular development and ovulation, or may develop cystic ovaries before weaning. Weaning is a random event relative to follicular development on the ovary. Therefore, variation in the weaning to oestrus interval in sows is caused by weaning at random stages of follicular development. Most sows experience a rapid period of follicular growth after weaning and return to oestrus within 3–7 days. Delayed intervals to oestrus after weaning are associated with inactive ovaries before weaning (follicles < 2 mm in diameter) or weaning during the regression phase of a follicular wave. An integrated model for follicular growth and oestrus in weaned sows should include endocrine mechanisms (that is, individual differences in insulin, insulin-like growth factor I (IGF-I), LH and FSH), behavioural mechanisms (relationship between follicular growth and the initiation of oestrus) and morphological mechanisms (that is, timing of weaning relative to ovarian follicular development).

### Introduction

Sows must return to oestrus after weaning so that they can be inseminated and establish a new pregnancy. The return to oestrus normally occurs within 3–7 days after weaning and ovulation occurs near the end of oestrus (Soede and Kemp, 1997). Even under optimal conditions, the variation in the interval to oestrus after weaning and the variation in the time of ovulation relative to oestrus are far too great (Flowers and Esbenshade, 1993; Kemp and Soede, 1997). Oestrous detection and insemination must be performed on each of the days that sows are expected to return to oestrus. Furthermore, sows must be inseminated twice to ensure an optimum conception rate and litter size because ovulation is not precisely timed relative to the onset of oestrus. The poor timing of reproductive events places significant demands on labour for oestrous detection and insemination, and wastes semen because sows are inseminated two or more times. Little is known about the factors that cause the variation in the

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weaning to oestrus interval or the weaning to ovulation interval in sows. If we could manage follicular populations in sows then it may be possible to reduce the variation in the interval from weaning to oestrus or ovulation. This reduction would benefit the producer by shortening the period of oestrous detection and insemination and possibly eliminate the need for double inseminations if a precise synchronization of ovulation can be achieved.

### Ovarian morphology before and after weaning in sows

#### *Follicular growth before weaning in sows*

Follicular growth before weaning in sows has been studied intensely and several authors have reviewed the literature (Edwards, 1982; Britt *et al.*, 1985; Varley and Foxcroft, 1990). There is a steady increase in the diameter of ovarian follicles, but few follicles are > 5 mm in diameter before week 3 of lactation. After week 3 of lactation, the number of 5 mm follicles increases, but follicles > 5 mm do not generally develop in lactating sows.

The classical data on follicular growth in sows were collected from sows killed at specific stages of lactation. Therefore, only one observation of the ovaries could be made on each sow. Gross changes in follicle size could be measured (that is, progressively larger follicles during later lactation), but patterns of follicular growth within a sow could not be elucidated. Ovarian ultrasonography revolutionized the study of ovarian function in cattle, sheep and horses because follicular growth could be studied each day in individual animals (Pierson *et al.*, 1988). Ultrasonography can also be performed each day in sows. The ovary of gilts or first parity sows can be examined by attaching a flexible handle to the ultrasound probe. Second or greater parity sows can be palpated manually through the rectum (similar to cattle). Owing to the large number of follicles, the examinations are videotaped and played later for frame-by-frame viewing, digitizing and data collection. The timing of hormonal events relative to follicular growth at oestrus (Soede and Kemp, 1997) and critical windows of insemination relative to ovulation have been described (Soede *et al.*, 1995).

Lucy *et al.* (1999) performed an ultrasonographic and endocrine study of 31 sows before and after weaning. Sows were examined by ultrasonography each day from day -7 before weaning until ovulation after weaning (weaning = day 0). Four patterns of ovarian follicular growth were observed. The first two patterns of development were ovulation before weaning and the formation of cystic follicles before weaning. The third pattern of follicular development (ovarian inactivity) was characterized by extremely small follicles (< 2 mm diameter). The follicles in sows with inactive ovaries may grow and regress, but their development cannot be tracked using ultrasonography because they are too small. The fourth pattern of follicular development is perhaps the most unique and interesting. On the basis of ultrasonographic examinations performed each day in individual sows, synchronized waves of ovarian follicular growth were observed before weaning. The wave consisted of a cohort of 20–30 follicles that grew from a diameter of 2 mm (limit of detection by ultrasonography) to 4–6 mm (co-dominant follicles). The co-dominant follicles then regressed and were replaced by a new wave of follicles. The ultrasonographic data were in agreement with classical observations that show that follicles rarely exceed a diameter of 5 mm before weaning. However, there did not appear to be a steady progression of larger and larger follicles. Instead, follicles grew in waves with each wave of follicular growth creating broad peaks of follicular activity. Anoestrous cattle also have non-ovulatory follicular waves during lactation (Ginther *et al.*, 1996). However, in anoestrous cattle, a dominant follicle is selected from the cohort and then after selection the dominant follicle fails to ovulate. In both pigs and cattle, the follicular cohort (pigs) or the dominant follicle (cattle) will ovulate once the suppressive effects of lactation are either removed (that is, weaning in sows) or reduced (later lactation in cattle).

### *Follicular growth after weaning in oestrous sows*

Sows typically have follicles of 2–5 mm in diameter when they are weaned. Weaning causes a rapid development of preovulatory follicles (Palmer *et al.*, 1965; Cox and Britt, 1982; Dyck, 1983). The follicles are maximally steroidogenic at 6–7 mm when sows come into oestrus (Liu *et al.*, 2000). Follicles grow slightly larger (7–8 mm) before ovulation (Soede *et al.*, 1998). Using ICI 33828, Day and Longenecker (1968) showed that pig follicles could grow from an immature stage to preovulatory size in 5 days. Numerous studies have demonstrated a rapid return to oestrus and ovulation in lactating sows treated with equine chorionic gonadotrophin (eCG), combinations of eCG and hCG, or GnRH (Esbenshade *et al.*, 1990). Although sows can be hormonally stimulated into oestrus, the interval from treatment to the onset of oestrus is variable. Likewise, there can be a large variation in the weaning to oestrus interval in the absence of any hormonal treatment. Some of the variability is caused by an imprecise relationship between oestrus and ovulation in pigs (Soede and Kemp, 1997). Therefore, the actual interval to ovulation after weaning may be more or less precise than the interval to oestrus. ten Napel *et al.* (1995) successfully shortened the duration of the weaning to oestrus interval by using genetic selection in primiparous sows. However, the decrease in the weaning to oestrus interval was due to a decrease in the number of sows with extremely long weaning to oestrus intervals. In other words, the mean interval from weaning to oestrus decreased because outliers with extremely long weaning to oestrus intervals were eliminated. The distribution of sows in oestrus within 1 week was essentially unchanged by genetic selection.

