Porcine prolactin levels during the periparturient period: The development of a porcine prolactin radioimmunoassay

by

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INTRODUCTION

A condition occurs in sows in which they fail to lactate shortly after parturition resulting in high baby pig losses due to starvation. For years, researchers have been trying to elucidate the cause of this hypoglactia or agalactia.

Bacterial mastitis has been implicated but more than one species of bacteria can cause the condition. Not all agalactic sows have mastitis but instead have sterile mammary glands. It is also believed that endotoxins produced by E. coli populations either in the mammary gland or other parts of the body such as the gut could result in agalactia. Furthermore, there are probably several factors that may contribute to the incidence of this condition, but in controlled experiments they have been unable to cause agalactia (Penny, 1970). Thus environment, diet, exercise and endocrine factors have been demonstrated to play only a minor role in the actual course of the disease.

In order to determine the cause or causes of lactation failure, it is necessary to understand hormonal events which are responsible for preparing the mammary gland for lactation, initiating lactation and maintaining lactation. Prolactin, a protein hormone produced by the anterior pituitary, is important in all of these stages of lactation for most mammalian species studied thus far.

Wagner (unpublished data, W. C. Wagner, Professor and Head, University of Illinois College of Veterinary Medicine, Urbana) treated lactating sows with single injections of ergocryptine (Sandoz, CB 154), a substance known to inhibit prolactin release, and was able to cause complete cessa-

tion of lactation within 24 to 36 hours. The symptoms closely resembled field cases of agalactia. This suggests that prolactin is important and necessary for lactation in the pig, although proof of this would depend on measurement of prolactin in these pigs.

This work concerns the development of a radioimmunoassay to measure plasma prolactin in pigs. With this assay, prolactin concentrations were measured for several days prepartum and postpartum. Knowledge of plasma prolactin concentrations during the periparturient period is a first step in determining the significance of prolactin for lactation in the pig. The new information presented here demonstrates that prolactin levels during the periparturient period follow trends similar to those in other species. Endotoxin induced agalactia could be caused at the hypothalamic level by interference in the synthesis or release of prolactin or at the target tissue by some interference there. Studies to elucidate these mechanisms will be greatly aided by the assay method and data presented here.

REVIEW OF THE LITERATURE

Prolactin

Prolactin (PRL) is a protein hormone synthesized, stored and secreted by lactotrophic cells of the anterior pituitary gland. The complete amino acid sequence for procine PRL has been determined (Li, 1973) and in comparison to ovine PRL 162 of 198 residue positions are occupied by identical amino acids. The molecular weight of porcine PRL is approximately 22,400 compared to 23,300 for ovine PRL (Bewley and Li, 1975). Bovine PRL differs from ovine PRL by only two amino acids.

Prolactin is a phylogenetically old hormone controlling a wide variety of physiological mechanisms (85 distinct and diverse effects for actions of PRL among vertebrates) in different species (Nicoll, 1974). Horrobin annually reviews and summarizes papers published on prolactin (Horrobin, 1976; Horrobin, 1977). His monographs disclose the many specific and individual actions of prolactin as well as its synergistic and permissive actions. Prolactin probably has many target organs (Horrobin, 1976, p. 46; Horrobin, 1977, p. 53). In some species PRL has metabolic effects similar to, but less pronounced than growth hormone (Horrobin, 1976, p. 56; Horrobin, 1977, p. 64). It has recently been reported that PRL is important in male reproduction although its precise actions remain to be determined (Horrobin, 1976, p. 60; Horrobin, 1977, p. 68). The role of PRL in female reproduction varies from the sheep where it seems necessary for normal ovarian function (Denamur and Martinet, 1961) to cattle where no such indication has been reported to date. Prolactin is a major

regulator of fluid and electrolyte metabolism in lower vertebrates and probably several mammals (Horrobin, 1976, p. 118; Horrobin, 1977, p. 126). This role of PRL may prove to be one of its most important roles in helping to maintain lactation.

Prolactin is one of a complex of hormones involved in mammogenesis, lactogenesis and galactopoiesis (Horrobin, 1976, p. 79; Horrobin, 1977, p. 93). Stricker and Grueter (1928) were first to recognize the importance of an anterior pituitary hormone in initiating lactation and Riddle et al. (1933) were the first to call it prolactin.

Specific prolactin receptors have thus far been found in the mammary gland, ovary, uterus, liver, kidney, pituitary, cerebral cortex, adrenal cortex, prostate, testis, epididymis, seminal vesicles, muscle and hair follicles (Horrobin, 1976, p. 46; Horrobin, 1977, p. 53). Many of the actions of PRL depend on the time of day, length of daylight, season and sex. Many factors (temperature, season, time of day, etc.) modify PRL levels and many stimuli are known to cause prolactin release. Prolactin was thought to be distinct from other hormones in having no feedback from its target organs on its secretion. Recently, however, it has been found that there could be a positive feedback from the mammary gland on PRL secretion (Northrup et al., 1975; Harada, 1976).

The numbers of lactotrophs in the AP vary within species according to physiological state and also between species. For example in man, PRL cells show progressive hyperplasia during pregnancy so that by the end of pregnancy and during lactation, lactotrophs are the predominant anterior

pituitary cell (Pasteels et al., 1972). In pigs, the number of prolactin cells increases in later stages of pregnancy (Anderson et al., 1972).

Steps involved in prolactin secretion have been described by Farquhar (1977) as follows:

(1) Synthesis of PRL on attached polyribosomes.

(2) Secretion in rough endoplasmic reticulum (RER).

(3) Transport from rough ER to Golgi by small vesicles located at the periphery of the Golgi apparatus.

(4) Concentration within the innermost one or two Golgi cisternae.

(5) Aggregation and further concentration within immature granules.

(6) Storage within mature granules.

(7) Discharge of granules containing PRL:

i) Extracellularly at the cell membrane, or

ii) Intracellularly into lysosomes where it is subsequently degraded (crinophagy).

Prolactin is stored until needed in secretory granules which vary in size (500-900A) according to how many Golgi-derived small packets have merged and pooled their contents. In lactating animals under continuous stimulation to discharge synthesized hormone, relatively few large granules accumulate (Farquhar, 1977).

Control of Prolactin

Prolactin secretion is primarily regulated by the hypothalamus and mediated by two factors: (1) Prolactin Inhibitory Factor (PIF) which decreases PRL secretion and (2) Prolactin Releasing Factor (PRF) which increases it. Unlike the situation for the other anterior pituitary hormones, PRL is under a predominantly inhibitory influence from the hypo-

thalamus in mammals. There are at least three substances present in the hypothalamus which inhibit PRL release (PIF, catecholamines, and acetylcholine) and four or more substances that can increase PRL release (PRF, serotonin, thyrotropic releasing hormone (TRH) and prostaglandins) (see Meites, 1977 for review). The structures of PIF and PRF are not yet known but it is believed that they could be small polypeptides.

Dopaminergic neurons in the basal hypothalamus are responsible for the majority of the inhibitory influence over PRL release. Dopamine terminals from the tubero-infundibular system end directly on the portal capillaries in the median eminence and on the end feet of the releasing hormone axons as axoaxonal presynaptic junctions (MacLeod and Login, 1977). Therefore, dopamine may either be delivered directly into the portal system or inhibit hormone release from its axoaxonal contacts. In this way, dopamine could have strategic importance in regulating the secretion of prolactin. Dopamine inhibits PRL release from rat anterior pituitaries (Koch et al., 1970; MacLeod and Lehmeyer, 1974; Samli and MacLeod, 1974). The administration of a precursor to dopamine, L-DOPA, rapidly increases the stores of brain catecholamines and decreases serum PRL. Administration of agents which reduce brain catecholamines (reserpine or α-methyldopa) stimulates the synthesis of PRL in the pituitary and increases serum PRL. Specific dopamine blocking agents increase serum PRL (MacLeod and Login, 1977). Voogt and Carr (1975) have also demonstrated that injection of a catecholamine synthesis inhibitor (α methyl-p-tyrosine) increased PRL levels and decreased accumulation of newly synthesized dopamine and norepinephrine in both nonsuckled and suckled lactating

rats. However, more evidence is required to prove that dopamine is the PIF of the hypothalamus. Dopamine may act to either increase PIF or it may act independently of PIF and directly on the anterior pituitary to decrease PRL release.

Greibrokk et al. (1974) isolated PIF and later reported that their porcine PIF extract had properties of a peptide rather than a catecholamine (Greibrokk et al., 1975). Dular et al. (1974) also found that purified preparations of PIF and PRF from bovine pituitary stalk and median eminence contained peptide material. In direct contrast, Schally et al. (1976) reported that the PIF activity present in extracts of pig hypothalami was due to the catecholamines, noradrenalin and dopamine. The influence of norepinephrine on PRL release has not been defined. Norepinephrine has been shown to decrease PRL release and recently Carr et al. (1977) reported it was important in the estrogen mediated increase in PRL release. Another material reported to possess PIF activity is gamma aminobutyric acid although it is unknown if its effects are physiological or pharmacological (Schally et al., 1977).

Another inhibitory influence on PRL exists as a "short feed back loop" in which PRL inhibits its own secretion (Meites, 1972). It is believed that PRL exerts this effect at the hypothalamic level by activating dopamine and thereby increasing PIF. There could also be a direct action of PRL on the pituitary since PRL receptors have been found on anterior pituitary cells (Frantz et al., 1975).

The controversy about the existence of a peptide PIF also exists for a peptide PRF. Thyrotropin-releasing hormone (TRH) acts directly on the

pituitary to stimulate PRL secretion *in vitro* and *in vivo* in a wide variety of species and was believed to be PRF. However, evidence against TRH as the hypothalamic PRF is that suckling is associated with a substantial rise in PRL secretion with little or no change in TSH. The evidence is now very strong for a PRF distinct from TRH. Kokubu et al. (1975) identified a PRF substance in bovine hypothalami which eluted before TRH, suggesting it is larger than TRH. Szabo and Frohman (1976), using porcine stalk/median eminence extracts, also distinguished PRF from TRH activity. Incubation of their extract with plasma, which is known to destroy TRH, destroyed TSH releasing action to a greater extent than the PRL releasing action.

It is well-known that administration of serotonin or serotonin precursors can stimulate PRL secretion. Blockade of serotonin secretion or action has no effect on basal PRL level but can prevent a PRL rise in response to suckling. Apparently serotonin dependent mechanisms play no part in basal regulation, but may control suckling release (Clemens et al., 1977; Horrobin, 1977; MacLeod and Login, 1977). The precise mechanism for this action of serotonin is unknown.

Estrogen stimulates PRL synthesis and release both *in vivo* and *in vitro*, potentiates responses to agents such as TRH or dopamine blocking agents, and enhances responsiveness to PRL releasing stimuli. The effect of estrogen is at the hypothalamic and at the pituitary levels. Estrogen binds specifically to nuclear components of pituitary cells, probably having the same mechanisms of action as in peripheral target tissue (Sulman, 1970; MacLeod and Lehmeyer, 1972; Farquhar, 1977).

A review of hypothalamic control of secretion and release of PRL by Tindal (1974) suggests the following conclusions. A PIF and PRF exist in the hypothalamus and are distinct from hypothalamic amines which regulate them. PIF is probably located in a diffuse area of the medial hypothalamus. Manufacture or storage of PIF may be in the arcuate nucleus, ventral part of the ventromedial nucleus and median eminence. PRF may be in the preoptic, lateral and posterior hypothalamus. There may be one mechanism involving inhibition of PIF which accounts for the major release of prolactin due to "natural" stimuli such as milking or suckling. Another mechanism involves a specific PRF for the more rapid trivial release associated with minor stresses and traumas. A PRL release pathway has been traced in the rabbit but apparently terminates at some distance from the median eminence. Therefore, there must be a "final" neuron chain between the PRL release pathway and neurons of PIF release. When electrically stimulated, this "final" pathway causes an increase in PIF and a decrease in PRL release. However, incoming stimuli along the PRL release pathway achieve PRL release by inhibiting this final neuron chain.

Appropriate incoming neural and humoral stimuli activate or depress hypothalamic amines. These amines are the final neurotransmitters in a chain of events leading to release of hypothalamic factors and hence trophic hormones. The balance between release and inhibition of release of PRL is mediated by hypothalamic amines acting via PIF.

There exists a dopaminergic innervation of the external layer of the median eminence originating in cells of the arcuate nucleus and anterior periventricular hypothalamic nucleus. There is also noradrenergic inner-

vation in the internal layer of the median eminence and throughout the hypothalamus and preoptic area. Cell bodies of noradrenergic neurons are probably located further away in the hypothalamus or possibly entirely outside the hypothalamus. A serotoninergic pathway from the mesencephalon terminates diffusely in the hypothalamus. Dopamine inhibits PRL release by maintaining secretion of PIF. Noradrenaline may act through PRF to achieve minor acute release of PRL. Serotonin and possibly melatonin inhibit or reduce release of PIF, leading to the major, prolonged increase in circulating PRL levels.

