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I am submitting herewith a thesis written by Heather L. McLane entitled "Relationships of Ovarian Characteristics to Endocrine, Metabolic and Nutritional Variables in the Cyclic Beef Cow." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science with a major in Animal Science.

F. Neal Schrick, Major Professor

We have read this thesis
and recommend its acceptance:

Accepted for the Council:

Associate Vice Chancellor and
Dean of The Graduate School

RELATIONSHIPS OF OVARIAN CHARACTERISTICS TO ENDOCRINE, METABOLIC AND NUTRITIONAL VARIABLES IN THE CYCLIC BEEF COW

A Thesis

Presented for the

Master of Science

Degree

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ABSTRACT

A two-year experiment was conducted to identify possible associations between common reproductive parameters and ovarian structures. Two, 3-, and 4-year old, lactating and nonlactating, cycling Polled Hereford cows (yr 1: n=20; yr 2: n=22) at random stages of the estrous cycle were treated with 25 mg of $PGF_{2\alpha}$ and two injections of a GnRH agonist to synchronize estrus (Day=0). Ovaries were removed on Days 5 (n=4), 9 (n=34) and 13 (n=4) of the cycle, measured, sectioned into four quarters and fixed in Bouin's solution. Eight 10 μm thick sections, 100 μm apart, were stained for light microscopic examination. Follicles were classified and recorded as primary, growing or vesicular (normal or atretic; small, medium and large) on a whole ovary basis. Cows were sampled via jugular venipuncture at the time of ovariectomy. Samples were assayed for follicle stimulating hormone (FSH), luteinizing hormone (LH), progesterone (P_4), estradiol-17B (E_2), prolactin (PRL), insulin, blood urea nitrogen and androstenedione. Cows were divided into low, medium and high groups for birth weight (BWT), weaning weight (WWT), yearling weight (YWT), body weight at ovariectomy (OWT) and the afore mentioned hormonal variables .

For cows in **Year One**, WWT (determined at 7-9 mo of age), OWT, age and lactational status were the most influential variables for ovarian follicle populations. Cows with medium WWT had more primary (P=0.03), growing (P=0.04), small normal (P=0.03) and small atretic (P=0.04) vesicular follicles than high and low WWT cows.

Body weight at ovariectomy influenced medium (P=0.02) follicular populations, with high OWT cows possessing more medium (P=0.02) and medium normal (P=0.04) follicles than medium OWT cows. High OWT cows had more large normal (P=0.03) and more large atretic (P=0.01) follicles than medium and low OWT cows. High P_4 cows had fewer large normal (P=0.04) and more large atretic follicles (P=0.06) than medium and low cows.

Follicle stimulating hormone and body weight at ovariectomy were the most significant variables for cows ovariectomized in **Year Two**. Age, status, Day, FSH and OWT interacted to influence all vesicular follicle populations except large (P=0.001-P=0.02). Most variation in these models was due to FSH concentrations, as high FSH cows had reduced quantities of small and medium vesicular follicles compared to cows with low and medium FSH concentrations. Cows with low insulin concentrations had fewer primary follicles (P=0.01) than medium and low. Number of growing follicles was influenced by WWT, as medium WWT cows possessed more follicles than low and high WWT cows. Size of the primary CL affected large follicles, as a large primary CL was associated with more large normal follicles (P=0.01). Cows with a small CL had more large atretic follicles (P=0.02) than those with a large or medium CL.

Luteal type influenced ovarian follicle populations for cows in both years. Primary follicles were located closer to the secondary (4.7 $\pm$ 1.3 mm) than the primary CL (8.8 $\pm$ 1.3 mm; P=0.04), and number of primary follicles was reduced in sections containing the primary CL (36.6 $\pm$ 7.3) compared to the secondary CL (53.9 $\pm$ 7.3;

P=0.03). More normal follicles tended to associate with the primary (2.5 ± 0.4) than the secondary CL (1.6 ± 0.4; *P*=0.06). The distance to the closest atretic follicle was shorter for the secondary (4.1 ± 0.7 mm) compared to the primary CL (8.4 ± 1.6 mm; *P*=0.03), while the size of the closest atretic follicle was smaller for the secondary (2.0 ± 0.7 mm) than for the primary (4.1 ± 0.7 mm). In conclusion, follicular populations in the bovine are highly variable and influenced by factors such as body weight, hormone concentrations, number and type of luteal structure. Knowledge of key interactions between these factors may help producers select for and manage reproductive efficiency.

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1. INTRODUCTION

Poor reproductive efficiency is a common enigma to a productive beef cattle operation. Reproductive efficiency may be defined as production of a calf each year by every cow bred (Osoro and Wright, 1992). Conception rates for beef cattle are estimated to lie between sixty and eighty percent annually (Azzam et al., 1989). Cows that fail to conceive and produce a live calf each year are a significant source of inefficiency. Twelve percent of the United States cattle population never calve at all (Ensminger, 1987).

Many reproductive delays are related to length of the postpartum anestrus. This anestrous period can last between thirty and one hundred and ten days in the beef cow (Hazan, 1986). Factors that influence this period include nutritional status and body condition (Wiltbank et al., 1962; Lishman et al., 1979), frequency of suckling (Wettemann et al., 1978), age (Wiltbank, 1970) and breed (Echternkamp et al., 1982). The hypothalamo-pituitary-ovarian axis influences these factors. Focusing our attention on interactions of this physiologic cascade with the afore mentioned factors may help to elucidate possible causes of infertility.