The variation in the weaning to oestrus interval is probably caused by the variation in pre-weaning follicular development in sows. The first two pre-weaning patterns of ovarian follicular development (pre-weaning ovulation and pre-weaning cystic ovary) occur in a small percentage of sows. Nevertheless, these pre-weaning patterns are important because sows that have ovulated before weaning or that have developed cystic ovaries before weaning will theoretically not show signs of oestrus after weaning. The third pattern of pre-weaning development (ovarian inactivity) probably causes a long interval from weaning to oestrus in sows. We have observed this type of development in 'normal' sows, but it may be more common in undernourished or low-body condition sows (Prunier and Quesnel, 2000), or in sows exposed to heat stress. A preliminary experiment was carried out using ten sows exposed to either thermoneutral conditions (21°C, 60% relative humidity;  $n = 5$ ) or heat stress (32°C, 60% relative humidity;  $n = 5$ ) during lactation. All sows were weaned at approximately 21 days and moved to thermoneutral conditions. Populations of follicles at weaning were distinctly different for thermoneutral and heat-stressed sows. At weaning, thermoneutral sows had nearly 40 follicles that were 4–5 mm in diameter (Fig. 1). On successive days after weaning, this group of follicles developed into progressively larger size classes (6–7 mm and then 8 mm). Heat-stressed sows did not have any 4–5 mm follicles at weaning. All of the follicles in the heat-stressed sows were < 2–3 mm in diameter. As a consequence, subsequent follicular growth was delayed in the heat-stressed sows. In fact, the heat-stressed sows required 4 days after weaning to develop the same number of 4–5 mm follicles that were found in the thermoneutral sows on the day of weaning. This delay in follicular development resulted in delayed oestrus in the heat-stressed sows compared with thermoneutral sows.

If follicles of a sow are developing in synchronized waves before weaning (fourth pattern), the interval to oestrus after weaning will depend on the stage of follicular development of the cohort at weaning. Sows weaned during the development of a follicular cohort will return to oestrus first because weaning is timed with the development of a group of follicles. Sows weaned when the cohort is regressing may return to oestrus later because a new wave of follicles must develop and replace the regressing cohort.

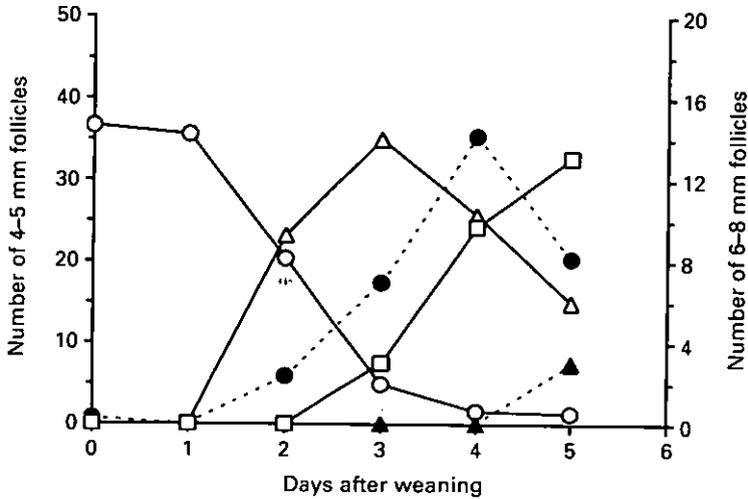
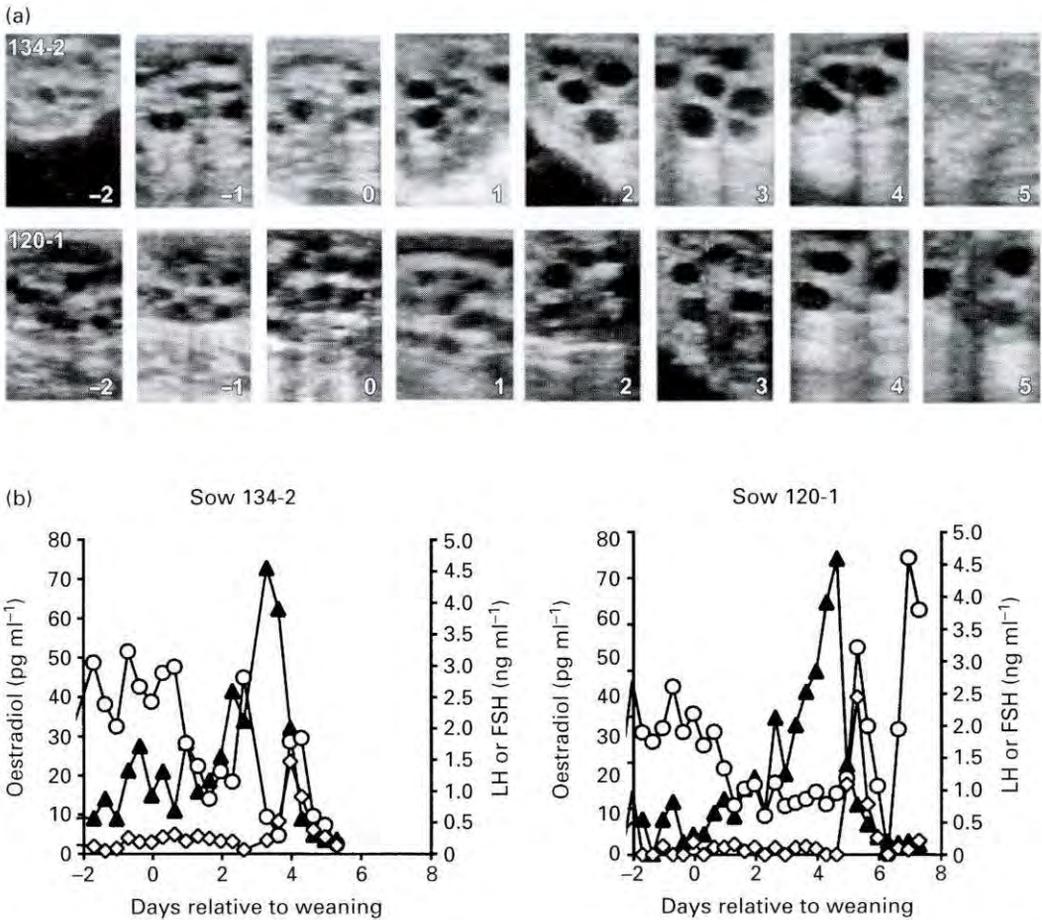