Prolactin exerts a negative feedback effect on its own secretion by raising PIF. Prolactin does so by activation of dopaminergic terminals in the external layer of the median eminence which in turn increases release of PIF by an axoaxonic effect. In contrast, estrogen causes release of PRL by acting at the hypothalamic level and reducing PIF content or by acting directly on the pituitary.

Biochemistry of Prolactin: Its Effects on the Mammary Alveolar Cell

This discussion is a summary of important and generally accepted effects of prolactin on the mammary alveolar cell. The information discussed here comes largely from that presented in Turkington (1972a), Turkington (1972b), Horrobin (1976) and Horrobin (1977).

Prolactin participates in the regulation of alveolar cell differentiation and induces the synthesis of milk proteins after parturition. The initial events in stimulation of the target cell involve specific PRL

receptors found in preparations of mammary plasma membranes (Turkington, 1972b; Shui and Friesen, 1974; Frantz et al., 1974). The receptor sites which bind PRL exhibit a high degree of specificity for PRL and have the greatest affinity for PRL. However, competitive displacement studies demonstrate that these sites can also bind other hormones with lactogenic activity (human growth hormone and human placental lactogen). Whether prolactin's effect is a result of a reaction at the outer cell membrane or a result of PRL or some "active" form of PRL inside the cell is unresolved. Prolactin can stimulate RNA synthesis in isolated nuclei (Chomczynski and Topper, 1974). But there are PRL receptors which seem to be mainly located on the alveolar surface of cells adjacent to the vascular supply. It is clear that there are many effects of PRL on explants of mammary gland pretreated with insulin and hydrocortisone.

Insulin causes division of nonsecretory cells giving rise to daughter cells which are identical to their undifferentiated parent cells. The daughter cells, when treated with hydrocortisone, develop a copious supply of granular endoplasmic reticulum. Hydrocortisone is essential for the formation of rough ER. Daughter cells treated with hydrocortisone and subsequently with insulin and PRL display an enlarged supranuclear golgi apparatus with nuclear and granular endoplasmic reticulum shifted to the basal portion of the cell. Secretory granules then appear in the golgi vesicles of these cells. RNA polymerase activity doubles after incubation with insulin with a further increase upon PRL stimulation of explants preincubated in a medium of insulin and hydrocortisone.

An early effect of PRL is an increase in phosphorylation of histones and certain nonhistone nuclear proteins. The de novo synthesis of proteins with which cAMP will interact is induced by PRL. Increase in the intracellular concentration of cAMP-activated protein kinase and cAMP-binding protein occurs rapidly in response to the addition of PRL. This protein-kinase complex exhibits a high specificity for histones. It appears to cause phosphorylation of specific nuclear proteins which in turn bind better to histones, removing histones from DNA and thus allowing for transcription of that portion of DNA to occur (Majumder and Turkington, 1972).

Prolactin's primary effect is at the transcriptional level to increase nuclear RNA, t RNA and r RNA (hydrocortisone and insulin are also required). Transcription of these multiple classes of RNA leads to induction of milk proteins. Prolactin and insulin induce both galactosyl transferase and α-lactalbumin in epithelial cells pretreated with insulin and hydrocortisone. Together these form a complex, lactose synthetase, an enzyme that catalyzes the terminal and rate limiting step in the biosynthesis of lactose. Since synthesis of α-lactalbumin can be inhibited by progesterone, the fall in progesterone levels at parturition is a key factor in initiation of normal lactation (Turkington, 1972b).

Subsequent to lactose synthetase induction, PRL initiates the formation of casein. The continuing presence of insulin is required for both casein and lactose synthetase induction.

Mammary alveolar cells are also capable of de novo synthesis of short chain fatty acids via the malonyl-CoA pathway. Prolactin preferentially

stimulates the pentose pathway for glucose oxidation and thus will supply the required NADPH. Prolactin also activates pyruvate dehydrogenase which may be the rate-limiting step in the synthesis of fatty acids from pyruvate. Prolactin, insulin and cortisol are required for maximum synthesis of fatty acids.

Action of Prolactin on Fluid and Electrolyte Movements across the Mammary Alveolar Cell

Another role of prolactin on the mammary alveolar cell is control of the movement of monovalent cations into milk. However, the way in which prolactin achieves this is controversial.

In the rabbit in late lactation (25-28 days), milk [Na^+] and [Cl^-] are high while [K^+] and [lactose] are low. Linzell et al. (1975) and Taylor et al. (1975) believe this is due to an increased permeability of a paracellular pathway. In the Dutch-type rabbit there is a small paracellular route throughout lactation. The change in milk composition in late lactation and the increased passage of Na^+ and Cl^- from blood to milk can be entirely accounted for by an increased paracellular movement. They also described the situation in the goat during late lactation when disaccharides can cross the epithelium suggesting that the tight junctions become "leaky" allowing exchange of Na^+ and Cl^- for K^+ and lactose between blood and milk by this route. This group found that the administration of PRL during late lactation caused milk composition to change to that obtained during normal mid-lactation with milk [Na^+] and [Cl^-] being significantly lower and milk [K^+] and [lactose] higher than in control groups

in late lactation. The entry of ^{14}C-sucrose into milk and the calculated paracellular movements of both Na^+ and Cl^- were significantly lower. They believe the mechanism by which prolactin does this is by affecting tight junctions between secretory cells acting to decrease the permeability of the paracellular pathway.

Falconer and Rowe (1977) believe prolactin modulates changes in intracellular ions by way of the Na^+/K^+ ATP-ase. In this way, prolactin controls the transport of Na^+ across the basal membranes of the mammary alveolar cell and thus intracellular Na^+ content and $Na^+:K^+$ ratio of the cells. They arrived at this conclusion through in vitro and in vivo experiments in which ouabain, a specific inhibitor of Na^+/K^+ ATPase was used. It reversed the effects of PRL to decrease whole tissue and calculated intracellular $[Na^+]$ and increase $[K^+]$ in rabbit mammary glands. They believe the presence of both Na^+/K^+ ATPase and PRL receptors in the basal region of the plasma membrane of alveolar cells is consistent with their suggestion. Their studies provided no information on the existence of "tight" or "leaky" cell junctions in mammary alveolar cells as described by Linzell et al. (1975) and Taylor et al. (1975).

Whatever the mechanism, it is clear that prolactin controls electrolyte movements across the mammary alveolar cell and in this way influences the aqueous composition of milk. Prolactin may be acting in a similar manner on the kidney. It is a major regulator of fluid and electrolyte metabolism in submammalian species but research to date on this effect in mammals is highly controversial.

Role of Prolactin in Lactation in Women

Mammogenesis

Hormonal control of mammary growth (mammogenesis) and initiation of milk secretion (lactogenesis) is still little understood in the woman. There is probably an essential, though permissive, role of PRL in the development of the human breast. However, its precise function as a mammotropic agent in human beings remains to be defined (Frantz, 1978). Pituitary hormones are probably involved in mammogenesis and there is an increased size of the pituitary gland throughout pregnancy in women. This is because lactotrophs are numerous and hypertrophied in pregnant and postpartum women (Pasteels et al., 1972). In laboratory animals, PRL and growth hormone acting in conjunction with ovarian hormones stimulate normal mammary growth. This may be the case in women. The ductular-lobular-alveolar growth during pregnancy is evoked by luteal and placental sex steroids (estrogen and progesterone). Prolactin, placental lactogen, growth hormones and probably chorionic gonadotropin support the lobular-alveolar tissue growth of the breast. Together with insulin and cortisol, these hormones contribute to the differentiation of glandular epithelial stem cells into presecretory mammary cells. Adrenal corticosteroids (mainly cortisol) also contribute to mammary cellular differentiation (Vorheer, 1974).

Lactogenesis

The synthesis of colostrum during late pregnancy is most likely due to effects of PRL, human placental lactogen and metabolic hormones. It

appears that during pregnancy the synthesis and secretion of milk into the alveolar lumen induced by PRL is inhibited directly at the mammary epithelium by estrogen and progesterone. Only with the postpartum withdrawal of these steroids is PRL fully capable of stimulating synthesis and secretion of milk into the alveoli. Thus, lactogenesis involves conversion of glandular cells from presecretory into secretory cells (synthesizing and releasing milk) and requires estrogen and progesterone withdrawal (Vorheer, 1974).

Prolactin levels

Throughout pregnancy, while growth and development of the mammary gland are taking place, PRL levels in the woman are increasing dramatically and reach a maximum at term (Tyson et al., 1972; Tyson and Friesen, 1973; Jaffe et al., 1973; L'Hermite et al., 1975b). Tyson and Friesen (1973) reported basal plasma PRL concentrations of 214 ng/ml at term and PRL levels rose even higher in response to intravenous TRH. This progressive rise in pituitary release of PRL is believed to be related to high estrogen secretion during gestation (Rigg et al., 1977). In other species, estrogen and PRL levels usually remain low until the last trimester or less of pregnancy.

After parturition, PRL concentrations decrease and basal levels are reached in the nonpregnancy range at about two weeks postpartum. Prolactin levels fluctuate widely during puerpheral lactation (Jaffe et al., 1973). This fluctuation is due to PRL release in association with suckling. Milk production remains relatively constant despite these wide fluctuations. As lactation advances, the amount of PRL released in

response to suckling decreases. This decrease in PRL release is not due to a reduction in the releasable pool of PRL in the pituitary, as the amount of PRL released in response to TRH is not altered.

In nursing mothers each suckling period induces a dramatic rise in plasma PRL. This increase in PRL secretion after suckling is important for initiation and maintenance of lactation. A correlation has been shown between an increase in milk yield and net increases of PRL in response to suckling. No correlation exists between milk yield and basal PRL levels (Aono et al., 1977). In this study, no post-nursing increases in PRL were found at 4 and 6 days postpartum in a poor lactation group. The periodic PRL increment and removal of milk from the alveolar lumen induced by regular suckling promote further milk production. It has been observed that an overall PRL secretion is greater in a successful lactator than an unsuccessful one.

Further proof that PRL is important for lactation in women comes from studies in which TRH has been used to induce PRL release. Tyson et al. (1975) have shown that an elevation of serum PRL induced by TRH was associated with a significant increase in mammary milk production in postpartum women. TRH promoted breast engorgement and a rise in both milk volume and milk fat concentration in these women.

The importance of PRL in milk secretion by women has been substantiated in several studies involving the suppression of PRL secretion by using 2-Br-α-ergocryptine (CB-154) in lactating women (del Pozo, 1972; del Pozo and Fluckiger, 1973; Brun del Re et al., 1973; Rolland and Schellekens, 1973; Rolland et al., 1975). CB-154 is known to inhibit PRL

release and possibly its synthesis at the pituitary level. In addition, CB-154 has been shown to reduce transmitter turnover in the tuberoinfundibular dopaminergic neurons in the hypothalamus (Floss et al., 1973; Clemens et al., 1975; Vaisrub, 1976; Fluckiger, 1978). In the immediate postpartum period, CB-154 causes a marked suppression of plasma PRL levels and completely inhibits puerperal lactation and breast engorgement (Brun del Re et al., 1973). This same group has found that CB-154 is also effective in suppressing an established lactation confirming the role of PRL in maintaining lactation in the woman. They also reported this compound inhibits the PRL peak that normally occurs in response to suckling.

Artificial induction of lactation

Successful artificial induction of lactation can also help clarify the role that hormones play in initiating lactation in the normal state. Tyson et al. (1975) have reported the initiation of lactation in two nonpuerperal women. These women each received 2.5 mg of conjugated estrogen twice daily and 0.35 mg norethindrone once daily for 14 days. TRH (100 µg) was injected at the beginning, middle and the end of the 14 day period. Following estrogen withdrawal, a PRL response to nipple stimulation appeared and PRL response to TRH increased. The nipple stimulation and subsequent PRL release resulted in milk secretion and ejection.

Role of Prolactin in Lactation in the Cow

Mammogenesis

Hormonal control of mammogenesis and the onset of lactation (lactogenesis) in cows has been reviewed by Convey (1974) and Erb (1977). In

the cow, as in other species, estrogen stimulates development of mammary ducts while both estrogen and progesterone stimulate proliferation of secretory tissue and synchronize secretory cells ready for differentiation before secretion begins. Alveoli do not appear before the first pregnancy since concentrations of estrogen and/or progesterone are too low or elevated too briefly to synergize lobulo-alveolar development. Development of the mammary gland to a degree capable of milk production appears to require high levels of estrogen and progesterone followed by their decrease at parturition. Progesterone is apparently the main inhibitor of lactation before parturition and until this progesterone block is removed PRL cannot initiate synthesis of α-lactalbumin and lactose. However, enzymes necessary for lactose and fatty acid synthesis and for hydrolyzing blood triglycerides for uptake of lipids by the mammary gland are present in the mammary gland well in advance of parturition (Convey, 1974).