Deficiencies in production or secretion of hypothalamic and pituitary hormones can delay return to cyclicity in the postpartum cow. Follicle stimulating hormone (FSH) and luteinizing hormone (LH) are pituitary gonadotropins responsible for follicular development and subsequent ovulation of the dominant follicle. These hormones are

released from the anterior pituitary after stimulation by gonadotropin releasing hormone (GnRH) from the hypothalamus. Infrequent pulses of LH are observed as a common endocrine pattern in the postpartum cow (Williams, 1990). Nett and associates (1988) detected sufficient hypothalamic GnRH stores to stimulate LH release early in the postpartum period. Nevertheless, quantities of LH contained in the anterior pituitary were low until Day 30 postpartum. Estradiol and GnRH pituitary receptors increase by Day 15 postpartum (Nett et al., 1988). This allows for subsequent increased LH synthesis and anterior pituitary receptivity.

The ovary plays a critical role in reproductive viability of the cow. Most cattle are born with an incredible stockpile of oocytes (Erickson, 1966). These oocytes are surrounded by flattened granulosa cells which subsequently develop into primary follicles. Follicles may be thought of as containers with an internal milieu that can nurture or destroy the oocyte. Cattle are a monovular species and ovulation may occur randomly from either ovary. Follicular development occurs as two or three waves of growth during each estrous cycle (Savio et al., 1988; Sirosis and Fortune, 1988; Ginther et al., 1989b; Fortune, 1994). This growth culminates with ovulation of a dominant follicle and expulsion of the oocyte (Espey, 1994).

Follicular development is highly variable and influenced by a multitude of factors (Erickson et al., 1976; Monniaux et al., 1984). Pituitary gonadotropins, as discussed previously, are critical for folliculogenesis (McNatty et al., 1985; Adams et al., 1992a; Scaramuzzi et al., 1993; Campbell et al., 1995). Vesicular follicle growth cannot occur in the absence of LH and FSH. Follicles larger than 4 mm in diameter are dependent on

gonadotropins for continued growth (McNeilly et al., 1991; Fortune, 1994). Suckling and neuroendocrine interactions also influence follicular development (Weiss et al., 1981; Williams, 1990; Williams and Griffith, 1995). Interactions between cow behavior, infrequent pulsatile LH secretion, opioid peptides and oxytocin can prevent ovulation (Williams and Griffith, 1995). Age of the cow plays a role as well. Follicular numbers gradually decline until approximately four years of age (Erickson et al., 1976). After that, numbers decline drastically until the cow reaches the end of her productive life.

Undernutrition can have a negative impact on reproduction. Energy restriction can delay growth of the dominant follicle (Jolly et al., 1995). Rhodes and co-workers (1993) showed that restricting dietary energy caused a decline in heifer body mass, which was associated with a linear decrease in the size of the dominant follicle and corpus luteum. Some of these effects may occur at the pituitary level. A decrease in substrates (glucose, amino acids) can reduce quantities of LH and FSH secreted (Downing and Scaramuzzi, 1991). Low concentrations of insulin may restrict ovarian substrate processing and follicular growth (Monget and Monniaux, 1995). Research efforts in this area have produced variable and conflicting results. Nutritional excesses can be harmful to reproduction as well. Diets high in crude protein increase plasma urea nitrogen (Canfield et al., 1990). Increases in urea reduces uterine pH (Elrod and Butler, 1993), which may be detrimental to gamete and embryo survival. Furthermore, *in vitro* studies have shown that urea can inhibit LH binding to its ovarian receptors (Canfield et al., 1990).

Ratios of the ovarian steroids, estrogen and progesterone, are useful indicators of

normal and atretic follicles (Spicer and Echternkamp, 1986). High concentrations of estrogen indicate the presence of healthy follicles (Ireland and Roche, 1982). Comparing concentrations of these hormones in the peripheral circulation of cattle to follicular populations may prove useful in identifying cows with increased quantities of healthy follicles. Unfortunately, removing ovaries for histological analysis is not a practical production tool. Blood sampling and subsequent radioimmunological hormone detection may prove to be a fairly non-invasive method of identifying follicular populations. Follicular populations may be related to other production variables. Identifying relationships between follicles and metabolism, nutrition, lactational status, body weight, and days postpartum may serve as useful indicators of reproductive efficiency.

Researchers and producers alike have often asked the question, "what are the characteristics of a reproductively efficient cow?" This study was undertaken in an attempt to identify physiological factors which could be used to define this animal. Ovarian steroids, pituitary gonadotropins, corpus luteum size and numbers, nutritional status, body weight, cow age, days postpartum and lactational status may show a useful relationship to follicular health and populations of the cyclic beef cow.

2. LITERATURE REVIEW

Physiology of the Hypothalamus and Pituitary

Proper function of the hypothalamo-pituitary-ovarian axis is important for reproductive function in the bovine. Secretion of gonadotropin releasing hormone (GnRH) by the hypothalamus acts at membrane bound receptors in the anterior pituitary. This stimulates cells in the anterior pituitary, referred to as gonadotrophs, to release luteinizing hormone (LH) and follicle stimulating hormone (FSH) from intercellular granules. These hormones act on the ovary to promote follicular development and steroid secretion. Hormones such as estradiol 17-β (E_2), progesterone (P_4), inhibin and activin are produced by ovarian structures and are involved in negative and positive feedback regulation of hypothalamic and pituitary hormones.