Fig. 1. The number of follicles within different diameter ranges for sows exposed to either thermoneutral conditions (O, 4–5 mm follicles; Δ, 6–7 mm follicles; □, 8 mm follicles; 21°C) or heat stress (●, 4–5 mm follicles; ▲, 6–7 mm follicles; 32°C) during lactation. Sows were moved to thermoneutral conditions after weaning and the number of follicles was counted using ultrasonography. Note the reduced number of 4–5 mm follicles in heat-stressed sows at weaning (day 0). Complete development of the 4–5 mm follicle pool required 4 days in heat-stressed sows. Subsequent follicular development (growth of follicles to 6–7 mm in diameter) was also delayed in heat-stressed sows.

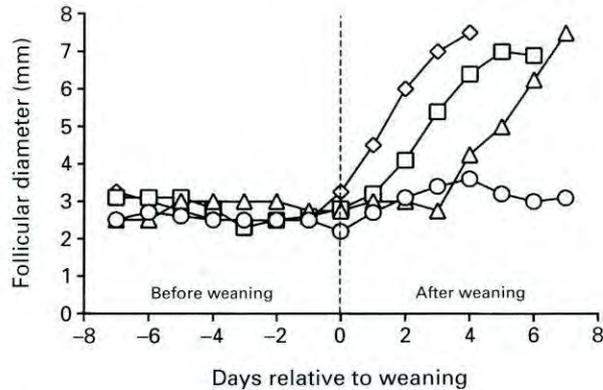
Ultrasound images and endocrine data that demonstrate the effect of pre-weaning follicular development on interval to ovulation after weaning are shown for two sows (Fig. 2). Sow no. 134-2 ovulated 5 days after weaning, whereas sow no. 120-1 ovulated 7 days after weaning. The ultrasound images show that sow no. 134-2 had smaller ovarian follicles 2 days before weaning (day -2) compared with sow no. 120-1. However, the cohort of follicles underwent rapid development for the next 2 days so that at the time of weaning (day 0), plasma oestradiol concentrations had already increased in sow no. 134-2. Although sow no. 120-1 had follicles of a similar size before weaning, the follicles were marginally oestrogenic and may not have responded to the increase in LH after weaning because they were atretic. Both sows had decreased FSH concentrations after weaning, an increase in oestradiol and an LH surge. However, the increase in oestradiol and the LH surge occurred earlier in sow no. 134-2 than in sow no. 120-1. Ovulation in sow no. 134-2 was completed by day 5.

The effect of pre-weaning follicular development and follicular development shortly after weaning on the interval to oestrus and ovulation can be clearly seen when populations of follicles are studied in weaned sows. Lucy *et al.* (1999) examined the average diameter of the follicular cohort in sows with different weaning to ovulation intervals (Fig. 3). The average follicular diameter for sows with different weaning to ovulation intervals was the same at the time of weaning. However, after weaning, follicular development occurred earlier in sows that ovulated on day 5 after weaning than in sows that ovulated on day 7 or day 9 after weaning. The analysis demonstrated that the growth curves for the preovulatory follicles were parallel. In other words, the rate of growth of the preovulatory follicular cohort was the same regardless of the weaning to ovulation interval. This pattern of development is different from



**Fig. 2.** (a) Daily ultrasonographic images of sows numbered 134-2 and 120-1 from day -2 to day 5 after weaning. (b) Plasma concentrations of oestradiol (▲), FSH (○) and LH (◇) from these sows (2 days before weaning (day -2) until ovulation). Sow number 134-2 ovulated on day 5 after weaning. Note the presence of follicles visible by ultrasonography on day 4 and the absence of visible follicles on day 5 (ovulation). Sow number 120-1 ovulated on day 7 after weaning. Preovulatory follicles were visible on day 5, but these follicles did not ovulate for another 2 days. Sow number 134-2 had smaller ovarian follicles 2 days before weaning compared with sow number 120-1. However, the cohort of follicles in sow number 134-2 underwent rapid development for the subsequent 2 days so that at the time of weaning, plasma oestradiol concentrations were already increased (day 0). An increase in plasma oestradiol was not observed in sow number 120-1. The follicles in sow number 120-1 may have been atretic co-dominant follicles that were unable to respond to the increase in LH after weaning. Both sows had decreased plasma FSH concentrations and an increase in oestradiol and an LH surge after weaning.