Lactogenesis

Erb (1977) postulates that sequential development of secretory cells requires: (1) insulin for one division of parent cells; (2) organelle formation in daughter cells requiring cortisol; and (3) secretory capability requiring PRL. Progesterone inhibits this process at step 2 by competing for high affinity receptors which bind both progesterone and glucocorticoids. Large amounts of PRL may be ineffective until step 2 is completed after which basal concentrations of hormones may be adequate to meet requirements for the onset of lactation.

Mammary explants from cows have been used to elucidate the hormones necessary for lactogenesis in the cow and are in agreement with Erb's

proposal (Collier et al., 1977a). The culture of explants in a medium containing insulin plus hydrocortisone resulted in alterations in alveolar cell cytology but no milk synthesis. Prolactin was required for induction of milk synthesis. Heifers must be pretreated with estrogen and progesterone before subsequent mammary explants can be maintained by other hormones. Growth hormone and thyrotropic hormones are also known to be lactogenic in cattle, probably due to their metabolic effects (Schmidt, 1971).

Prolactin may be an important component of the hormonal milieu concerned with initiation and maintenance of lactation, although there is still a paucity of information as to its in vivo role in mammary function in the cow. Consistent periparturient increases in prolactin suggest it may be needed for lactogenesis in cows. Approximately five days before parturition, PRL concentrations begin increasing and reach a peak one day before parturition. Prolactin levels then decrease to values characteristic of pregnancy by two days. They stabilize until day 9 and then decline again until day 26 postpartum (Ingalls et al., 1973). Elevated PRL levels at parturition may be unrelated to subsequent lactation as basal levels alone may meet requirements when the inhibiting effects of progesterone are removed. The role of PRL in the lactating cow may not be solely in facilitation of milk secretion but in coordination of the use of nutrients from food and/or body reserves (Swan, 1976).

Lactogenesis is depressed and the composition of colostrum is altered if CB-154 is administered before parturition (Schams et al., 1972).

Treatment of cows with TRH during the onset of lactation has been shown to increase milk yield (Karg and Schams, 1974).

Galactopoiesis

Teat stimulation is a specific stimulus for PRL release in cattle (Reinhardt and Schams, 1974). It seems logical that the release of prolactin at milking or nursing in cows must be responsible for maintaining lactation. However, the role of PRL in galactopoiesis in the cow is still not understood. Koprowski and Tucker (1973) reported little correlation between serum PRL levels measured 2 to 4 hours before milking and milk yield during weeks 1-44 of lactation. There was only a small, but significant, positive correlation between milk yield and PRL levels immediately after and 1 hour after milking. Suppression of PRL with CB-154 does not significantly inhibit milk production (Karg et al., 1972; Smith et al., 1974). However, results of these experiments should not be interpreted as evidence that PRL is not required for galactopoiesis. Even after treatment with CB-154, a low basal level of PRL (1 ng/ml) was evident and may have been adequate to maintain lactation in the cow.

Artificial induction of lactation

Hormone induced lactation in cows substantiates what has already been discussed about hormonal control of mammogenesis and lactogenesis. Cows have been treated to induce lactation with 17β-estradiol plus progesterone (days 1 to 7) and dexamethasone (days 17 to 19) (Croom et al., 1975; Collier et al., 1975). However, not all cows responded. Those which were successful underwent critical periods of cellular proliferation and were undergoing lactogenesis and fatty acid synthesis by day 8, while unsuc-

cessful cows did not. Using a similar treatment regimen, Erb et al. (1976) found that the major differences associated with inferior lactations were high titers of estrogen in plasma on the last day of treatment (day 7) and failure to maintain above average titers through day 14 to 17 concurrent, with rapid decreases in progesterone. The inferior lactators also had chronically low mean PRL concentrations after day 17. Bauman et al. (1977) have shown that reserpine treatment in the cow caused a prolonged elevation of plasma PRL and could potentially be used for in vivo studies designed to delineate the role of PRL in dairy cows. They later reported that reserpine administration during hormonally induced lactation elevated serum PRL and caused higher peak milk yield and greater milk production (Collier et al., 1977b). Results were consistent with their hypothesis that PRL is the limiting factor in those cows which fail to lactate following estrogen-progesterone treatment to induce lactation.

Prolactin levels in postparturient cows are probably higher than needed to maintain milk secretion. The possibility also exists that PRL has no role in galactopoiesis in the cow. Prolactin has a more fundamental role in metabolism than just its involvement in mammary physiology. This is reflected in the fact that such a wide variety of stimuli can elicit its release. Serum prolactin concentrations in cows fluctuate with a circadian periodicity (Koprowski et al., 1972) and are also influenced by daylight hours and season (Schams and Reinhardt, 1974) and temperature (Wetteman and Tucker, 1974; Tucker and Wetteman, 1976; McMurtry et al., 1974). Stressful stimuli and changes in metabolic events also alter serum PRL concentrations.

Role of Prolactin in Lactation in Sheep and Goats

General

In sheep and goats as in cows, the mammary gland apparently grows in response to sustained high serum concentrations of estrogen and progesterone during pregnancy. However, there is some question whether estrogen and progesterone are required in the goat. Prolactin, GH and glucocorticoids are of sufficient quantity to support this mammary growth during pregnancy. There is also good evidence for the involvement of placental lactogen in mammary growth in sheep and goats but the exact role of placental lactogen is not known. Development of the goat udder begins in mid-pregnancy with rapid changes in the formation of lobulo-alveolar tissue between days 70 and 80 which coincides with increasing placental lactogen concentrations and continued low PRL concentrations (Buttle et al., 1972). In sheep, pregnancy is unaffected by hypophysectomy after day 30 and since some mammary development and a transient lactation occur, the placenta is believed to be substituting for the pituitary in promoting udder growth (Denamur and Martinet, 1961). Placental lactogen of sheep can be detected in plasma samples by day 60 of gestation and thereafter it increases as pregnancy advances, reaching peak concentrations on days 95 to 114 of gestation, followed by a decline (Kelly et al., 1974).

The sheep, like other ruminants, experiences increases in PRL at parturition (Arai and Lee, 1967; Davis et al., 1971; McNeilly, 1971; Kelly et al., 1974; Lamming et al., 1974; Burd et al., 1976). The more recent studies agree with the trends of PRL levels as published earlier although striking differences in actual PRL concentrations occur. Pro-

lactin levels are probably low throughout most of pregnancy with a great increase around 3 days prepartum and highest peaks on the day of parturition. Prolactin levels decrease in early lactation, reaching basal levels within about four weeks. However, reports are also in disagreement here. During the immediate postpartum period there is substantial release of PRL at suckling. Thereafter, however, the PRL response to the suckling stimulus declines (Lamming et al., 1974). The role of PRL released at parturition and the effect on milk secretion of PRL release during milking have not been determined.

Lactogenesis in sheep

Secretory activity of mammary tissue has been detected on the 90-100th day of pregnancy in the ewe. At this time some researchers report basal levels of PRL (Arai and Lee, 1967; Davis et al., 1971; Kelly et al., 1974), while other researchers report substantial increases in PRL are beginning to occur (McNeilly, 1971; Lamming et al., 1974). Since placental lactogen and PRL are both increasing at this time they may both be important in mammogenesis and lactogenesis. Anytime after 100 days, injections of glucocorticoids can induce precocious milk secretion in the ewe (Denamur, 1971). This is consistent with the situation in the cow where it is suggested that glucocorticoids may be overcoming the progesterone block by competing successfully for receptors.

Prolactin undoubtedly plays an important role in lactation in the ewe but as one hormone of a lactogenic complex. Prolactin and adrenal steroids are not effective alone in hypophysectomized ewes but the addi-

tion of thyroxine and growth hormone result in rapid secretion of significant quantities of milk (Denamur, 1971).

Studies in which specific areas of the hypothalamus, known to control PRL secretion, were sectioned lend support to an important role of PRL in mammogenesis and lactogenesis (Wolinska et al., 1977). The anterior medial basal hypothalamus (MBH) is the center for stimulatory action on production and release of PRL. The caudal MBH is the inhibitory center of PRL release. Lesions made in the anterior MBH in pregnant or lactating ewes caused lack of development of the mammary gland and depressed milk yields. A decrease in plasma PRL and structural changes in PRL cells as manifested by lack of expected degranulation (lack of hormone release) also occurs. Lack of development of the mammary gland and depressed milk yields suggest PRL was required in these processes.

The essential role of prolactin for lactogenesis has been determined in vitro (Kann and Denamur, 1974) using mammary tissue from pregnant primiparous ewes with biopsies performed between day 80 and 90 of gestation. If tissues contained well-formed acini with nonsecretory alveoli, then insulin, cortisol and PRL induced milk secretion after 5 days of incubation.

Mammogenesis in the goat

Cowie (1971) has shown that mammogenesis in the goat proceeds as a rapid growth of lobulo-alveolar tissue between the 70th and 100th day of pregnancy. Lactogenesis in the pregnant goat is indicated by secretions containing lactose and fat globules occurring in the alveoli approximately day 86 to day 111 of gestation. Since low levels of PRL are found in the

goat during pregnancy and high levels of placental lactogen have been detected in the second to last trimester of pregnancy (Buttle et al., 1972), this mammary gland growth and early secretion may occur in response to the placental lactogen. Currie et al. (1977) have confirmed the existence in goats of a prolactin-like hormone derived from the placenta. This caprine placental lactogen has lactogenic and GH-like activities and probably provides a powerful trophic stimulus to the udder during pregnancy.

There is controversy about whether or not mammary gland growth occurs in response to sustained high levels of estrogen and progesterone during pregnancy. Cowie et al. (1968) have suggested that a substantial part of mammary growth response to ovarian and placental steroids in the goat may be associated with their ability to cause release of the mammogenic hormone complex from the anterior pituitary. In the absence of the pituitary, estrogen and progesterone fail to stimulate growth of the mammary gland or to prevent regression of the already developed mammary gland (Cowie et al., 1966). Prolonged application of the milking stimulus, known to cause release of anterior pituitary hormones, was shown to bring about mammary growth and initiation of lactation in ovariectomized virgin goats (Cowie et al., 1968). This mammogenic and lactogenic response to the milking stimulus was completely abolished by pituitary stalk transection. Since stalk transection would allow only PRL to be released, apparently PRL alone cannot support mammogenesis and lactogensis. Hart (1976) agrees that the ability of estrogen and progesterone to stimulate mammary gland growth and induce lactation in virgin goats is mediated by enhanced secretion of PRL by the pituitary. He observed a significant and

accumulative increase in plasma PRL and a considerable increase in the size of the mammary gland when goats were injected daily for 77 days with estradiol and progesterone. However, no increase in udder size was observed when CB-154, a PRL blocker, was also given simultaneously. The onset of lactation occurs soon after abortion induced by section of the pituitary stalk in pregnant goats, even as early as day 44 of pregnancy when mammogenesis and lactogenesis have not normally occurred (Cowie et al., 1964a). Stalk section allows high levels of PRL to be secreted while gonadotrophic and luteotrophic function of the anterior pituitary have been depressed so that ovarian steroids decrease. Presumably there is sufficient GH in the circulation for a time to aid PRL in mammogenesis and lactogenesis in the case of this abnormal situation.

Lactogenesis in goats

Fleet et al. (1975) have reported two stages of lactogenesis in goats. At about 81 days of gestation there is a progressive rise in udder volume with the fluid in the teats changing from being like extracellular fluid to having high concentrations of lactose and immunoglobulins (lactogenesis I). Then 2-3 days before parturition there is a substantial rise in citrate concentration which seems to herald the massive increase in flow of milk at parturition referred to as lactogenesis stage II. Correlating these changes with hormonal changes, they inferred that, in the presence of progesterone and placental lactogen, mammary growth is largely determined by the level of estrogens. Prolactin secretion appeared to correlate with the secretory ability of the cells. The systemic trigger for lactogenesis stage II is in question. Estrogen, progesterone and

placental lactogen fall precipitously at term while PRL and cortisol rise. Plasma PRL rises between 5 and 37 hours before parturition, reaching concentrations 2.6 to 4.5 times higher than the lowest concentration of PRL found earlier in gestation (Hart, 1972; Hart, 1974). In the presence of PRL, estrogen and progesterone may promote growth but depress secretion. Prolactin alone primarily promotes secretion.

Hormonal control of mammary development was studied by Skarda and Bilek (1975) using mammary gland explants from 63-70 day pregnant goats. After addition of insulin, marked proliferation could be seen along with hyperplasia of the epithelium. On culture with insulin, cortisol and PRL the parenchymal cells enlarged and became regularly arranged around the alveolar lumens which became engorged with secretion. There was greater secretory response to culture with PRL than with GH and GH was shown to have little if any effect on stimulation of secretion. This is in contrast to *in vivo* studies where hypophysectomized goats showed synergism between PRL and GH in initiating mammary growth and secretion (Cowie et al., 1964b).