The hypothalamus is developed from the anterior column of fornix and the third ventricle of the brain (Racadot, 1990). The pituitary gland lies in close proximity to the base of the hypothalamus (Figure 1). A portal system consisting of the median eminence, infundibular stem and the pars tuberalis connects the two glands (McCann, 1987). Axons of the paraventricular nuclei extend from the rostral preoptic area of the brain into the anterior pituitary (adenohypophysis). Supraoptic nuclei axons lead to the posterior pituitary (neurohypophysis). Hormones such as GnRH, thyrotropin-releasing hormone

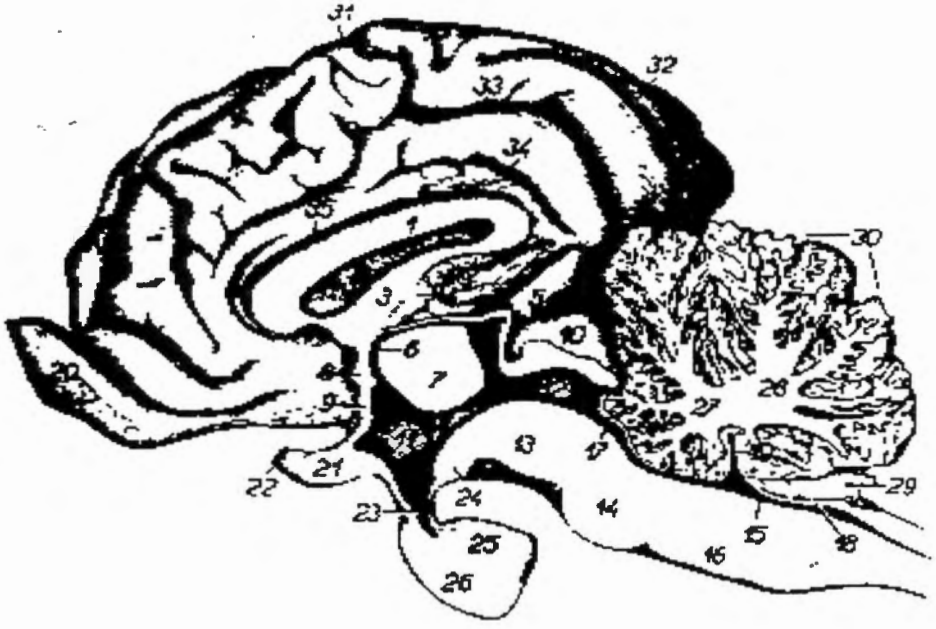

Figure 1. Sagital section through the bovine brain. Structures shown include the fornix (3), third ventricle (11), adenohypophysis (26), and the neurohypophysis (25) (from Popesko, 1977).

(TRH) and corticotropin-releasing hormone (CRH) are transported to the median eminence via the paraventricular nuclei. These hormones are then released into the portal system and carried to the anterior pituitary.

The anterior pituitary consists of the pars anterior (anterior lobe), pars intermedia (intermediate lobe) and the pars tuberalis. In terms of size and weight, the anterior pituitary is the largest component of the pituitary. It is composed of cords, which contain layers of hormone secreting cells. These cells are often distinguished based on staining qualities of their secretory granules. The gonadotrophs are basophilic cells stimulated by GnRH to secrete FSH and LH. Estimates of pituitary cell populations show that gonadotrophs make up 10-15% of pituitary cells.

Gonadotropin Releasing Hormone

GnRH is a decapeptide purified in 1973 (Matsuo et al., 1974; Labrie, 1990). Neurons releasing GnRH originate in the medial preoptic area and the rostral-most anterior hypothalamus in the bovine (Leshin et al.,1988). The gene that encodes for GnRH can be influenced by ovarian steroids. Studies by Weesner and associates (1993) have determined that ovarian hormones produced during the estrous cycle can influence the number and activity of GnRH-expressing neurons. Decreased quantities of GnRH mRNA were detected during the mid-luteal phase of postpubertal heifers.

Administration of GnRH causes the anterior pituitary to expel its contents of FSH and LH via a second messenger system. Binding of GnRH to receptors on gonadotropin cell membranes causes adenylate cyclase to be activated. This action causes ATP to be

converted into the second messenger cAMP, stimulating hormone release. Maximal levels of LH and FSH are seen by 30 and 60 minutes, respectively, after stimulation (Kletzky et al., 1977). Stored LH is released in pulses, while FSH is secreted continuously in the ovine (Clark et al.,1986) and bovine (Rowland, 1994) species. Numbers of pituitary receptors for GnRH increase in the pituitary gland during the follicular phase of the estrous cycle (Nett, 1990). Receptor numbers are regulated by ovarian steroids and GnRH itself (Nett, 1990).