an alternative possibility in which the rate of preovulatory follicular growth is simply slower in sows that ovulate later after weaning (that is, growth curves have different slopes for sows with different intervals to ovulation). There appears to be a mixed population of follicles on the ovary at weaning. Some of the follicles will participate in the preovulatory follicular wave, whereas other follicles will regress. We assume that healthy follicles (non-atretic) participate in the follicular wave. The average diameter of the healthy follicle pool may be impossible to determine unless the ovary is monitored carefully for several days before ovulation. When



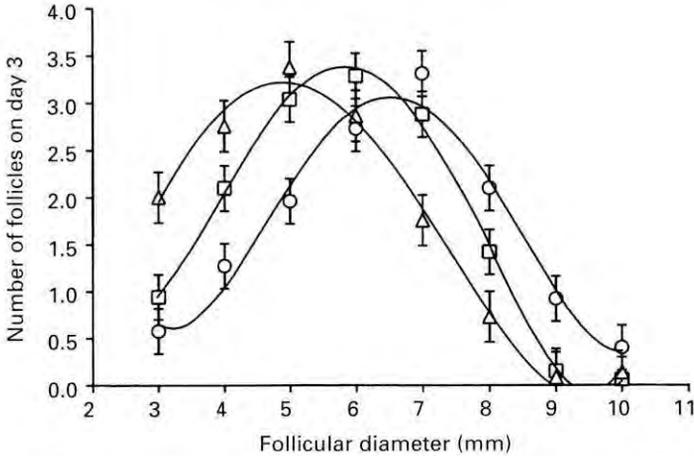
**Fig. 3.** Least square means for follicular diameter after weaning in sows. Sows were grouped on the basis of the number of days to ovulation after weaning (5 days: ◇ (SEM = 0.5), 7 days: □ (SEM = 0.3), 9 days: △ (SEM = 0.5) and anoestrous: ○ (SEM = 0.3)). Sows ovulating at 5 days after weaning initiated a follicular wave soon after weaning. There was a delay in the initiation of follicular growth in sows ovulating later. Anoestrous sows failed to develop preovulatory follicles.

plasma oestradiol concentrations were studied, it was found that follicles of sows with the shortest intervals to ovulation had greater oestrogenic activity before weaning and an earlier increase in preovulatory oestradiol concentrations (Lucy *et al.*, 1999). Thus, the entire process of preovulatory follicular development begins before a sow is weaned.

The population of preovulatory follicles that will ultimately participate in ovulation is clearly established by day 3 after weaning. Sows in a commercial herd were studied to determine factors affecting follicular populations and the interval to ovulation after weaning (Bracken *et al.*, 1999). Ovaries were examined by ultrasonography each day beginning on day 3 after weaning and twice each day from day 4.5 until ovulation. Sows with short intervals to ovulation ( $\leq 6.5$  days) had follicular populations on day 3 after weaning that were more advanced (comprised larger follicles) when compared with sows with long ( $\geq 9$  days) intervals to ovulation (Fig. 4). Follicular populations in sows with intermediate (7–8 days) intervals to ovulation were intermediate in size when compared with sows with short or long intervals to ovulation. Production factors (that is, parity and body condition score) known to influence the interval to oestrus and ovulation had a predictable effect on follicular growth within 3 days after weaning in sows (that is, sows with low body condition score and first parity had populations of follicles shifted toward smaller sizes on day 3). Thus, follicular populations by day 3 after weaning are predictive of the time of ovulation in sows.

#### *Follicular growth in anoestrous sows*

Ovarian follicular development in anoestrous sows can be monitored each day using ultrasonography. On the basis of ultrasonographic data, anoestrous sows can be classified as either type I anoestrus or type II anoestrus. In type I anoestrus, sows have 1–2 mm follicles at weaning. However, a follicular cohort does not develop after the sow is weaned. Instead, ovarian follicular populations remain at approximately the same size throughout the first week after weaning. Sows fail to come into oestrus because they do not have preovulatory follicles.



**Fig. 4.** The average number of 3–10 mm follicles on day 3 after weaning for each follicular diameter in sows with short (○,  $\leq 6.5$  days;  $n = 48$ ), medium (□, 7–8 days;  $n = 48$ ) or long ( $\Delta \geq 9$  day;  $n = 39$ ) intervals to ovulation. Symbols and error bars represent least square means and standard errors. The lines represent the fourth-order polynomial regression curves that were fitted to the original data.

Type II anoestrus is an alternative pattern of follicular growth in anoestrous sows. Sows with type II anoestrus initiate follicular growth after weaning. The follicles grow to approximately 5 mm and then fail to progress to a preovulatory size (7–8 mm). The cohort that develops after weaning eventually regresses and a second wave of follicular growth begins. Langendijk *et al.* (2000) studied follicular growth using ultrasonography and reported type II anoestrus in a population of weaned sows. Oestrous sows had follicular growth from 2.3 mm (day 0) to 5.4 mm in diameter (day 4) after weaning. Type II anoestrous sows had follicular growth from 2.4 mm (day 0) to 4 mm in diameter (day 4) after weaning but failed to develop preovulatory follicles.

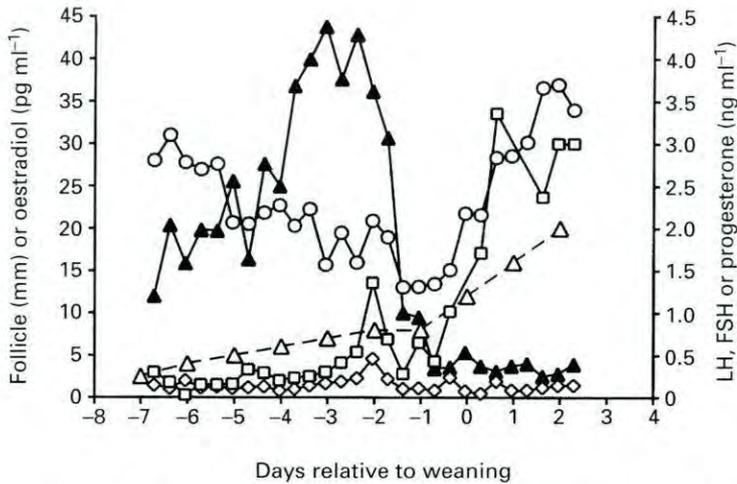
#### *Follicular growth in sows with cystic ovaries*