Infusion of synthetic adrenocorticotrophic hormone (ACTH) on day 125 of gestation caused a marked increase in udder size of goats and induced lactation within 6 days which coincided with parturition. This indicates that in goats as well as sheep and cattle, glucocorticoids overcome a progesterone block of lactation.

Galactopoiesis in the goat

Partly successful hormonal replacement in the hypophysectomized lactating goat was found to consist of PRL, GH, insulin, T_3 and fluoro-

cortisol (Cowie and Tindal, 1961). Complete restoration and maintenance of milk yield was then achieved with a combination of ovine PRL, bovine GH, T_3, insulin and dexamethasone (Cowie et al., 1964b). The presence of PRL was important for the restoration of milk yield to the pre-hypophysectomy level but it was not always necessary for the maintenance of lactation at that level (Cowie, 1969).

CB-154 was found to block release of PRL from the anterior pituitary of the goat during milking. It also decreased normal basal circulating levels of PRL in lactating goats. Milk yield in these goats remained unchanged (Hart, 1974). However, the use of CB-154 in these studies did not completely eliminate PRL from the circulation and sufficient residual PRL may have remained to maintain the milk yield. Thus, PRL cannot be excluded as a galactopoietic hormone. In an experiment on induced lactation, much greater concentrations of CB-154, which completely inhibited PRL increases at milking, resulted in no milk secretion (Hart, 1976). McMurtry and Malven (1974) have shown that during chronic CB-154 inhibition of plasma PRL, milk concentration of PRL was greater than that of plasma reflecting an accumulation of PRL in the mammary gland against a concentration gradient. This should have an impact on how we interpret results of CB-154 studies when only plasma PRL levels are determined. It could be that during mid-lactation the endocrine demands of the lactating mammary gland are met by GH and other hormones. These CB-154 studies in the goat only indicate that high circulating levels of PRL are unnecessary to maintain milk yield at mid-lactation. Perhaps an increase in PRL receptor affinity occurs which is known to take place in rabbit mammary

glands during postpartum lactation. This decreased need for PRL may account for the lack of correlation between average concentrations of PRL found at milking with milk yield and the lack of correlation between average concentrations of PRL released during early lactation with milk yield (Hart, 1975).

There is a large concentration of PRL released during milking in the goat as in other species (Bryant et al., 1970; Hart, 1974; Hart, 1975). While this ensures that the mammary gland is exposed to high levels of prolactin, the significance of this is unknown. The mammary gland apparently does not require these high PRL levels.

The degree of tactile stimulation at milking determines the amount of PRL released. However, the amount of PRL released does not seem to determine the milk yield. Experiments to investigate the relative importance of the tactile, conditioned and possible metabolic components of the milking stimulus on the release of PRL and GH have been described (Hart and Linzell, 1977). The actual importance of this release of PRL and GH remains to be answered.

Role of Prolactin in Lactation in the Mare

Thus far nothing is known about hormones in relation to mammary development and initiation and maintenance of milk secretion in mares.

Lactose and triglycerides are present in the mammary secretions at least as early as one week prepartum and progressively increase in their concentration in colostrum and in milk. This indicates the secretory capacity of the udder of the mare is well-established before parturition

(Forsyth et al., 1975). However, mammary gland bioassay results did not confirm lactogenic activity in the plasma of mares suggesting that plasma levels of PRL may remain less than 100 to 200 ng/ml (the sensitivity limit of their assay) during late pregnancy, parturition and early lactation in the mare. No evidence of a positive secretory response in the co-culture of mammary explants which would indicate placental lactogen secretion was observed at any stage of gestation in the mare by Forsyth et al. (1975).

Nett et al. (1975a) measured prolactin by radioimmunoassay in mares during pregnancy and found PRL levels to vary but not to change significantly during gestation. This is unlike the increase seen in women throughout pregnancy or the peak occurring just before parturition in other species. Another study by Nett et al. (1975b) indicated serum PRL levels were extremely variable in the postpartum period and did not increase due to suckling. The role of prolactin in lactation in mares must await further research.

Role of Prolactin in Lactation in Rodents and Rabbits

Most biochemical and ultrastructural studies, as well as bioassays, for study of prolactin's role in lactation have used rabbit, rat or mouse mammary tissue. The following articles have been consulted for this discussion: Denamur, 1971; Floss et al., 1973; Forsyth, 1973; Cowie, 1974a; Cowie, 1974b; Cowie and Forsyth, 1975; Horrobin, 1976; Horrobin, 1977.

Rats and mice

In marked contrast to the rabbit, the mouse and rat need a lactogenic complex of hormones as PRL exerts its mammotropic effects only in conjunction with other hormones.

In rats, self-stimulation of the nipples, possibly inducing PRL secretion, is essential for normal mammary development during pregnancy. Animals in which self-stimulation was prevented had mammary glands which were only half the normal size. In triply-operated rats (removal of adrenals, ovaries and pituitary gland) normal mammary duct growth can be induced with GH + estrone + adrenal steroids. Addition of progesterone and PRL are necessary for lobulo-alveolar development. Growth hormone and PRL in the absence of ovarian and adrenal steroids can cause moderate lobulo-alveolar development in triply-operated rats.

Slightly different hormonal requirements for mammogenesis in the mouse have been found. Some duct growth occurs in triply operated mice in response to a combination of estrogen and adrenal steroids. It has been found that while some strains of mice require PRL for lobulo-alveolar development other strains do not.

Prolactin and GH play the major role in mammogenesis in rats and mice while steroids probably sensitize the alveolar cell to respond. In the intact rat, it is clear that estrogen can have an indirect action on mammogenesis by causing release of PRL. However, it is believed that ovarian hormones have a direct effect on the mammary parenchyma, sensitizing it to the action of pituitary hormones. Pretreating mice with estrogen and progesterone before explants were taken caused marked

stimulation of the rate of synthesis of RNA, protein and DNA and activated DNA polymerase suggesting end-bud cells entered a phase of rapid proliferation providing a pool of precursor cells for subsequent lobulo-alveolar differentiation. As in other species, during mammary growth secretory activity is inhibited by a direct action of ovarian steroids on the mammary parenchyma. The sudden fall of blood progesterone at the end of gestation permits the lactogenic hormone complex to exert its effect on the mammary gland.

Placental lactogen is known to be produced in rats and mice which probably supplements or synergizes with hormones from the anterior pituitary in mammary growth. Mice and rats hypophysectomized at mid-pregnancy show mammary gland development and transient lactation at parturition.

Hormonal requirements for mammary growth in mammary tissue explants are in agreement with those obtained in vivo in triply-operated animals. Glands taken from 5 to 7 week old mice maintained as explants on a synthetic medium containing insulin, estradiol, progesterone, aldosterone, PRL and GH demonstrated lobulo-alveolar development. Younger mice must first be pretreated with estradiol or progesterone for these same results. This stimulates mammary duct growth and end-bud formation. Minimal hormone requirements with this procedure are insulin, aldosterone and PRL. Insulin causes one cycle of cell division in mouse mammary explants while PRL must be present in rat mammary explants in order for active cell division to occur. In mid-pregnant mouse mammary gland cultures, basic maintenance requirements are insulin, hydrocortisone and PRL. Addition of T_4 or T_3 enhanced α-lactalbumin synthesis.

The precise hormonal mechanisms that control the initiation of milk secretion have been determined in the rat. A fall in the level of blood progesterone enables PRL and placental lactogen to exert their lactogenic effects on the mammary cells. The minimum hormonal requirements for lactogenesis in rat and mouse are prolactin or GH in conjunction with a glucocorticoid. In some strains of mice, GH can replace PRL.

Studies on replacement therapy after hypophysectomy have been used to analyze the hormonal requirements required for galactopoiesis. In the rat, prolactin + ACTH or glucocorticoids are necessary. If the release of prolactin at suckling is inhibited by CB-154, milk secretion is rapidly inhibited in the rat. The lactogenic and galactopoietic responses to estrogen depend largely on its ability to release PRL from the anterior pituitary.

Rabbit

The rabbit is the only animal which does not require a lactogenic complex of hormones. As early as 1928, Stricker and Grueter found that aqueous extracts of the anterior pituitary injected into ovariectomized pseudopregnant rabbits initiated lactation. In the classic work of Lyons (1942), prolactin injected into the teat galactophores of pseudopregnant rabbit mammary gland sectors produced localized milk secretions only in those sectors. The ultrastructural changes in the mammary alveolar cells of the pseudopregnant rabbit in response to PRL, either intraductally or systemically administered, are quite comparable to the changes observed at the onset of normal lactation. In the triply-operated rabbit (removal of adrenals, ovaries and pituitary gland) PRL alone is sufficient to initiate

lactation. Removal of the pituitary causes a rapid inhibition of milk secretion in the rabbit. The yield and composition of milk can be restored by PRL alone. CB-154 treatment markedly decreases or abolishes milk yield in the rabbit but the milk yield is recovered by administering ovine PRL. Although the milk yield remains relatively high after 18 days of lactation, there are marked changes in milk composition which PRL treatment reverses or prevents.

The biochemical changes occurring in the pseudopregnant rabbit mammary gland in response to PRL have also been studied. Within 12 hours PRL induces an increase in total RNA and by 24 hours an increase in DNA and in the number of aggregated polyribosomes which become bound to rough ER has occurred. The synthetic activity of the polyribosomes is also increased with the rate of incorporation of leucine doubling and that of proline tripling within 24 hours. Casein and lactose contents of those glands rises after 48 hours.

Role of Prolactin in Lactation in the Sow

There is a dearth of information on endocrine physiology of the puerpheral sow which is responsible for mammary growth and development and milk secretion. There are only recently a few reports in the literature on prolactin levels in periparturient sows (Threlfall et al., 1974; Bevers et al., 1978; Landeghem and Wiel, 1978). We can surmise from what is known to occur in other species, that prolactin plays a role in mammogenesis and lactogenesis. It is important to know and understand the

endocrine mechanisms responsible for lactation in order to determine why lactation may fail.

In the pig, progesterone levels begin to decline several days before parturition while estrogen levels increase to high values in late pregnancy and decline after the onset of parturition (Molokwu and Wagner, 1973; Ash and Heap, 1975). This is consistent with what happens in other animal species. While Ash and Heap have reported no consistent changes in corticosteroids at the time of parturition, Molokwu and Wagner have reported corticoid levels rising on day 3 prepartum and reaching a peak on day 0. This rise in plasma estrogen and corticoid level well-above gestation levels and the substantial decline in progesterone may be an important change in the hormonal environment permitting lactogenesis to occur.

Anderson et al. (1972) have found that PRL cells increased in the adenohypophysis in later stages of pregnancy. In the lactating pig there was a marked decrease in the percent of PRL cells whereas chromophobes were the predominant cell type. These chromophobes were believed to be active acidophils which were synthesizing and secreting PRL and not storing it, thus accounting for the low concentration and content of PRL found in the adenohypophysis during this time. Perhaps there was a hypersecretion of PRL by the adenohypophysis with no storage soon after parturition. This would correspond to a time when milk production was maximal.

Threlfall et al. (1974) reported no significant difference between plasma PRL levels of virgin gilts, sows at mid-gestation, sow at 112th day of gestation and sows during the suckling period. In contrast to this, recent studies have shown a significant rise in PRL after parturition,

during the lactation period and a significant drop postweaning (Bevers et al., 1978; Landeghem and Wiel, 1978). Unfortunately no one has included the prepartum period where trends may resemble the prepartum rise reported in other animals which is believed necessary for lactogenesis. A single injection of ergocryptine causes complete cessation of lactation in the sow (unpublished data, W. C. Wagner, Professor and Head, University of Illinois College of Veterinary Medicine, Urbana). This and the fact that there are high levels of PRL during the periparturient period are support for prolactin being an important hormone for lactogenesis and possibly galactopoiesis in the sow.

MATERIALS AND METHODS

General

Fifteen periparturient gilts were used in this experiment. They were housed at an experimental environment building at Iowa State University in standard farrowing crates at constant temperature and humidity. They were exposed to a constant low level of lighting throughout each 24 hour period. Four gilts were brought into the experimental building for farrowing and blood sampling in November of 1975, five more in January of 1976 and six in May of 1976.

Jugular vein catheters were surgically placed one day before sampling was started. They were then sampled daily from 1:30 pm to 4:30 pm at 15 minute intervals. All samples were collected with heparinized saline to prevent clotting. Ten to twelve ml of blood were collected for each sample. Blood samples were then centrifuged and plasma removed to plastic vials and frozen on dry ice. They were stored at -20°C until the time of assay. The greatest time span for sampling of any animal was 8 days prepartum to 8 days postpartum.

Surgery

One jugular catheter was surgically placed the day before onset of sampling and exteriorized at the top of the shoulder. Catheterization was done under general anesthesia (sodium thiopental) administered via the ear vein. Silastic tubing (ID 1.016 mm OD 2.032 mm) was inserted by exposing the jugular and removing connective tissue to expose the wall of the

vessel. A nick was then made in the vessel with iris scissors, the tubing was inserted and sutured into place. The end of the catheter was tied to the eye of an aluminum rod and the rod was used to pull the catheter along the fascia of the shoulder and out the dorsum of the animal for easy access to the cannula. Cannulas were kept patent by flushing with heparinized saline (400 u/ml) after each collection.