Luteinizing Hormone and Follicle Stimulating Hormone

The gonadotropins (FSH and LH) are critical for control of recruitment, selection and dominance of vesicular follicles (Driancourt, 1991). FSH and LH are glycoprotein hormones which share structural similarities (Gharib et al., 1990). Both are heterodimers with common α-subunits. Transcription of the α-subunit gene is controlled by cAMP (Gharib et al., 1990). This subunit is coded for by only one gene, while several genes encode the β–subunit. Biological specificity is conferred by the β-subunit, which is distinct for each species. Membrane receptors for FSH and LH traverse the plasma membrane with seven transmembrane domains (Johnson and Dhanasedren, 1989). These receptors have long amino termini and extracellular loops which specifically bind these hormones (Sprengel et al., 1990).

N-linked oligosaccharides are associated with gonadotropin subunits (Gharib et al., 1990). Variations in these oligosaccharides make up the different isoforms of LH and FSH (Cooke et al., 1996). These isoforms vary in their isoelectric points, potencies, and

receptor binding affinity (Kojima et al., 1995). Various isoforms of these hormones may potentially have distinct effects at different time points of the estrous cycle (Phillips et al., 1994). Carbohydrate residues on α and β subunits play a role in signal transduction. Deglycosylated hormones are not as efficient as the glycosilated forms in elevating cAMP levels in target cells (Sairam and Bhargavi, 1985).

Plasma half-life of FSH is approximately 170 minutes (Labrie, 1990). Follicle stimulating hormone has a molecular weight of 33,000 kd (Labrie, 1990). The FSH molecule has five sialic acid residues per molecule. Target cells for FSH are granulosa and Sertoli cells. These two cell types are believed to be the only cells which contain the FSH receptor (Griswold et al.,1995). FSH influences follicular populations and ovulation rates. Waves of follicular growth are associated with fluxuations in FSH concentrations (Adams et al., 1992a; Ginther et al.,1994). Observed variations in FSH levels are associated with development and regression of large ovarian follicles (Fortune et al., 1991). Turzillo and Fortune (1993) determined that suppressing the second plasma FSH surge during the first wave of follicular development in the heifer interrupted growth and development of the dominant follicle. This is due to the FSH dependency of follicles at this stage of growth.

Follicle stimulating hormone may play a role in increasing ovulation rate in prolific breeds of sheep, such as the Booroola. McNatty and Henderson (1987) found that homozygous Booroola carriers of the F gene have higher mean plasma levels of FSH and LH. The frequent exposure to elevated FSH levels increases activity of aromatase in the granulosa cells of antral follicles. Studies of follicular dynamics in highly prolific

breeds are important because these breeds share similar follicular growth and development patterns with nonprolific breeds (Hunter et al., 1992). Understanding follicular selection in breeds with multiple ovulations may help elucidate mechanisms of dominant follicle selection and survival capability.

The half-life of LH is about 30 minutes (Labrie, 1990). Luteinizing hormone has a molecular weight of 28,000 kd with approximately 2 sialic acid residues per molecule (Labrie, 1990). Maturation and ovulation of the dominant follicle is stimulated by LH. A secondary function is to stimulate the formation and maintenance of the corpus luteum. The theca interna is a major target tissue for LH. LH receptors are found on thecal cells of large healthy and atretic follicles. Plasma LH levels are fairly low throughout most of the estrous cycle. Small pulses of LH occur throughout the cycle. As progesterone decreases late in the cycle, LH secretion begins to increase. Approximately 27 hours before ovulation, a surge of LH occurs. This peak of LH stimulates ovulation and luteinization of follicular granulosa and thecal cells. The LH surge causes an alteration in follicular steroid production and resumption of meiotic divisions in the oocyte.

LH is responsible for more than just ovulation. The role of LH in follicular development has been explored numerous times. Scaramuzzi and colleagues (1993) developed a functional model of folliculogenesis. Acquisition of LH receptors is an important criteria for gonadotropin-responsive and gonadotropin-dependent follicles. Ovulatory follicles must have high concentrations of functional LH receptors for maximal response to the LH surge. LH stimulation throughout follicular development increases the diameter of the follicle selected to ovulate. Secretion of estrogen from this ovulatory

follicle triggers GnRH and subsequent LH release (Caraty et al., 1989).

Physiology of the Ovary

The ovary can be divided into histological zones. Van Wezel and Rodgers (1996) describe five zones from the ovarian surface to the medulla. The first zone consists of a single layer of cuboidal cells called the surface epithelium. Zones two and three are just below the surface and consist of collagenous tissue. The fourth zone contains many different cell types. Primary follicles are located between zones three and four. Zone five includes the stroma of the ovary. Vesicular follicles are most likely to be located in this region of the ovary, as evidenced by the numerous blood vessels.

Ovarian structures (Figure 2) help to regulate endocrine/paracrine steroid and peptide hormone production and feedback control of pituitary and hypothalamic hormones. Estrogen and progesterone are the main ovarian steroid products. Steroid hormones arise from cholesterol derived from acetate or taken up from the blood (HDL/LDL) (Niswender et al.,1994). Production of steroids depends on a constant supply of their cholesterol precursor. The cholesterol side chain cleavage enzyme system (cP450) on the inner mitochondrial membrane fuels the conversion of cholesterol to pregnenolone (Liscum and Dahl, 1992). Biosynthesis of androgens occurs through the $\Delta 4$ or $\Delta 5$ pathway. These androgens are then converted in different steps to estrogen and progesterone. Steroid hormones are bound to carrier proteins in the blood. They must be removed from these proteins so that they may cross the plasma membrane and bind to their nuclear receptors.