Cystic ovaries develop rapidly in sows (Fig. 5). Generally, follicles predetermined to become cysts grow to a diameter of 8–9 mm (slightly larger than expected preovulatory size follicles in sows), stop growing (plateau phase) and then fail to ovulate on the day of expected ovulation (approximately 2 days after reaching mature size). The plasma oestradiol concentrations decrease and plasma FSH concentrations increase before the follicles undergo rapid growth culminating in the cystic condition (20–30 mm ovarian follicles). The period of cystic follicle development was associated with progesterone secretion from the ovary perhaps arising from the cysts themselves or from corpora lutea formed after the ovulation in some follicles within the follicular cohort. Lucy *et al.* (1999) reported that cysts regressed approximately 1 week after formation and a new cohort of follicles was formed.

### **Endocrine mechanisms controlling follicular growth**

#### *Gonadotrophins before weaning*

The inhibition of LH secretion by lactation prevents preovulatory follicular development in sows before weaning (Britt *et al.*, 1985; Varley and Foxcroft, 1990). Weaning soon after

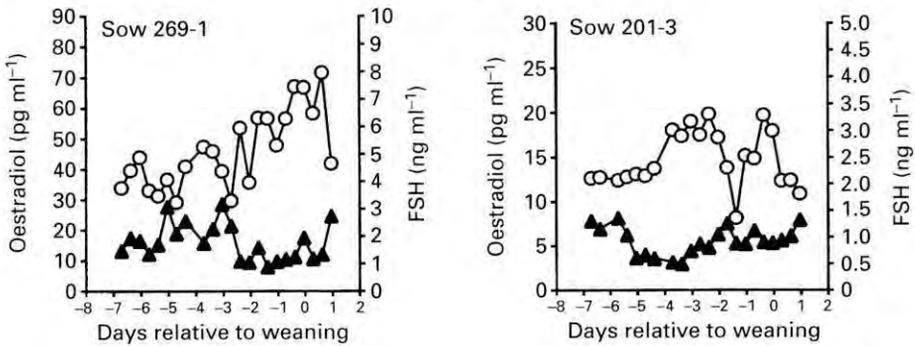


**Fig. 5.** Follicular diameter ( $\Delta$ ), and plasma concentrations of oestradiol ( $\blacktriangle$ ), progesterone ( $\square$ ), LH ( $\diamond$ ) and FSH ( $\circ$ ) in a sow developing cystic ovaries before weaning. The sow had follicular development before weaning and an increase in oestradiol. There was an LH surge on day  $-2$  that was associated with the ovulation of some follicles and a subsequent increase in progesterone. Other follicles formed cysts on the ovary.

farrowing (zero-weaning) leads to increased LH secretion and enhanced (although cystic) follicular growth (DeRensis *et al.*, 1993). In suckled sows, LH secretion initially increases after farrowing, but then decreases (DeRensis *et al.*, 1993; Sesti and Britt, 1994). During this time, the releasable pool of LH remains constant (Sesti and Britt, 1994). However, in time, LH secretion increases (Edwards and Day, 1984), perhaps because of reduced suckling stimulus as the litter matures (Edwards, 1982; Britt *et al.*, 1985; Quesnel and Prunier, 1995). The increase in LH secretion is believed to stimulate follicular growth and may explain why sows have progressively larger follicles during late lactation. Follicles that develop during lactation are capable of preovulatory follicular growth and ovulation when stimulated with GnRH or eCG (Britt *et al.*, 1985).

The secretion of LH can be modified by nutritional and environmental inputs (Einarsson and Rojkittikhun, 1993; van den Brand *et al.*, 2000), which can lead to decreased follicular growth (associated with reduced LH secretion) in sows that are under-fed (Koketsu *et al.*, 1998; Quesnel *et al.*, 1998) or exposed to high environmental temperatures (Armstrong *et al.*, 1984). In contrast to LH, the secretion of FSH is not inhibited during lactation (Britt *et al.*, 1985). Therefore, as with other farm animals, FSH shows some autonomy and appears to be regulated more tightly by ovarian factors (possibly oestradiol, inhibin or both).

Changes in plasma FSH and oestradiol concentrations coincide with the development of follicles before as well as after weaning. Plasma concentrations of FSH and oestradiol are shown for two sows before weaning (Fig. 6). In each sow, follicular development is coincident with an increase in plasma oestradiol concentrations and a decrease in plasma FSH concentrations. Greatest concentrations of oestradiol in the vena cava were associated with co-dominant follicles during the pre-weaning follicular wave. Regression of the follicular wave led to a decrease in plasma oestradiol and an increase in plasma FSH concentrations. The peaks of follicular activity were preceded by an increase in FSH that presumably stimulates a new wave of follicular development. In this sense, follicular waves before weaning in sows



**Fig. 6.** Plasma oestradiol (▲) and FSH (○) concentrations on the days before weaning (day -7 to day 0) in two sows (numbers 269-1 and 201-3). In sow number 269-1, the maximum diameter of the cohort of follicles was from day -6 to day -2 and then the follicles regressed on day -1 and day 0. The oestradiol and FSH concentrations indicate that the follicles on day -2 had become atretic co-dominant follicles because oestradiol decreased and FSH increased. The maximum diameter of the cohort of co-dominant follicles in sow number 201-3 was from day -7 to day -5 and these follicles regressed and a new wave began on day -3 to day 0. Again, the decrease in oestradiol and the increase in FSH on day -5 indicate that the cohort of co-dominant follicles had become atretic (loss of dominance).

are similar to those observed in cattle because an increase in FSH precedes the follicular wave (Ginther *et al.*, 1996).