Hormone Assay

Antigen

Purified porcine prolactin was obtained from A. E. Wilhelmi, Emory University, and was used as standard for the prolactin assay, iodinated with I^{125} for use in the assay and was used to develop antibody against this porcine prolactin in rabbits.

Buffer

A 0.10 M phosphate buffer (pH 7.1) was used for the iodination reaction. A 0.01 M phosphate buffered saline with 0.1% gelatin (pH 7.0) was used as the diluent buffer and to prepare the Sephadex G-25 and Sephadex G-100 columns.

Prolactin antiserum production

Rabbits were used to develop antibody against porcine prolactin. Five mg porcine prolactin powder were mixed with 5 ml tris-HCl buffer (0.1% gelatin) and then with 5 ml Freunds complete adjuvant to produce a stiff emulsion. Each rabbit was then injected with 1 mg of this antigen given in four 0.5 ml aliquots. Injections were deep IM in the upper area of both hind legs. One month later the rabbits received 0.5 mg porcine

PRL IM dissolved in a 2 ml emulsion of saline and complete Freunds adjuvant. Each dose was split into 2 injection sites per hind leg and 0.5 ml given per injection site. After a second period of one month, rabbits were given an injection of 0.1 mg of porcine prolactin in saline and complete Freunds adjuvant in the form of 10 injections given subcutaneously on the rabbit's back. One week later the rabbits were bled via the ear artery. Forty-eight days after this last series of 10 injections a final series of injections were given subcutaneously. A total of 1 ml was given containing approximately 45 µg porcine prolactin. Rabbits were bled one week later. Plasma from rabbit 017 was chosen for use in the assay. It was diluted to 1:1000 with 1:400 normal rabbit serum (NRS) giving a titer which would bind ^{125}I PRL 20% to 50% depending on the particular assay. (NRS was diluted in 0.05 M PBS [pH 7.0] with EDTA).

Sheep anti-rabbit gamma globulin

Sheep anti-rabbit gamma globulin was obtained from Antibodies Incorporated, Davis, California. It was diluted in 0.01 M PBS gel (pH 7.0) to 1:50 for use in the double antibody system to separate free from bound hormone.

Iodination reaction - I^{125} labelling of porcine prolactin

The lactoperoxidase radioiodination procedure was used as adapted from Niswender, Colorado State University. All reagents and reaction solutions were maintained at 4°C in an ice bath with all reactions carried out under a hood designed for iodination purposes. Porcine PRL hormone was prepared previous to iodination by dilution with buffer to a concen-

tration of 10 µg/5 µl, then placed in a 2 ml vial and stored at -20°C until use.

A stepwise procedure for lactoperoxidase radioiodination is as follows:

1. Preparation
   a. Let Sephadex G-25 slurry warm to room temperature.
   b. Pour G-25 column (18 cm), added #42 filter paper to top.
   c. Add 1 ml phosphate buffer with 0.1% gelatin (0.01 M, pH 7.0) to each of twenty tubes used to collect I^{125}-PRL from the G-25 Sephadex column.
   d. Thaw PRL hormone and keep the vial on ice (4°C).
   e. Thaw: 1) 0.1 M phosphate buffer
      2) transfer solution (16% sucrose w/v)
      3) rinse solution (8% sucrose w/v)
      keep all of these on ice (4°C)
   f. Thaw the concentrated lactoperoxidase (2 µg/µl).
   g. Dilute lactoperoxidase to a new concentration of 1 µg/5 µl (50 µl lactoperoxidase + 450 µl of 0.1 M phosphate buffer pH 7.1).
   h. Dilute stock solution of H_2O_2 (250 µl of 30% H_2O_2/100 ml): add 20 µl of stock solution to 4 ml of deionized water to obtain the working H_2O_2 solution (40 ng in 10 µl H_2O_2).
2. Procedure for iodination
   a. Add 5 µl of porcine PRL hormone solution (10 µg/5 µl) to the bottom surface of vial.

b. Using a micropipet, add 30 µl of 0.1 M phosphate buffer pH 7.1; mix by tapping vial gently.

c. Add 5-10 µl of lactoperoxidase enzyme (1 µg/5 µl) to the vial containing prolactin hormone.

d. Add 20 µl of 0.1 M phosphate buffer pH 7.1 to 1 mCi of I^{125} in the vial in which it was shipped.

e. Remove the solution from the radiation vial with a Hamilton syringe and add it to the buffer surface of the PRL hormone solution to be iodinated.

f. Stopper the reaction vial (parafilm) and mix - (finger tap).

g. Add 10 µl of hydrogen peroxide working solution to the reaction vial with a Hamilton syringe and let it react for 4 minutes.

h. Add 100 µl (0.1 ml) of transfer solution (using a tuberculin syringe) to the reaction vial.

i. Using this same tuberculin syringe, transfer the entire solution onto the G-25 sephadex column.

j. Add 70 µl rinse solution to the reaction vial and transfer this onto the G-25 sephadex column.

3. Procedure for separation by sephadex G-25 column

a. After the iodination reaction solution has been transferred to the G-25 column (18 cm), open the column until there is a slow steady drip.

b. When the solution has descended to a level just above the filter paper on the top of the column, slowly add 1 ml eluent buffer (0.01 M PBS-gel, pH 7.0); DO NOT disturb the column.

c. Let this descend until just above the level of the filter paper and add another 1 ml of eluent buffer.

d. Repeat this procedure until 1 ml has been collected in each of 2 tubes (previously prepared by adding 1 ml buffer to each) total = 2 ml/tube.

e. Ten µl aliquots from each tube are then counted on a gamma counter.

f. Tubes with the 1st peak of activity from the G-25 column are saved.

g. To each of these peak tubes another 1 ml of 0.01 M PBS-gel (pH 7.0) is added to bring the total volume in each tube to 3 ml.

h. Five 0.6 ml aliquots from each tube are put into separate tubes, frozen and stored at -20°C until purification on a sephadex G-100 column.

Purification of I^{125}-PRL

Prior to assay, iodinated porcine PRL was purified on a 1 x 30 cm sephadex G-100 column. The second peak was pooled and used in the assay. Pooled ^{125}I PRL was diluted with 0.1 M PBS-gel (pH = 7.0) to give approximately 15,000 to 25,000 CPM per 0.1 ml.

Prolactin assay

Control tubes were set up for each assay. Blank 1 which established percent ^{125}I-PRL bound to rabbit antiserum (% Bo):

0.1 ml diluent buffer

0.1 ml ^{125}I-PRL

0.1 ml blank serum

0.1 ml AB 017

0.1 ml 2nd antibody

Blank 2 and Blank 3 established nonspecific binding to the assay tube and to the 2nd antibody respectively. Blank 3 was identical to Blank 2 so it was concluded there was no nonspecific binding of ^{125}I-PRL to 2nd antibody and it was eliminated in later assays. Blank 2 and Blank 3 were set up as follows:

Blank 2 = 0.3 ml diluent buffer

0.1 ml ^{125}I-PRL

0.1 ml blank serum

Blank 3 = 0.2 ml buffer

0.1 ml ^{125}I-PRL

0.1 ml blank serum

0.1 ml 2nd antibody

Standards were run in quadruplicate for each assay. A known concentration of porcine PRL (800 ng PRL per 0.2 ml diluent buffer) was diluted in blank serum to different nanogram amounts of PRL per 0.1 ml. Each standard tube contained:

0.1 ml of 0.75 ng, 1.0 ng, 1.5 ng, 2.0 ng, 3.0 ng, 5.0 ng, 7.0 ng or 10.0 ng

0.1 ml ^{125}I PRL

0.1 ml diluent buffer

0.1 ml AB 017

0.1 ml 2nd antibody

Two different volumes from each unknown plasma sample were run in the assay (25 µl and 50 µl). The total amount of plasma in each tube of unknowns was brought to 0.1 ml by addition of 75 µl and 50 µl, respectively, of blank plasma. Each tube of unknown contained:

0.1 ml unknown plasma + blank plasma

0.1 ml ^{125}I-PRL

0.1 ml diluent buffer

0.1 ml AB 017

0.1 ml 2nd antibody

The blank plasma referred to above was obtained from a bromoergocryptine (CB-154) treated sow. This plasma had previously been compared with hypophysectomized porcine plasma and found to result in identical % Bo and standard curves.

The assay protocol was carried out according to the following schedule:

Day 1: Added diluent buffer, standard or unknown plasma, and AB 017 to tubes. Mixed on a vortex. Incubated 24 hours at 4°C.

Day 2: After purifying I^{125}-PRL on a G-100 sephadex column, 0.1 ml was added to each tube (15,000-20,000 cpm/tube). Mixed on a vortex. Incubated 24 hours at 4°C.

Day 3: Added 0.1 ml 2nd antibody (sheep anti-rabbit gamma globulin diluted 1:50 in 0.01 M PBS-gel pH 7.0) to tubes. Mixed on a vortex. Incubated 72 hours at 4°C.

Day 6: Added 2 ml of cold 0.01 M PBS (pH 7.0) to each assay tube. Centrifuged for 30 minutes at 3000 RPM. Determined total CPM by

counting six random tubes on a Beckman gamma counter. Poured supernatant off and counted precipitates in a gamma counter.

Data from the assays were analyzed by a computer program RAD-ASS developed by Animal Genetics Department at the University of Illinois. The standard curves were fitted with a modified cubic equation. A standard curve using porcine PRL is shown in Figure 1.

Validation of porcine prolactin assay

This porcine prolactin assay was validated by the following procedures.

a. Samples of porcine plasma known to contain no prolactin were used in the assay to confirm zero prolactin levels compared to a standard curve. This plasma was from a hypophysectomized pig and from a CB-154 treated animal.

b. Samples containing high concentrations of porcine PRL (TRH-stimulated PRL release) were serially diluted to demonstrate parallelism to the standard curve. Alternatively, a known concentration of PRL was added to PRL-free plasma and serially diluted to obtain the same results.

c. Cross reactivity studies were carried out using 10^0, 10^1 ... 10^5 ng of porcine ACTH, GH, FSH and LH to determine percent cross reaction in this assay system.

Steroid assay

Steroids (progesterone, total estrogen and corticoids) were assayed by technical personnel in the laboratory to monitor and ensure normal values for these components.

Corticoid assay Corticoids were assayed using a competitive protein binding method as described by Wagner et al. (1977). Briefly, this consisted of pre-extraction with hexane to remove progestins, extraction with dichloromethane and assay of aliquots of this extract using adrenalectomized dog plasma for the CBG source and not of ^{3}H-cortisol as the labelled hormone. Separation of free and bound hormone was done using dextran-coated charcoal.

Progesterone assay Progesterone was assayed as described by da Rosa and Wagner (1979). The assay involved extracting plasma samples with petroleum ether, freezing the plasma and decanting off the ether to separate it from the plasma. The ether was then evaporated and absolute methanol was added. Three different volumes of this methanol solution were then added to different assay tubes and evaporated to dryness. Following this, ^{3}H progesterone and antiserum specific to progesterone were added and charcoal was used to separate bound from free hormone after a 24 hour incubation.

Estrogen assay Total estrogens were assayed as described by da Rosa and Wagner (1979). Plasma unknowns were extracted twice with diethyl ether. After freezing, the ether phase was transferred to a different tube and evaporated. Methanol was added to the dried extracts and two different volumes of this methanol solution were added to two different tubes and evaporated. Following this, ^{3}H estrogen and rabbit anti-estrone antibody were added to the dried extracts. After incubation, dextran-coated charcoal was used to separate bound from free hormone by centrifugation and the supernatant was counted.

RESULTS

Prolactin

A typical PRL curve is shown in Figure 1. Generally the standard curve was able to detect PRL in amounts ranging from 0.5 ng to 7.0 ng per assay tube.

Samples from animals treated with ergocryptine were found to contain no PRL as measured by this assay system. Serial dilution of porcine plasma containing high concentrations of PRL resulted in a curve parallel to the standard curve.

Cross reactivity of this antiserum with other porcine hypophysial hormones was essentially nonexistent (Figure 2). Addition of amounts up to 10^5 ng of porcine ACTH, STH, LH or FSH did not result in any significant binding with the porcine PRL antiserum. Additionally, the fact that ergocryptine-treated sows were found to have no detectable PRL would also suggest little or no cross reactivity with substances in porcine plasma other than PRL.