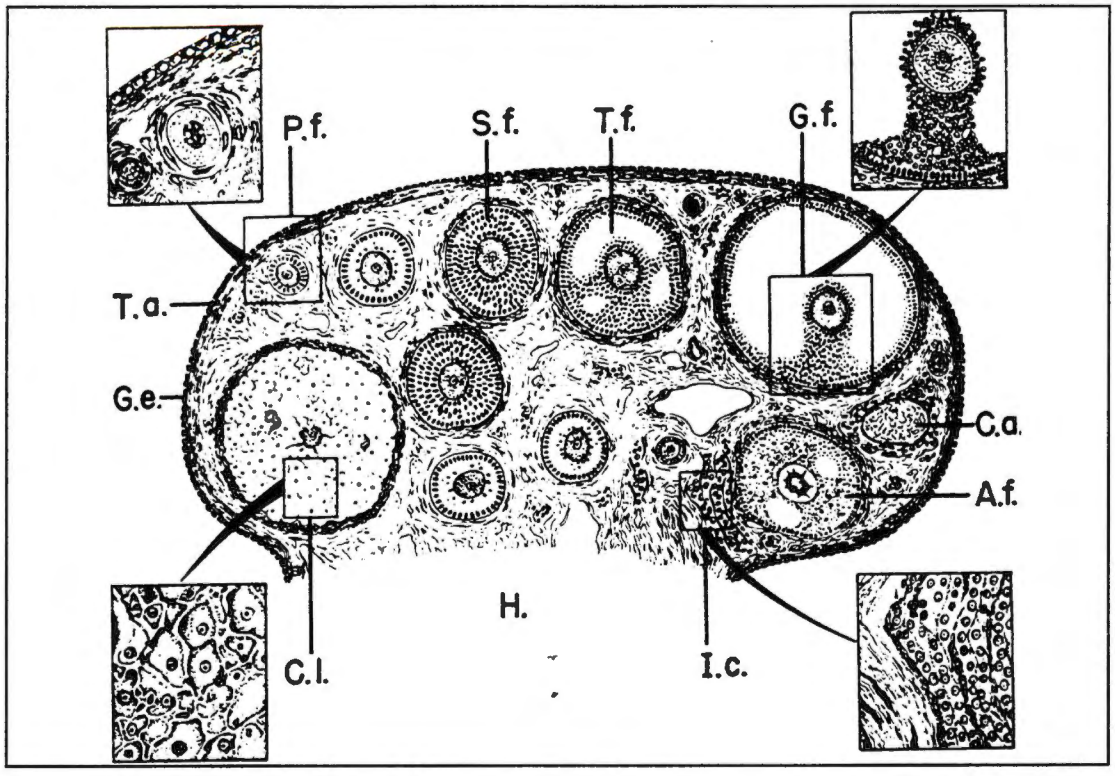

Figure 2. Composite diagram of the mammalian ovary. Structures identified are the corpus albicans (C.a.), corpus luteum (C.l.), germinal epithelium (G.e.), graafian follicle (G.f.), hilus (H), interstitial cells (I.c.), primary follicle (P.f.), secondary follicle (S.f.), tunica albuginea (T.a.), and tertiary follicle (T.f.) (from Hafez, 1993).

Several regulatory proteins, such as sterol carrier protein 2, steroidogenesis activator polypeptide (SAP) and the steroidogenic acute regulatory (StAR) protein may play a role in steroid production. Protein synthesis (translation) is an important step in hormone stimulated steroid production (Stocco and Clark, 1996). The StAR protein has been widely researched. Translocation of cholesterol across the membrane of the mitochondria can be stimulated by the StAR protein (Stocco and Clark, 1996). StAR protein expression has been located in the ovary, testis and adrenal gland of the mouse (Clark et al., 1995) and StAR mRNA in the corpus luteum of the sheep and cow (Juengel et al., 1995).

Both the theca and granulosa layers of the follicle are involved in steroid production. These cells must work in concert due to the fact that aromatase, which is necessary for steroid production, is absent from thecal cells. Thecal cells have receptors for LH. LH binds to the theca cells and induces production of androgen from cholesterol. Androgen is then transferred to the granulosa layer where it is aromatized to estrogen (Fortune, 1986). This process is commonly referred to as the two-cell, two-gonadotropin theory.

Estradiol-17β

Estradiol-17β is the primary biologically active estrogen produced by the ovary. Estrone and estriol are metabolites of estradiol and are produced in smaller quantities. The structure of estradiol consists of the cholesterol rings with an OH group at carbon 18. Both the granulosa and thecal layers are involved in estrogen synthesis. Androgen,

produced by the thecal cells in reaction to LH, is transported to the granulosa cells (Fortune,1994). Under the control of FSH, aromatase in the granulosa layer converts androgen into estradiol. Estradiol is then secreted into follicular fluid of developing antral follicles and the ovarian vein.

Plasma concentrations of estradiol are low throughout most of the estrous cycle. Approximately four days before estrus, estradiol begins to rise until a peak occurs at the time of estrus. This increase in estradiol is correlated with the increase in size of the ovulatory follicle. Studies have shown increases in the concentrations of estradiol with first wave dominant follicles on Day 5 of the bovine estrous cycle (Badinga et al., 1992; Sunderland et al.,1994). Estrogen is involved in the feedback regulation of recruitment and selection of follicles. Estrogen and inhibin production by the dominant, estrogen-active follicle reduces FSH secretion by negative feedback (Roche, 1996). Aromatase activity is maximal in this dominant follicle. Thus, one of the first signs of functional maturation of the dominant follicle is the start of estrogen secretion (Hirshfield, 1991a).