### Gonadotrophins after weaning

Basal LH concentrations and the number of LH peaks increase after weaning (Cox and Britt, 1982; Edwards, 1982; Shaw and Foxcroft, 1985). The increase in LH pulsatility is believed to drive follicular growth toward ovulation. Concentrations of FSH initially increase after weaning, but then decrease as preovulatory follicles develop (Edwards, 1982; Shaw and Foxcroft, 1985).

We have also observed a decrease in FSH coincident with an increase in follicular growth and an increase in oestradiol in weaned sows (Fig. 2). Although LH is important for follicular growth, there is variability in the patterns of LH secretion in sows before and after weaning, and a high correlation between intensity of LH secretion and interval to oestrus has not been found. In studies of weaned sows, neither Shaw and Foxcroft (1985) nor DeRensis and Foxcroft (1999) obtained a correlation between characteristics of LH secretion after weaning and interval to oestrus or follicular growth. Similarly, FSH before or after weaning was not correlated with the interval to oestrus after weaning. However, these findings do not exclude the possibility that there are differences in gonadotrophin secretion between oestrous and anoestrous sows. In a study of FSH and inhibin, Trout *et al.* (1992) found increased FSH and inhibin in sows that underwent oestrus after weaning. Both oestrous and anoestrous sows had decreasing concentrations of FSH and increasing concentrations of inhibin after weaning. However, in anoestrous sows these changes were not associated with maturation of follicles and expression of oestrus.

### Metabolic hormones controlling follicular growth

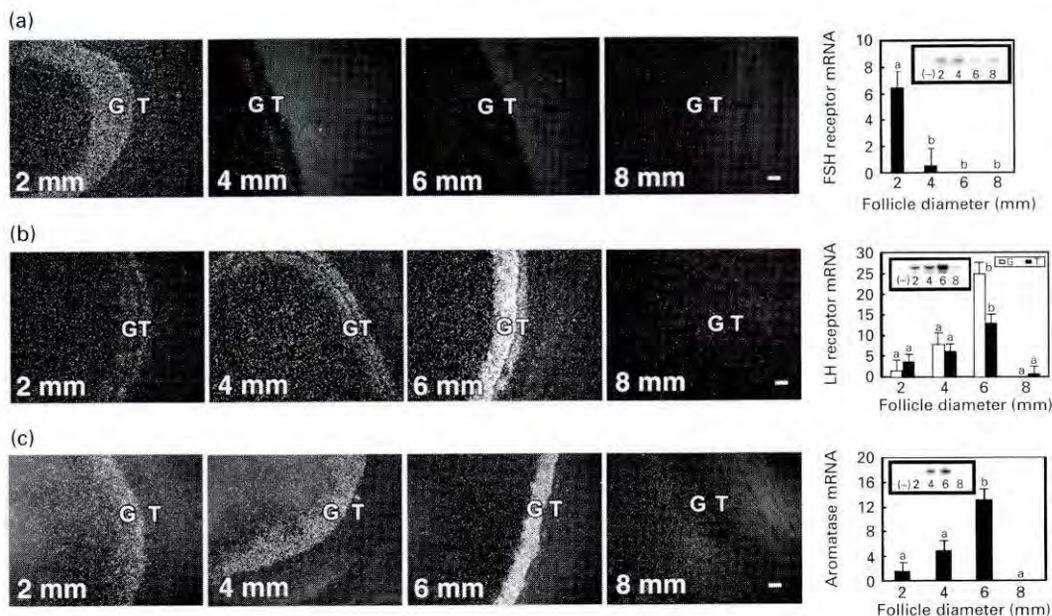
Most reproductive studies of the weaned sow have focused on LH and FSH because gonadotrophins play a role in preovulatory follicular development (Flowers *et al.*, 1991).

Metabolic hormones like insulin, IGFs and leptin are also involved by either directly influencing hypothalamic–pituitary function (Barb *et al.*, this supplement) or by affecting gonadotrophin action at the ovary. IGF-I and -II are the ligands for the IGF system (Jones and Clemmons, 1995) and both are structurally related to insulin. Their actions are similar to each other because they are similar in structure and amino acid sequence. However, IGF-II has lower potency than IGF-I for tyrosine kinase signalling through the type I IGF receptor. Neither IGF-I nor IGF-II has insulin-like metabolic effects unless present at extremely high concentrations. Likewise, insulin does not have IGF-like effects unless present at extremely high concentrations. Nevertheless, both insulin and the IGFs share some functional similarity in terms of their effects on ovarian cells. Granulosa and theca cells express insulin receptors as well as type I and type II IGF receptors (Liu *et al.*, 2000; Prunier and Quesnel, 2000). Thus, insulin, IGF-I and IGF-II can act directly on ovarian cells. The pig follicle also expresses IGF-I (granulosa cell layer) and IGF-II (theca cell layer). The intraovarian expression of the IGFs adds an additional component to the ovarian IGF system (see below). When acting alone, IGF-I, IGF-II and insulin cause growth, differentiation and survival of ovarian cells (Poretsky *et al.*, 1999; Schams *et al.*, 1999; Lucy, 2000). However, the most important actions of ovarian IGFs as well as insulin, are observed when the IGFs and insulin act synergistically with the gonadotrophins (LaVoie *et al.*, 1999; Sekar *et al.*, 2000; Zhang *et al.*, 2000). The synergistic relationship between the IGFs and insulin with gonadotrophins is observed for a variety of cellular functions, including mitogenesis and steroidogenesis. The synergism is caused by the ability of IGFs and insulin to increase the number of gonadotrophin receptors and increase the activity of gonadotrophin receptor second messenger systems. At the same time, gonadotrophins increase type I IGF receptor expression and may increase IGF-I synthesis in granulosa cells. Both insulin and IGF-I may play a role in follicular development during periods of under-feeding or weight loss because undernutrition causes a decrease in plasma concentrations of IGF-I and insulin (IGF-II is influenced less by undernutrition). According to the hypothesis, plasma insulin and IGF-I act in an endocrine manner to affect ovarian cells. The decrease in plasma IGF-I and insulin concentrations during undernutrition decreases the responsiveness of the ovary to gonadotrophins and ultimately leads to a decrease in follicular growth (Cox, 1997; Prunier and Quesnel, 2000). Mao *et al.* (1999) decreased plasma insulin and IGF-I concentrations with feed restriction and then failed to find a correlation between LH and follicular growth in sows treated with GnRH. Likewise, Cosgrove *et al.* (1992) found that re-alimentation of feed-restricted gilts could increase follicular growth in the absence of a change in LH secretion. The change in follicular development was associated with greater plasma IGF-I and insulin concentrations in the re-alimented group. Thus, feeding increases the responsiveness of the ovary to LH through its effects on insulin and IGF-I.