Plasma PRL levels for individual sows eight days prepartum until seven days postpartum are shown in Table 1 in the Appendix. Figures 3a and 3b depict data from two individual sows. Although samples were collected from gilts every 15 minutes from 1:30 pm until 4:30 pm daily, only alternate 30 minute samples were assayed. Marked increases in PRL over basal levels began as early as 3 days prepartum for some sows while in others PRL increases began later at 2 days prepartum, 1 day prepartum or on the day of farrowing. Peak PRL concentrations occurred on day 1 pre-

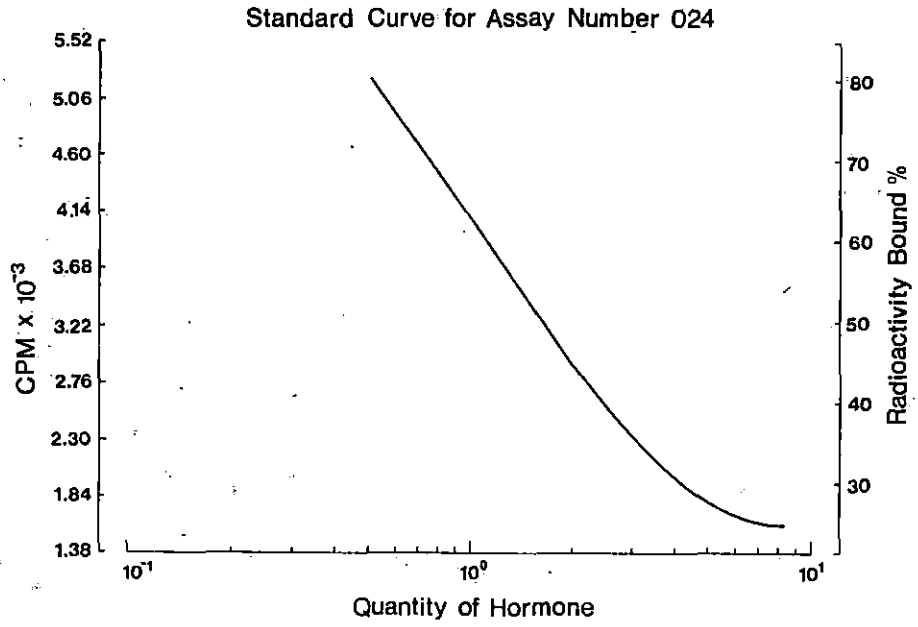

Figure 1. Indicates a representative standard curve for a prolactin assay with antibody 017.

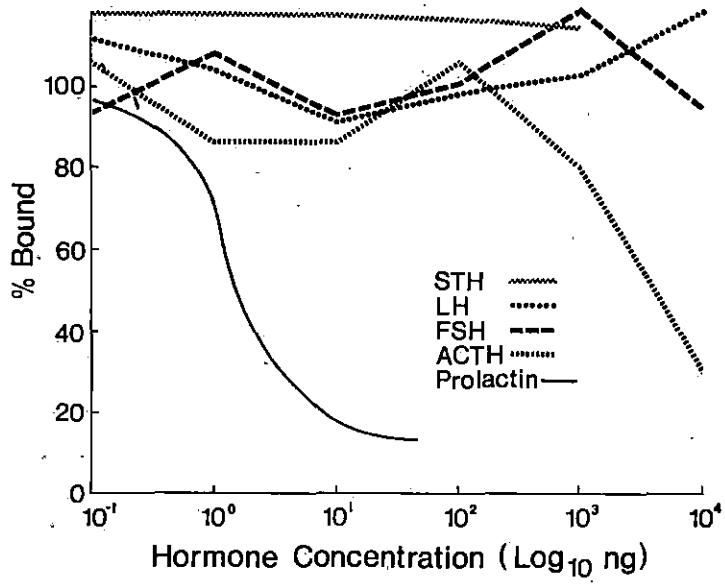

Figure 2. Cross reactivity of other pituitary hormones with the ACTH antiserum.

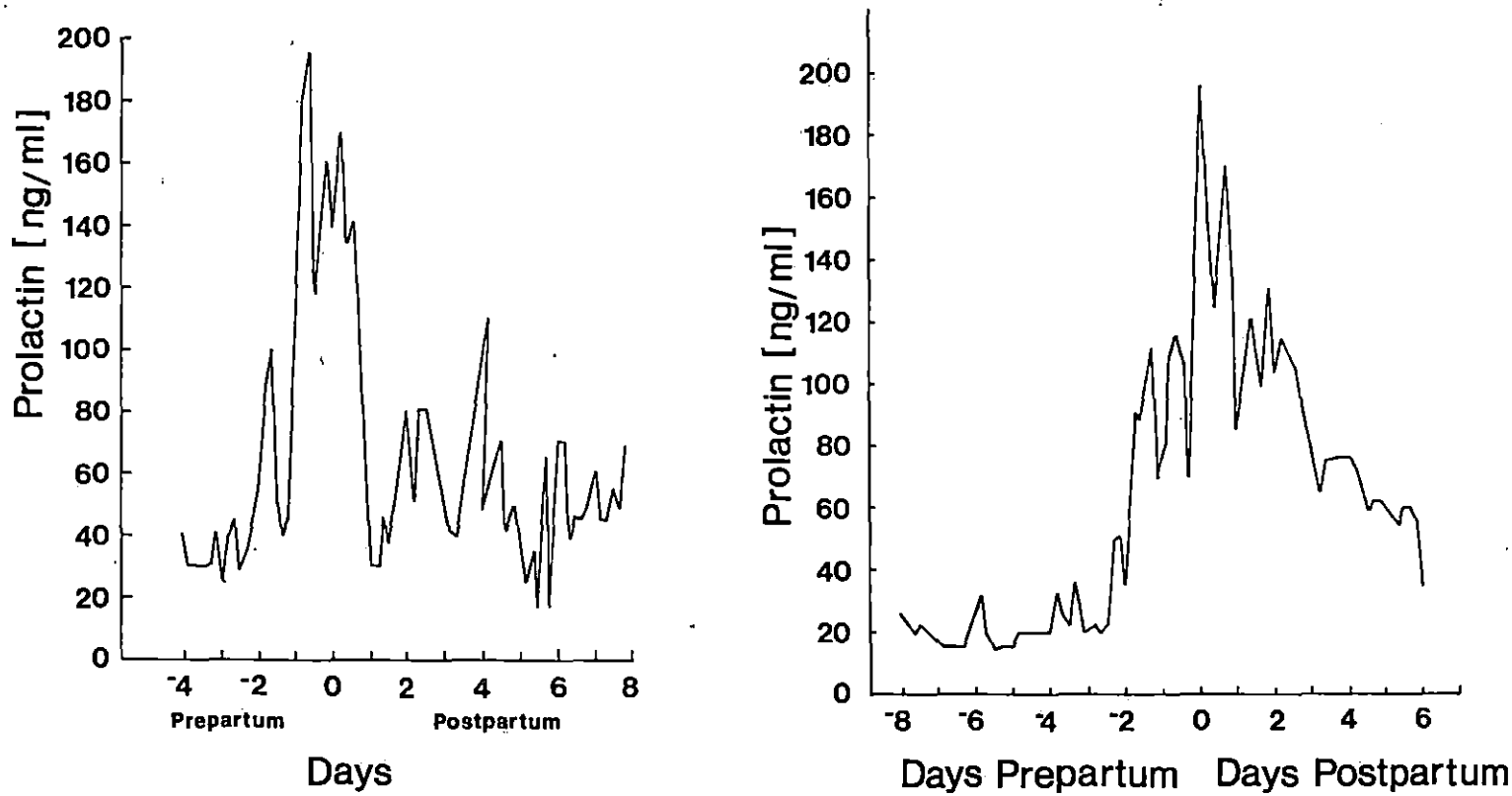

Figure 3. Prolactin concentrations in two individual animals. Values represent 30 min interval sampling from 1.

partum for seven sows, the day of farrowing for two sows, 1 day postpartum for two sows and two days postpartum for one sow. Peak concentrations ranged from 56 ng/ml in a sow which had low PRL throughout the periparturient period to a comparatively high value of 260 ng/ml. Prolactin values for individual sows in the prepartum period before marked increases occurred ranged from 15 ng/ml to 50 ng/ml. In the postpartum period, PRL levels varied from day to day within an individual sow and between different sows.

Prolactin values were averaged for each day over all sows and the results are shown in Figure 4. Prolactin increased from 22 ng/ml on Day -8 to 42 ng/ml on Day -3. A sharp increase occurred on Day -2 with the peak prolactin value (128 ng/ml) coming on the day before farrowing (Day -1). High prolactin continued on the day of farrowing through Day +2 and began to decline on Day +3 (81 ng/ml). A gradual decrease in PRL levels occurred in the postpartum period through Day +7 reaching concentrations of 52 ng/ml. Statistical analysis using the t test revealed that values for Days -1, 0, +1, and +2 ($\overline{X}$ = 116.94) were significantly higher than all other days ($\overline{X}$ = 62.70) ($P < 0.0005$).

Corticoids

The mean plasma corticoids for all sows are depicted in Figure 5. The corticoid levels rose sharply from Day -2 to -1 reaching peak concentrations (52.1±7.8 ng/ml) at this time. High levels were maintained on the day of farrowing (47.2±7.2 ng/ml), then declined sharply reaching basal levels of 18.9±2.2 ng/ml on Day +3. Plasma corticoid levels on Days

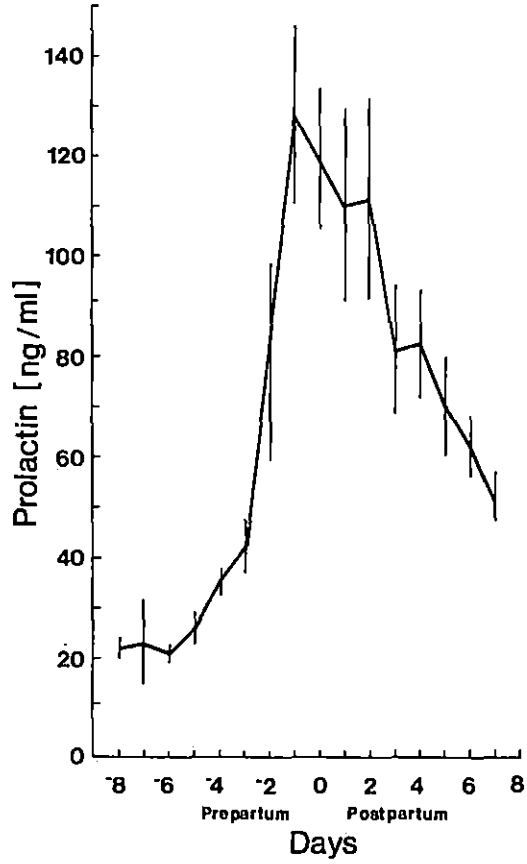

Figure 4. Mean prolactin concentrations for all sows sampled. Bars represent SEM.

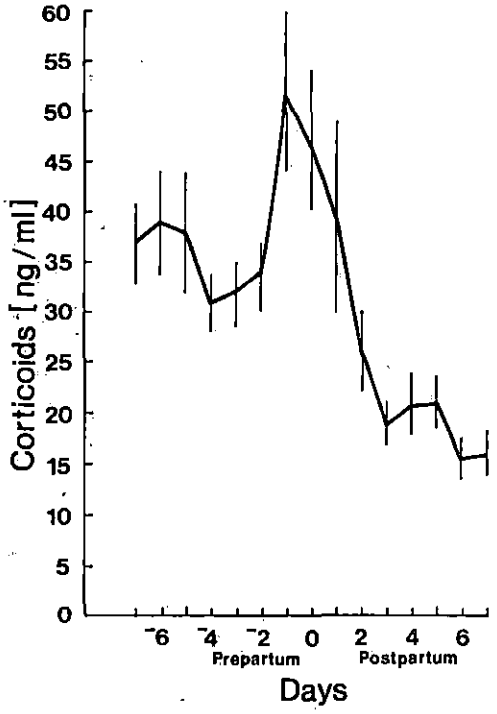

Figure 5. Mean and SE for glucocorticoids in jugular plasma of sows. Day 0 is day of farrowing.

-1, 0, +1 were significantly higher ($P < 0.01$) than levels for all other days sampled. Individual sow corticoid data are given in Table 2 in the Appendix.

Progesterone

The mean progesterone level (Figure 6) started to decline on day -5. This decline was very rapid from Day -2 (5.83±0.86 ng/ml) to Day +1 (0.49± 0.05 ng/ml). Plasma progesterone showed only a slight decline from Day +2 (0.29 ng/ml) to the end of the experimental period (0.13 ng/ml). Individual values are in Table 3 in the Appendix.

Total Estrogen

The mean levels of plasma total estrogens are shown in Figure 7 while data for individual sows are given in Table 4 in the Appendix. An increase in total estrogen was seen from Day -7 until Day -1 with peak values occurring on this day (2632 pg/ml). Total estrogens decreased through farrowing (1162 pg/ml on Day 0) reaching levels of 174 pg/ml on Day +1 with a more gradual decrease to low levels of 23 and 11 pg/ml during the postpartum period of Days +3 to +8, respectively.