Estrogen plays an important role in follicular health. Follicular fluid can often be used to determine follicular health due to the fact that fluid from atretic follicles has an increased androgen to estrogen ratio (Carson et al., 1981). In hypophysectomized rats, granulosa cell DNA degradation was prevented by estrogen treatment (Billig et al., 1993). Estrogen increases capillary permeability and vascularization, and this is an important determinant for selection of the preovulatory follicle (Bruce and Moor, 1976). This increased permeability of capillaries and dilation of arterioles in the vascular tissue of the preovulatory follicle are important for substrate and hormone transport.

Progesterone

Progesterone is secreted by luteal tissue, the adrenal gland, and the placenta. Plasma concentrations of progesterone begin to increase about Day 4 of the bovine estrous cycle. Progesterone concentrations reach a maximum on Day 8-9, and then remain high until Day 17-18 (Peters and Ball, 1987). Concentrations decline as the corpus luteum (CL) begins to regress (Niswender et al., 1994). Regression of the CL is necessary for an increase in LH pulse frequency leading up to the LH surge.

Follicular health can also be assessed by progesterone levels. Interfollicular ratios of estrogen and progesterone may be used to indicate follicular health (Ireland and Roche, 1982). Levels of progesterone increase as the number of atretic follicles increase. Granulosa cells from atretic follicles loose their aromatase function and actively secrete progesterone (Spicer and Echternkamp, 1986; Spicer et al., 1987). Progesterone can also inhibit the development of the dominant follicle (Adams et al., 1992). Luteal phase concentrations of progesterone suppress LH secretion which the dominant follicle depends on for growth. Progesterone accumulates in the follicular fluid of subordinate follicles (Lucy et al., 1992). This accumulation may lead to atresia of follicles which are not destined to ovulate. Growth factors such as the insulin-like growth factors (IGF-1,-2) and insulin may play a role in the control of progesterone synthesis. Insulin-like growth factor-I levels regulated by insulin may in turn regulate progesterone biosynthesis (Spicer et al., 1988; Wathes et al., 1995). IGF-1 may also play a role in mediating gonadotropin suppression of atresia (Hsueh et al.,1994).

Progesterone also impacts fertility rate, as cows with increased conception rates at

first postpartum insemination exhibited increased concentrations of progesterone (Fonseca et al., 1982). Helmer and Britt (1987) detected higher concentrations of progesterone throughout the estrous cycle of human chorionic gonadotropin (hCG) treated heifers. This treatment resulted in increased luteal cell hypertrophy and thus an increase in progesterone and a decrease in estrogen production. Subsequent improved fertility may have been the result of decreased estrogen secretion and subsequent delayed luteolysis (Helmer and Britt, 1987). Luteal concentrations of progesterone are inhibitory to the LH surge (Peters and Ball, 1987). Estrogen increases late in the cycle (Day 17) as the corpus luteum regresses. Low progesterone concentrations at this time allows LH pulse frequency to increase, eventually resulting in an LH surge 24 hours later (Sirois and Fortune, 1990). Prolonged, diminished concentrations of progesterone can lead to the persistence of an estrogen-active, dominant follicles. Increased estrogen secretion from these persistent follicles negatively impact oocyte maturation and implantation in cattle, sheep (Breuel et al., 1993; Ahmad et al., 1995; Johnson et al., 1996).

Embryonic Development of the Ovary

Primordial germ cells (PGC) are established by Day 7 of gestation. PGCs migrate from the yolk sac to the caudal mesonephros by Day 12 of gestation. The embryonic gonad develops as an appendage on the mesonephros. The genital ridge is a visible thickening on the medial surface of the mesonephros by the 10 mm stage of embryo development. The PGCs are large, rounded cells with a diameter of 10-20 μm. PGCs contain granular chromatin, 2 nucleoli and very few organelles. After migration to the

genital ridge at approximately Day 32 of gestation (Erickson, 1966), the PGCs differentiate into oogonia and are located in the cortical region of the developing ovary. Mitotic divisions help the oogonia to proliferate into primary oocytes. Meiosis begins in the cow (Erickson, 1966) and human (VanWagenen and Simpson, 1965) ovary between Days 75 to 80 of gestation. The majority of oogonial divisions occur before birth. Oocytes (germinal vesicles) remain arrested in metaphase II of the first meiotic division until ovulation. Each oocyte is contained in a primary follicle. This follicle consists of a flattened layer of granulosa cells. Evidence exists supporting the idea that thecal cells are present around these primary follicles. Hirshfield (1991b) infused rats with tritiated thymidine and observed labeled, squamous cells near the granulosa cell basement membrane of primary follicles. These cells had characteristics very similar to thecal cells.

As the follicle begins to grow, so does the oocyte. It doubles in diameter and develops a zona pellucida. A cow can be endowed with a substantial number of germ cells during embryonic development (1-2 million). Many of these oocytes degenerate well before birth. In the bovine, quality of germ cells can remain fairly high in the ovaries up to about 180 days of age and then decline swiftly until the population is nearly depleted in an older cow (Erickson, 1967; Erickson et al.,1976). If germ cell quality limits reproductive capacity of an animal, it is imperative to determine relationships between these cells and other factors (endocrine, management, heritability).