## **Gene expression within follicles as a mechanism to control follicular growth**

### *Gonadotrophin receptors*

The ability of gonadotrophins to act on ovarian follicles depends on the expression of gonadotrophin receptors in ovarian cells. In the preovulatory follicles, the LH receptor is expressed in both granulosa and theca cell layers (Yuan *et al.*, 1996; Liu *et al.*, 2000). The amount of LH receptor mRNA increases as follicles develop from 2 mm to 6 mm in diameter (Fig. 7). In 8 mm follicles (after the LH surge), LH receptor mRNA is not expressed. The increasing intensity of LH receptor signals reflects the increased dependence of the preovulatory follicle on LH and is also associated with an increase in aromatase mRNA in the granulosa cell layer (Fig. 7). In contrast, FSH receptor mRNA is markedly reduced in 4 mm



**Fig. 7.** *In situ* hybridization of (a) FSH receptor, (b) LH receptor and (c) aromatase in follicles 2, 4, 6 and 8 mm in diameter collected from sows after weaning (Liu *et al.*, 2000). The images were obtained using darkfield microscopy. G: granulosa cells; T: theca cells. Least square means ( $\pm$  SEM) for quantification of mRNA signal from *in situ* hybridization are shown in the far-right panel. <sup>ab</sup>Within mRNA and cell layers, bars with different superscripts were significantly different at  $P < 0.05$  (Duncan's multiple range test). The inset shows the autoradiograph of the ribonuclease protection assay (20, 10 and 10  $\mu$ g RNA for FSH receptor, LH receptor and aromatase, respectively). (-): negative control. The amount of LH receptor mRNA increased as follicles developed from 2 mm to 6 mm in diameter (b). The LH receptor mRNA was not expressed in 8 mm follicles (after the LH surge). The increasing intensity of LH receptor signal reflects the increased dependence of the preovulatory follicle on LH and was associated with an increase in aromatase mRNA in the granulosa cell layer (c). In contrast, the FSH receptor mRNA was markedly decreased in 4 mm follicles and absent in follicles larger than 4 mm (a). Therefore, the preovulatory follicles switched from FSH- to LH-dependence. Scale bars represent 120  $\mu$ m.

follicles and absent in follicles of  $> 4$  mm in diameter (Fig. 7). Therefore, the preovulatory follicle releases itself from its dependence on FSH. Smaller follicles on the ovary continue to express FSH receptor, but FSH concentrations are reduced as larger follicles (growing independently of FSH) suppress FSH secretion to basal values.

The molecular analyses are in agreement with classical studies of FSH dependence of preovulatory follicles. Guthrie *et al.* (1988) treated gilts with highly purified pig FSH and found that the FSH-treated gilts had greater numbers of 3–6 mm follicles, but the number of 7–8 mm follicles and plasma concentrations of oestradiol were not increased when compared with controls. Esbenshade *et al.* (1990) reviewed studies showing that purified pig LH could induce oestrus in sows and gilts, but only when higher doses were administered. Lower doses of LH could induce oestrus when a combination of LH and FSH were used. These data indicate that pig follicles are dependent on FSH and LH for follicular growth. The follicles switch from FSH- to LH-dependence at the 2–4 mm stage.

Liu *et al.* (2000) measured gene expression in follicles from sows after weaning. To our knowledge, gene expression in ovarian follicles has not been measured before weaning. We propose that gene expression in follicles is developmentally regulated and is similar in sows

before and after weaning. If this is the case, then follicular growth in lactating sows is initially FSH dependent. At the peak of follicular development during lactation (5 mm follicles), the follicles switch from an FSH-dependent state to an LH-dependent state. The LH-dependent follicles are oestrogenic and can suppress FSH secretion (dominance phase) (Fig. 6). However, inadequate LH pulsatility during lactation leads to atresia of the LH-dependent large (5 mm) follicles (loss-of-dominance phase). Atresia in the large follicle pool leads to an increase in FSH that triggers a new follicular wave. If sows are weaned at the peak of the follicular wave then the LH-dependent follicles continue to grow (that is, develop from 5 mm follicles to the preovulatory stage) because LH pulsatility increases immediately after weaning. We predict that the weaning to ovulation interval in these sows is short, because most the follicular development is completed before weaning. Sows weaned at the beginning of a follicular wave (shortly after the large follicle pool becomes atretic) would be expected to have a long interval from weaning to ovulation.

### *Expression of ovarian growth factor and its role in follicular development*

IGF-I and IGF-II, their receptors (type I and type II IGF receptors) and IGF-binding proteins (IGFBP-1 to -6) regulate growth, differentiation and apoptosis of ovarian cells (Poretsky *et al.*, 1999; Schams *et al.*, 1999; Lucy, 2000). IGFs can originate from endocrine sources or they may be produced locally within the follicle. Under normal physiological conditions, a threshold of IGF-I protein in follicular fluid may be met by local (paracrine/autocrine) and endocrine sources of IGF-I. In general, IGFs and IGFBPs are localized to specific cell layers (granulosa and (or) theca) within developing follicles. The location of specific components of the IGF system corresponds to the location of LH receptors and FSH receptors within the developing follicles. The co-localization of IGF and gonadotrophin receptor genes indicates a coordination of gonadotrophin and IGF action within the ovary that may control growth, differentiation and steroidogenesis of theca and granulosa cells.