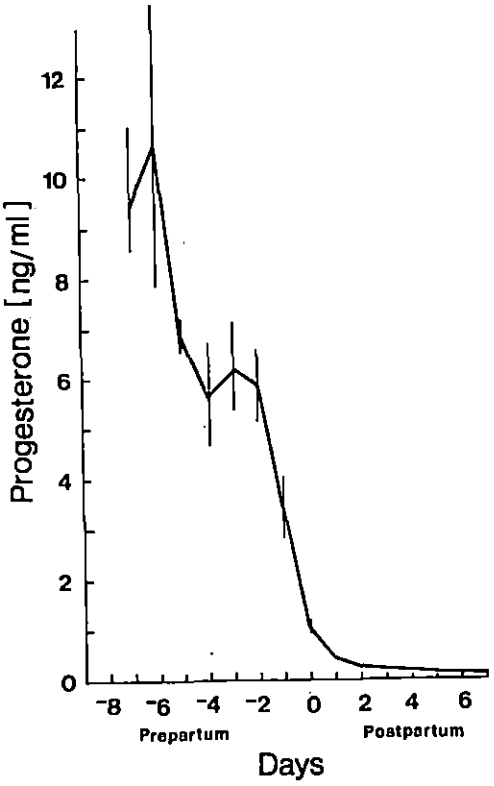

Figure 6. Mean and SE for progesterone in jugular plasma of sows. Day 0 is day of farrowing.

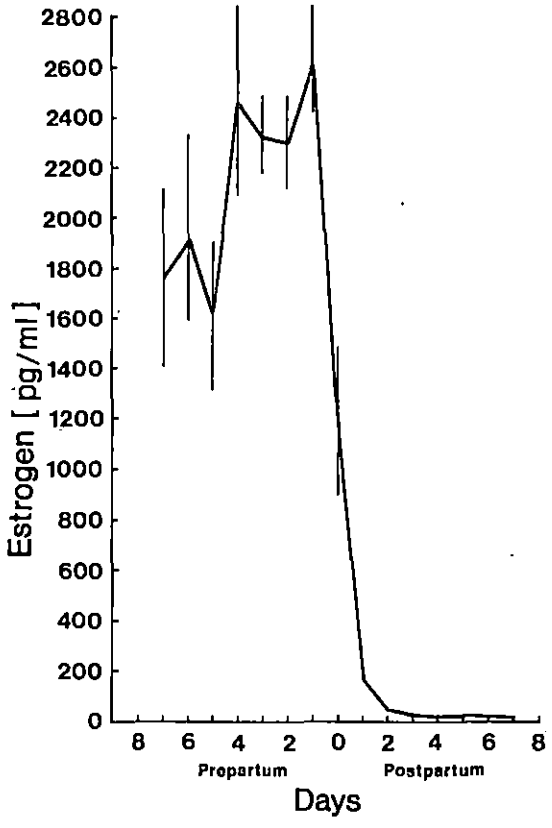

Figure 7. Mean and SE for total free estrogen in jugular plasma of sows. Day 0 is day of farrowing.

DISCUSSION

A valid porcine PRL radioimmunoassay was developed and porcine PRL concentrations were measured in fifteen gilts during the periparturient period. The radioimmunoassay was based on antiserum raised in rabbits against purified porcine PRL. For validation criteria, one is interested in specificity, sensitivity, and repeatability. Although repeatability was not tested as thoroughly as is optimum, the assay system clearly satisfied the other components for assay validity. The almost complete lack of cross-reactivity with other pituitary protein hormones as shown in Figure 2 and the lack of measurable hormone content in plasma from ergocryptine treated animals is strong support for the specificity of the antiserum. The validity of the system for measurement of plasma content of porcine PRL was further shown by the fact that serial dilutions of plasma containing a high concentration of PRL exhibited parallelism with the standard curve.

It has been well-established that PRL levels rise dramatically during the periparturient period in other species in preparation for lactation. The material presented here confirms the same trend in the porcine species and is in agreement with recent reports in the literature that PRL levels are high in the immediate postpartum period (Bevers et al., 1978; Landeghem and Wiel, 1978).

In the woman, PRL levels gradually increase throughout pregnancy and reach a maximum at term (Tyson et al., 1972; Tyson and Friesen, 1973; Jaffe et al., 1973; L'Hermite et al., 1975a,b). Studies with CB-154 have confirmed the essential role of prolactin in both the initiation of

lactation in the woman and in maintaining an already established lactation (Brun del Re et al., 1973). During late stages of pregnancy, bovine serum PRL is maintained at a relatively low level with a conspicuous rise in circulating PRL level occurring at the end of pregnancy. Peak values have been observed one day before the day of parturition and the PRL surge around parturition continues for 2-3 days (Ingalls et al., 1973). In goats, serum PRL levels are low during late pregnancy and increase 2-3 days before parturition. A peak value of PRL is obtained on the day before parturition (Buttle et al., 1972; Hart, 1972, 1974). A marked elevation of PRL concentration in the blood near parturition has also been observed in sheep (Lamming et al., 1974). Prolactin is important in onset of copious lactation in ruminants but its role is less critical in maintenance of lactation. Suppression of PRL secretion by CB-154 around the time of parturition prevents lactogenesis but once established, lactation appears insensitive to inhibition of PRL secretion (Schams et al., 1972; Karg et al., 1972; Smith et al., 1974). In fact, an established lactation in the goat is believed to occur independently of PRK (Hart, 1974).

This study showed that porcine PRL levels began to rise 5 days prepartum with a sharp increase occurring 2 days before parturition. Peak PRL levels were reached on the day before farrowing. Prolactin levels remained high on the day of farrowing through 2 days postpartum (Day +2) and began to decline on Day +3. Complete suppression of lactation in pigs occurs with inhibition of PRL by CB-154 (Fluckiger, 1972; Wagner, unpublished data, Professor and Head, University of Illinois College of Veterinary Medicine, Urbana). This indicates PRL is essential for a

normal lactation in the pig. The increases reported here during the periparturient period are probably essential for the initiation of lactation. It remains to be found what role a PRL deficit plays in clinical agalactia or hypogalactia in the sow.

There is a myriad of hormones responsible for preparation of the mammary gland for lactation and the actual initiation of lactation in animals. However, the hormones required to do so probably vary for different species and the same hormone may actually perform different functions in different species. Recently there is great interest in placental lactogen having an important role in mammary growth and in metabolic function in pregnant ruminants. During later stages of pregnancy, high placental lactogen concentration in the blood may suppress PRL and GH secretion of the pituitary gland in cows and goats (Johke, 1978). As placental lactogen declines, rising PRL concentrations may take over its function. Rising PRL levels may be due to increased estrogen which is a known stimulus to PRL synthesis and release. Estrogen is important for normal ductal growth and estrogen and progesterone together stimulate proliferation of secretory tissue. However, in the goat, it is now believed that this function may be due only to enhanced secretion of PRL by the pituitary caused by these steroids. Progesterone inhibits organelle formation in daughter cells by competing with corticoids for receptors. Progesterone also inhibits α-lactalbumin synthesis. The decline in plasma progesterone at parturition is a key factor in initiation of normal lactation. Glucocorticoids are important for organelle formation in secretory alveolar cells and may stimulate the synthesis of messenger RNA for a

number of key enzymes necessary for milk formation. It is now believed glucocorticoids also induce PRL receptors in lactogenesis and that the induction of these receptors is inhibited by progesterone (Kohmoto and Sakai, 1978). Prolactin is important for preparing the mammary gland cell for lactation and in the composition of milk itself. Prolactin allows transcription of DNA, stimulates RNA synthesis, stimulates synthesis of cell organelles and enzymes, regulates metabolic pathways for synthesis of all milk components and controls electrolyte movements into milk. Its effects on mammary gland function are profound.

Data reported here indicate there is a great increase in PRL and corticoids prepartum and peak levels at or near parturition in the sow. The high, increasing levels of estrogen may be the stimulus for increased PRL secretion. Stress of parturition and suckling may keep PRL levels high in the postpartum period. Declining levels of progesterone with the dramatic drop at parturition remove this block to lactation allowing corticoids and PRL to function in their essential roles in induction of lactation.

SUMMARY AND CONCLUSIONS

A sensitive radioimmunoassay for porcine prolactin was developed and used to measure prolactin levels in the periparturient period in fifteen gilts. Blood was collected every fifteen minutes over a four hour period and half hour samples were assayed. Blood collection was facilitated by a surgically implanted jugular catheter. Prolactin levels were shown to rise a few days prepartum, reach a peak 1 day prepartum and remain at high levels in the immediate postpartum period. During this time the mammary gland is undergoing changes to initiate copious lactation. A specific hormone environment is required at this time.

Based on results obtained using this porcine prolactin radioimmunoassay, it can be concluded that prolactin levels rise dramatically from the basal levels of pregnancy during the periparturient period. This is a trend consistent with what has been reported in other animal species and suggests an important role for prolactin in lactation of the sow.

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APPENDIX

Table 1. Plasma prolactin in periparturient sows

Sow No.	Day -8	Day -7
587-020		
589-021		
591-023		
594-010		
595-011		
596-012		
597-009		
598-007		
600-013		
601-014		
601-024	25 18 24 $\bar{x}$ = 22.3 s.e. = 2.2	15 15 15 15 $\bar{x}$ = 15
603-016		40 27 26 $\bar{x}$ = 31 s.e. = 4.5
604-017		
605-018		

Day -6	Day -5	Day -4
		28 33 27 32 36 $\bar{x}$ = 31.2 s.e. = 1.7
	35 24 15 20 11 17 12 $\bar{x}$ = 21 s.e. = 4.1	
	23 24 31 26 21 25 ± 1.7	57 32 31 24 30 30 $\bar{x}$ = 34 s.e. = 4.7
		40 33 29 32 42 $\bar{x}$ = 35 s.e. = 2.5
	51 38 29 23 35 ± 6.1	34 34 33 32 33 $\bar{x}$ = 33.2 s.e. = 0.4
	19 20	49 41 30 51 50 67 $\bar{x}$ = 48 s.e. = 5.0
33 18 14 15 $\bar{x}$ = 19 s.e. = 3.6	15 19 19 $\bar{x}$ = 18 s.e. = 1.3	21 33 27 24 35 30 $\bar{x}$ = 28 s.e. = 2.2
19 13 23 29 19 $\bar{x}$ = 22 s.e. = 2.4	31 39 30 33 31 $\bar{x}$ = 33 s.e. = 1.6	35 37 38 32 27 $\bar{x}$ = 34 s.e. = 2.0

Table 1. (Continued)

Sow No.	Day -3	Day -2
587-020		
589-021	79 85 53 36 $\bar{x}$ = 66 s.e. = 9.3	151 193 231 268 203 $\bar{x}$ = 209 s.e. = 19.5
591-023	10 14 11 $\bar{x}$ = 11.7 s.e. = 1.2	34 21 11 11 131 11 $\bar{x}$ = 36.8 s.e. = 19.6
594-010	59 79 29 29 29 $\bar{x}$ = 45 s.e. = 10.3	65 74 64 75 68 64 $\bar{x}$ = 68 s.e. = 2.0
595-011		43 57 42 31 31 22 $\bar{x}$ = 38 s.e. = 5.0
596-012	25 40 47 30 36 $\bar{x}$ = 36 s.e. = 3.8	57 88 101 51 42 45 $\bar{x}$ = 64 s.e. = 10.0
597-009	37 53 46 34 31 $\bar{x}$ = 40 s.e. = 4.0	42 34 39 47 41 48 $\bar{x}$ = 42 s.e. = 2.1
598-007		42 41 37 30 24 $\bar{x}$ = 35 s.e. = 3.4
600-013		
601-014	43 22 66 121 104 $\bar{x}$ = 71.2 s.e. = 18.4	193 177 134 $\bar{x}$ = 168 s.e. = 17.6
601-024	20 23 18 23 48 52 $\bar{x}$ = 31 s.e. = 6.2	36 92 89 110 70 $\bar{x}$ = 79 s.e. = 12.6
603-016	68 56 26 27 28 $\bar{x}$ = 41 s.e. = 8.8	114 88
604-017	107 104	18 11
605-018	44 45 45 40 31 42 $\bar{x}$ = 41 s.e. = 2.2	44 39 65 44 60 $\bar{x}$ = 50 s.e. = 5.1