Follicular Development

Folliculogenesis is a process in which less differentiated cells become committed to a particular developmental pathway (Hirshfield, 1991a). These cells develop together to become a distinct functional structure. Folliculogenesis involves development of a follicle from the primordial stage to a mature, ovulatory follicle. Follicle growth continues without interruption prior to birth or until the animal fails to cycle. Vesicular follicles are apparent by Day 250 of gestation in the bovine (Erickson, 1966).

The rate at which primordial follicles enter the growing pool appears to be dependant on the number of follicles stockpiled in the ovary (Hirshfield, 1994). Many possible explanations attempt to explain a mechanism for the development of these primordial follicles. Possibilities include the actions of local follicular growth factors or FSH. The number of primordial follicles may not be tightly regulated. Hirshfield suggests that follicular growth is not just a preprogrammed event. Outside influences are able to modify this system. In Hirshfield's 1994 study, rats were exposed to busulphan in utero. Busulphan causes death in proliferating cells by cross-linking their DNA. This treatment destroyed most of the primary follicles. The remaining follicles were recruited into the growing pool very quickly, exhausting follicular reserves by 2 months of age. Even though these treated rats expended most of their primary follicles very early in life, numbers of large antral follicles were similar to control rats (7 vs. 9). It is interesting to observe this increased rate of follicular loss. One would think the ovary would try to conserve its follicular stores instead of hastening its expenditure.

Antral follicles develop in "waves". Most cattle have two or three waves of

follicular growth per cycle (Savio et al., 1988; Sirois and Fortune, 1988) culminating in the ovulation of a dominant follicle. Cattle with two waves of growth show wave emergence on Days 2 and 11 of the estrous cycle, and new waves of growth emerge in cattle with three waves of development on Days 2, 9, and 16. Cows with three waves have luteal phases that are about 2 days longer (Ginther et al., 1989b). The ovulatory follicle develops out of the final wave of follicular growth. Follicular waves are apparent in heifers as early as two weeks of age (Evans et al., 1994). The authors propose that an early rise in gonadotropin secretion is important in stimulating follicular growth in these calves. Ovine follicles grow and develop continuously with lack of follicular dominance (Schrick et al., 1993). Cattle, sheep, and horses develop follicles of ovulatory size in the luteal and follicular phases, while rats, swine, and primates only grow ovulatory size follicles during the follicular phase (Fortune, 1994).

Dominance is supported by ovarian negative feedback on gonadotropins (FSH) (Fortune, 1994). However, the precise explanation of why a particular follicle is selected to become dominant and ovulate is not quite known. Unfortunately, the explanation that the follicle just happens to be in the correct place at the correct time is not adequate. That particular follicle has to have all of its cellular mechanisms in place and operating properly. These mechanisms include acquisition of granulosa cell LH receptors, binding of LH in the thecal and granulosa cell layers and aromatase activity (Ginther et al., 1996). Since the dominant follicle has escaped "programmed cell death", its DNA must have an intrinsic getaway plan. The granulosa cells of this follicle may have better access to substrates, more mitochondria to utilize ATP, decreased activity of endonucleases, or

may be able to survive the disruption of ionic gradients (Hirshfield, 1991a).

Vesicular follicle development consists of recruitment, selection, and dominance (Fortune, 1994). Recruitment involves the growth of a cohort of follicles. This stage is correlated with small increases in circulating FSH (Adams et al.,1992a). Unfortunately, not all of these follicles can grow and become ovulatory follicles. The dominant follicle(s) from the selected group secretes inhibin and estradiol (Campbell et al.,1990) which negatively feedback on FSH. Reduced levels of FSH do not appear to effect the follicle selected for dominance. The granulosa cells of this follicle become much more sensitive and responsive to FSH (increased cAMP production). Follicular fluid of maturing follicles have reduced concentrations of tissue inhibitor of metalloproteinase (TIMP) and insulin-like growth factor binding proteins (IGFBP's) and high inhibin: activin ratios (Roche, 1996). These factors interact in an effort to increase granulosa estrogen production. Local regulators, such as IGF-1, EGF, FGF, TGF-β, PDGF, and cytokines, increase follicular responsiveness to gonadotropins (Echterkamp et al.,1994; Monget and Monniaux, 1995).

As the dominant follicle continues to grow and differentiate, development of LH receptors on the granulosa cells is of critical importance. LH secretion helps to maintain high intercellular levels of cAMP in the follicle (Driancourt, 1991). The granulosa cells aromatize more androgen to estradiol. This estradiol feeds back and causes GnRH release from the hypothalamus and LH release from the pituitary.

Estrogen and inhibin produced by the dominant follicle can decrease the amount of FSH released by the anterior pituitary by reducing expression of the genes which code

for the FSH β–subunit (Roche, 1996). Reduction of FSH secretion suppresses growth of other follicles in the developing cohort. As these other follicles regress, the dominant follicle secretes less gonadotropin surge-attenuating factor (GnSAF) and more estradiol (Fowler and Templeton, 1996). GnSAF is a non-steroidal ovarian factor that reduces pituitary responsiveness to GnRH, calcium mobilization and protein kinase C activation.