Liu *et al.* (2000) measured the mRNA expression of gonadotrophin receptors, steroidogenic enzymes and IGF system genes during the maturation and differentiation of preovulatory follicles in the sow. Four developmental patterns of gene expression were observed for the ovarian mRNA. The first developmental pattern of gene expression was correlated with the oestrogenic capacity of the follicle and included genes for oestrogen biosynthesis, IGF-II and LH receptor. The second developmental pattern of gene expression was inversely correlated with follicular growth and included genes with decreased expression in large follicles (FSH receptor, IGFBP-2 and GH receptor). The third developmental pattern was constitutive (that is, no obvious developmental pattern; IGF-I and type I IGF receptor) and the fourth developmental pattern was increased expression after oestrus and the LH surge (IGFBP-4 and type II IGF receptor).

The IGF-I and type I IGF receptor mRNA were expressed constitutively (that is, no change in expression for 2–8 mm follicles). The pattern of IGF-I and type I IGF receptor gene expression is in agreement with studies in several species (Poretsky *et al.*, 1999; Lucy, 2000) and supports the observation that IGF-I expression in follicles is not correlated with follicular growth. The lack of a correlation between IGF-I and follicular growth does not mean that IGF-I is not physiologically important within the preovulatory follicle. Indeed, numerous gonadotrophin-dependent and gonadotrophin-independent actions of IGF-I have been described for both granulosa and theca cells. The lack of developmental regulation for type I IGF receptor gene expression indicates that IGF-I is required throughout the preovulatory period and highlights the importance of IGF-I and the type I IGF receptor for all phases of follicular growth.

Expression of IGF-II was high in the theca of 6 mm follicles when the greatest concentrations of oestradiol were observed in follicular fluid. The increase in IGF-II as well as the increase in steroidogenic enzymes was associated with an increase in LH receptor mRNA in both granulosa and theca interna cells of 6 mm follicles (Fig. 7). The steroidogenic enzyme and LH receptor mRNA underwent a precipitous decrease in follicles at the 8 mm stage after sows were observed in oestrus and apparently had an LH surge. However, IGF-II mRNA remained high in 8 mm follicles. The maintenance of IGF-II gene expression indicates that IGF-II may have additional functions in either ovulation or luteinization.

The availability of IGF in biological systems is modulated by IGFbps. Six different IGFbps, as well as four different IGFbp-related proteins, have been characterized (Jones and Clemmons, 1995). Each IGFbp has a unique pattern of tissue distribution and regulation. The IGFbps modulate the interaction of IGF-I and IGF-II with their receptors and may regulate cell growth and differentiation by titrating the trophic effects of IGF (Poretsky *et al.*, 1999; Lucy, 2000). Liu *et al.* (2000) examined the regulation of IGFbp-2 and -4 in the sow ovary. Expression of IGFbp-2 was inversely correlated with the diameter of the follicles (that is, IGFbp-2 mRNA decreased in large preovulatory follicles). Thus, the potential for IGF-I or IGF-II action in large follicles is enhanced by the coordinated decrease in IGFbp-2 gene expression because IGF-I action in preovulatory follicles may be greater when IGFbp concentrations are decreased (Spicer and Chamberlain, 1999). The IGFbp-4 mRNA was similar in 2, 4 and 6 mm follicles and increased in both granulosa and theca interna cells of 8 mm follicles. LH stimulated IGFbp-4 mRNA expression *in vitro* (Armstrong *et al.*, 1998) and the increase in IGFbp-4 that was observed may have been caused by the LH surge in sows with 8 mm follicles. Given the large increase in IGFbp-4 mRNA immediately preceding ovulation (8 mm follicle), we predict that IGFbp-4 may play a role in the luteinization of ovarian cells and (or) ovulation.

## Conclusions

There are numerous factors that control follicular growth, and ultimately the weaning to oestrus interval and the weaning to ovulation interval in sows. Pre-weaning follicular growth is heterogeneous because sows can experience near complete ovarian inactivity, wave-like patterns of follicular growth, cystic ovaries, or they may develop preovulatory follicles and ovulate. Gonadotrophins (LH and FSH) and metabolic hormones (insulin and IGF-I) act synergistically to promote follicular growth. Ovarian inactivity may be the result of a combination of low IGF-I and insulin concentrations, and low LH pulsatility during lactation. Conversely, the development of cystic ovaries or pre-weaning ovulation may be caused by a combination of high IGF-I and insulin concentrations, and high LH pulsatility. In many sows, follicles grow in cohorts that reach 5 mm in diameter. Follicles may cease growing at about 5 mm because they are LH dependent and LH pulsatility is low during lactation. Eventually, the 5 mm follicles regress because they cannot survive physiologically without LH. Regression of the follicular cohort results in an increase in FSH that triggers the development of a new group of follicles. LH pulsatility increases within 4 h after weaning and the increase in LH stimulates follicular development. Diameters of ovarian follicles after weaning increase beyond the 5 mm stage because LH pulsatility increases and the increase in LH rescues an LH-dependent cohort of follicles. Growth of ovarian follicles after weaning leads to an increase in oestradiol and a decrease in FSH. The decrease in FSH prevents the development of small follicles during the preovulatory period and ensures that only LH-dependent follicles (> 5 mm) will continue development. Sows with ovarian inactivity (all follicles < 2 mm) may not respond to the increase in LH after weaning because they express only low amounts of LH

receptor in their follicles. An integrated model that includes gonadotrophins, metabolic hormones and growth factors, cyclical patterns of follicular growth before weaning, and developmental regulation of gonadotrophin receptors, growth factors, and growth factor receptors may be necessary to explain follicular growth, interval to oestrus and interval to ovulation after weaning in sows.

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