Day -1	Day 0	Day +1
		202 201 44 146 157 $\bar{x}$ = 150 s.e. = 28.8
114 117 87 109 75 40 $\bar{x}$ = 90 s.e. = 12.1	150 260 228 201 $\bar{x}$ = 210 s.e. = 23.3	179 198 234 192 149 199 $\bar{x}$ = 192 s.e. = 11.4
93 73 73 77 112 $\bar{x}$ = 86 s.e. = 7.6	99 71 93 83 86 66 $\bar{x}$ = 83 s.e. = 5.2	80 92 133 90 85 85 $\bar{x}$ = 84 s.e. = 8.0
166 154 143 218 159 111 $\bar{x}$ = 158 s.e. = 14.3	109 104 96 107 96 108 $\bar{x}$ = 103 s.e. = 2.4	139 127 91 93 117 108 $\bar{x}$ = 113 s.e. = 7.7
104 86 91 107 $\bar{x}$ = 100 s.e. = 3.7	89 73 84 $\bar{x}$ = 82 s.e. = 4.7	
179 196 118 161 $\bar{x}$ = 164 s.e. = 16.8	139 168 136 142 $\bar{x}$ = 146 s.e. = 7.4	32 30 46 37 51 $\bar{x}$ = 39 s.e. = 4.0
55 60 51 56 57 54 $\bar{x}$ = 56 s.e. = 1.2	57 56 40 49 47 70 $\bar{x}$ = 53 s.e. = 4.2	55 48 49 56 42 45 $\bar{x}$ = 49 s.e. = 2.2
43 31 23 25 35 47 $\bar{x}$ = 34 s.e. = 3.9	166 166 102 166 $\bar{x}$ = 150 s.e. = 16	29 26 25 24 $\bar{x}$ = 26 s.e. = 1.1
190 221 188 252 $\bar{x}$ = 213 s.e. = 15.1		275 277 272 215 $\bar{x}$ = 260 s.e. = 15
79 109 115 107 61 156 $\bar{x}$ = 105 s.e. = 15.1	196 153 126 151 619 134 $\bar{x}$ = 138 s.e. = 10.5	86 105 122 110 100 132 $\bar{x}$ = 109 s.e. 6.6
101 125 128 124 107 $\bar{x}$ = 117 s.e. = 5.4	79 107 90 137 $\bar{x}$ = 103 s.e. = 12.6	96 85 73 56 $\bar{x}$ = 78 s.e. = 8.6
164 152 270 116 $\bar{x}$ = 176 s.e. = 33	74 90 75 79 108 $\bar{x}$ = 85 s.e. = 6.4	112 79 80 $\bar{x}$ = 90 s.e. = 10.8
186 182 263 291 $\bar{x}$ = 231 s.e. = 27.5	190 135 170 87 164 157 $\bar{x}$ = 151 s.e. = 14.6	107 127 115 157 125 $\bar{x}$ = 124 s.e. = 8.8

Table 1. (Continued)

Sow No.	Day +2	Day +3
587-020	152	103 28 93 78 132 $\bar{x}$ = 87 s.e. = 17.1
589-021	240 256 249 186 213 $\bar{x}$ = 260 s.e. = 23	164 163 200 196 173 191 $\bar{x}$ = 181 s.e. = 6.7
591-023	73 80 82 57 $\bar{x}$ = 73 s.e. = 5.7	73 49 53 42 43 $\bar{x}$ = 52 s.e. = 5.6
594-010	77 98 100 83 99 $\bar{x}$ = 92 s.e. = 3.9	48 77 71 75 44 $\bar{x}$ = 63 s.e. = 7.0
595-011		
596-012	80 54 82 82 $\bar{x}$ = 75 s.e. = 6.9	52 44 41 $\bar{x}$ = 46 s.e. = 3.3
597-009	60 41 46 31 $\bar{x}$ = 47 s.e. = 3.9	30 37 35 23 37 36 $\bar{x}$ = 33 s.e. = 2.3
598-007	62 42 68 $\bar{x}$ = 57 s.e. = 7.9	27 57 55 49 39 $\bar{x}$ = 45 s.e. = 5.6
600-013	12 132 151 85 107 $\bar{x}$ = 97 s.e. = 24.1	103 124 95 154 $\bar{x}$ = 119 s.e. = 13.2
601-014	331 219 228 192 201 $\bar{x}$ = 234 s.e. = 25	209 183 167 65 $\bar{x}$ = 153 s.e. = 34.2
601-024	105 114 105 92 $\bar{x}$ = 104 s.e. = 4.5	65 76 77 $\bar{x}$ = 73 s.e. = 3.8
603-016	55 61 81 58 87 $\bar{x}$ = 60 s.e. = 4.4	31 36 31 62 37 $\bar{x}$ = 39 s.e. = 5.8
604-017	115 98 89 109 84 $\bar{x}$ = 96 s.e. = 5.7	64 72 75 49 66 $\bar{x}$ = 65 s.e. = 4.5
605-018	125 172 129 $\bar{x}$ = 142 s.e. = 15	124 98 96 81 65 75 $\bar{x}$ = 90.0 s.e. = 8.5

Day +4	Day +5	Day +6
194 57 38 53 60 $\bar{x}$ = 80 s.e. = 28.7	64 69 57 74 75 $\bar{x}$ = 68 s.e. = 3.3	52 68 35 45 $\bar{x}$ = 50 s.e. = 6.9
147 162 144 124 145 125 $\bar{x}$ = 141 s.e. = 5.9	157 93 149 114 143 $\bar{x}$ = 131 s.e. = 12.0	
53 55 56 60 57 75 $\bar{x}$ = 59 s.e. = 3.3	54	64 49 62 $\bar{x}$ = 58 s.e. = 4.7
91 93 98 83 88 69 $\bar{x}$ = 87 s.e. = 4.1	71 74 64 86 84 $\bar{x}$ = 76 s.e. = 4.1	
50 110 70 45 48 $\bar{x}$ = 65 s.e. = 12.2	38 27 35 19 66 20 $\bar{x}$ = 34 s.e. = 7.1	71 69 40 47 45 49 $\bar{x}$ = 54 s.e. = 5.4
31 30 24 28 37 26 $\bar{x}$ = 29 s.e. = 1.9	32 31 35 41 30 $\bar{x}$ = 34 s.e. = 2.0	
40 56 46 31 $\bar{x}$ = 43 s.e. = 5.3	73	
95 55 98 70 72 $\bar{x}$ = 78 s.e. = 8.1	107 87 106 60 75 $\bar{x}$ = 87 s.e. = 9.0	61 114 109 61 $\bar{x}$ = 86 s.e. = 14.6
151 176 186 176 131 122 $\bar{x}$ = 157 s.e. = 10.8	90 119 90 112 $\bar{x}$ = 103 s.e. = 7.5	
77 73 61 63 63 $\bar{x}$ = 67 s.e. = 3.2	54 60 61 57 35 $\bar{x}$ = 53 s.e. = 4.8	
57 82 81 78 49 55 $\bar{x}$ = 67 s.e. = 6.1		
82 71 71 55 $\bar{x}$ = 70 s.e. = 5.6	58 75 55 69 48 54 $\bar{x}$ = 60 s.e. = 4.1	45 51 49 46 63 61 $\bar{x}$ = 53 s.e. = 3.1
107 129 136 135 143 101 $\bar{x}$ = 125 s.e. = 7.0	42 72 64 68 29 $\bar{x}$ = 55 s.e. = 8.3	83 75 63 55 $\bar{x}$ = 69 s.e. = 6.2

Table 1. (Continued)

Sow No.	Day +7
587-020	30 19 33 59 39 $\bar{x}$ = 36 s.e. = 6.6
589-021	
591-023	
594-010	
595-011	
596-012	62 45 45 56 48 68 $\bar{x}$ = 54 s.e. = 3.9
597-009	
598-007	47 47 51 $\bar{x}$ = 48 s.e. = 1.3
600-013	
601-014	
601-024	
603-016	
604-017	43 54 56 91 41 59 $\bar{x}$ = 57 s.e. = 7.3
605-018	48 62 74 50 79 $\bar{x}$ = 63 s.e. = 6.2

Table 2. Corticoids summary data

Sow	-8	-7	-6	-5	-4	-3	-2	-1
586								
587								
589					15	32	22	25
590						21	33	29
591				24	30	19	22	28
592								151
593								50
594				57	40	39	45	61
595						25	23	31
596					36	52	43	45
597		45	39	40	37	26	36	48
598							39	65
600								40
601	33	35	48	43	33	36	44	40
602								
603		32	31	25	23	30	28	48
604						36	25	56
605						36	49	64
$\bar{x}$ =	22.4	37	39	38	31	32	34	52.1
s.e. =	3.08	4	5	6	3	3	3	7.8
n =	129	3	3	5	7	11	12	15

0	+1	+2	+3	+4	+5	+6	+7	+8
77	17	37	35	21	27	23	33	
60	51	11	18	26	15	16	15	16
29	16	43	8	9	14	13	10	8
90	43	7	17	15	12	13	15	
29	6	13	12	23	14	10	13	
5	7	13	15	11	16	13	14	
63								
53	46	45	23	44	31	29	21	
25								
109	144	55	18	6	6	4	5	
88	30	7	5	0	19	16	5	
20	18	29	29	31	32	23	30	
47	96	42	18	19	30	28	12	
14	16	21	14	39	13			
32	37	25	28	22	32	5	19	
31	33	15	13	17	26	9	15	
30	31	29	30	35	29	15	16	
47.2	39.4	26.1	18.9	21.2	21.1	15.5	15.9	
7.2	9.5	4.0	2.2	3.2	2.7	2.1	2.1	
17	15	15	15	15	15	14	14	

Table 3. Progesterone summary data

Sow	-8	-7	-6	-5	-4	-3	-2	-1
586								
587								
589					6.6	6.7	5.3	3.5
590						8.5	9.9	2.2
591				6.2	7.4	7.3	6.9	2.6
592				6.5	2.1	0.7	0.5	0.5
593								10.6
594				8.2	8.2	10.2	7.3	3.3
595						3.0	6.1	2.5
596					6.8	7.3	6.2	2.7
597		12.3	16.3	6.3	5.7	8.6	8.5	2.1
598							2.7	2.0
600								6.6
601	4.6	6.3	6.7	7.3	7.9	3.0	4.6	5.0
602								
603		9.2	8.9	6.5	0.5	9.3	9.5	2.5
604						3.4	4.0	1.8
605						6.5	4.5	2.8
$\bar{x}$ =		9.27	10.63	6.83	5.65	6.21	5.85	3.38
s.e. =		1.73	2.90	0.32	1.00	0.86	0.74	0.63
n =		3	3	6	8	12	13	15

0	+1	+2	+3	+4	+5	+6	+7	+8
1.1	0.4	0.3	0.3	0.2	0.1	0.1	0.1	
1.6	.6	.5	.3	.2	.2	.1	.1	.1
0.6	.2	.1	.1	.1	.1	.1	.1	.1
0.6	.7	.2	.1	.1	.1	.1	.1	
0.7	.4	.1	.1	.1	.1	.3	.1	
0.2	.2	.1	.2					
0.7								
2.1	.7	.5	.3	.4	.3	.3	.2	
2.2								
1.4	1.0	.4	.2	.2	.2	.1	.1	
1.8	.6	.3	.2	.2	.2	.2	.2	
0.8	.5	.3	.2	.2	.2	.1	.1	
1.4	.5	.3	.1	.2	.2	.1	.1	
1.0	.5	.5	.3	.2	.1			
0.5	.4	.3	.4	.3	.1	.3	.2	
0.4	.3	.2	.3	.3	.2	.2	.2	
0.5	0.4	.3	.2	.1	.1	.1	.1	
1.04	0.49	0.29	0.22	0.20	0.16	.16	.13	
0.15	0.05	0.04	0.02	0.02	0.02	.02	.01	
17	15	15	15	14	14	13	13	2

Table 4. Estrogens summary data

Sow	-8	-7	-6	-5	-4	-3	-2	-1
586								
587								
589					2100	1923	2023	1730
590						2467	1546	1623
591				1697	2367	2866	2450	3450
592								3403
593								2620
594				46	4666	2950	3217	4300
595						1577	1987	2417
596					1793	1800	1965	2237
597		1597	1750	852	2400	1787	2163	2287
598							1760	1497
600								2302
601	1953	2450	2687	2273	2043	2917	3020	3053
602								
603		1213	1273	1620	1873	2323	1650	2350
604						2867	3536	3833
605						2100	2303	2380
$\bar{x}$ =		1753	1903	1610	2463	2325	2301	2632
s.e. =		365	415	292	377	156	184	211
n =		3	3	4	7	11	12	15

0	+1	+2	+3	+4	+5	+6	+7	+8
2453	225	55.6	43.8	33.8	16.8	22.5	23.2	
1522	359	43	32	18	23	24	15	15
1553	67	43	28	6	20	12	16	31
735	499	57	20	25	18	19	19	
390	40	25	21	22	22	35	8	
421	219	102	28	19	57	54	8	
598								
4233	260	83	40	11	14	15	11	
597								
1768	95	23	18	9	10	9	6	
1515	339	262	13	12	15	0	0	
337	30	22	12	10	10	10	14	
2420	127	20	12	13	3	4	4	
457	74	20	17	10	6			
175	66	20	14	13	21	15	13	
382	157	7	24	16	21	6	9	
312	52	14	24	16	17	11	6	
1168	174	53	23	16	18	17	11	
264	36	16	3	2	3	4	2	
17	15	15	15	15	15	14	14	