Studies performed by Lussier and associates (1987) have determined that it takes 27 days for a follicle to grow from 0.13 to 0.67 mm, 6.8 days to grow from 0.68 to 3.67 mm, and 7.8 days to grow from 3.68 to 8.56 mm. In cattle, two estrous cycles are required for a follicle to grow from antrum formation to preovulatory size. Ovulatory sized follicles are $\geq$ 10 mm, as this is the size of bovine follicles when LH receptors develop on granulosa cells (Lucy et al., 1992).

Atresia

Atresia is the final destination for more than 99% of ovarian follicles. These follicles never ovulate, and thus are a source of ovarian wastage. Ovarian germ cells undergo at least six waves of degeneration during their ovarian development (Hsueh et al., 1994). Three of these stages involve atresia of the germ cells or oocytes and occur during the prenatal period. The remaining 3 stages of atresia are found in preantral, growing, and preovulatory follicles.

Apoptosis, commonly referred to as programmed cell death, is thought to be the underlying mechanism of atresia (Hsueh et al., 1994). Some of the degenerative changes that occur are cell shrinkage and chromatin condensation. Pycnotic cell bodies are often

observed in the granulosa cells of early atretic follicles. Granulosa cells are the primary site of follicular atresia (Billig et al., 1993). The theca and granulosa layers become increasingly fragmented, and gel electrophoresis analysis shows a ladder-like pattern of fragmented DNA. This is often considered the "hallmark" of apoptosis.

Many different chemical markers of healthy and atretic follicles have been identified. Ireland and Roche (1982) determined that healthy growing bovine follicles have a high intrafollicular ratio of estrogen: progesterone. High concentrations of androstenedione (Spicer et al., 1987), inhibin and activin (Roche, 1996) and IGFBP-4 (Erickson et al., 1994) are associated with atretic follicles. GnRH has been shown to be atretogenic in rats (Billig et al., 1994) and cattle (Macmillan and Thatcher, 1991). Administration of GnRH agonists can inhibit many of the follicular maturation events associated with LH and FSH. An increase in medium atretic follicles seen approximately 6 days after GnRH treatment may be due to a lack of FSH support (Twagiramungu et al., 1995). Treatment with a GnRH agonist can directly cause apoptosis in granulosa cells by increasing intracellular calcium in granulosa cells and inhibiting endonuclease activity (Billig et al., 1994).

Immunology may play a role in atresia as well. Activation of the complement system may be involved in atresia (Hirshfield, 1991a). Complement destroys infectious particles and can damage the integrity of granulosa cell membranes. Cytokines such as interleukin-6 and tumor necrosis factor α may play roles in stimulating atresia.

The Corpus Luteum

The corpus luteum (CL) is a transient endocrine organ (Niswender et al., 1994). In the nonpregnant animal, its regression allows ovulation of a dominant follicle. Ovarian follicles are not required to maintain the CL in sheep and cattle (unlike rabbits) (Karsch et al., 1970). Interovarian relationships exist between the CL and follicles (Dufour et al., 1972). Ginther and co-workers (1989a) have found that the CL has an effect on follicular quantity, but no effect on selection, development or regression of dominant follicles. A study by Daily et al. (1982) found no relationship between diameter of follicles and distance from the corpus luteum. This same study also found that the ovary bearing the corpus luteum contained a follicle with a greater diameter than the ovary without the CL. Local secretion and communication between cells may explain positive relationships between follicles and the CL (Pate, 1996).

In pregnant animals, large follicles were least apparent during early gestation in cattle and swine (Rexroad and Casida, 1975). Thatcher and associates (1989) found that large follicles were found more often on the ovary without the CL in early gestating animals. An increase in atresia of large follicles was observed as well. A decline in large, estrogen-active follicles provides a preferable environment for maintenance of the early pregnancy corpus luteum. This implies that estradiol from large follicles may be involved in luteal regression (Al-Gubory and Abdennebi, 1996).

GnRH Agonist Treatment

Numerous studies have been performed employing the GnRH agonist Buserelin to improve fertility in cattle (Macmillan et al., 1985; Macmillan and Thatcher, 1991; Rettmer et al., 1992a,b; Twagiramungu et al., 1992). GnRH does not work directly at the ovarian level, as no receptors for GnRH are located on the bovine ovary (Ireland et al., 1990). Thus, effects of GnRH on follicles or the CL are mediated through the release of LH and FSH (Twagiramungu et al., 1992). After release of these hormones, large follicles are ovulated and small and medium sized follicles are luteinized. This helps to shift the complexion of the follicular population to include more medium sized follicles. A second administration of the GnRH agonist results in a more uniform population of follicles for ovulation and an improved synchronization of estrus. A Buserelin-induced short-term increase in progesterone has been said to act as a "primer" to initiate normal cycles in postpartum, anestrous cows (Macmillan et al., 1985; Twagiramungu et al., 1992).

Dairy cows that were treated with a GnRH agonist 12 hours after the onset of estrus had only a limited effect on follicular stocks and the attributes of follicular waves (Pursley et al., 1993). Studies in dairy heifers by Rettmer and co-workers (1992a) found luteoprotective effects of a GnRH agonist. Follicular estrogen secretion was reduced and associated with enhanced luteal function. GnRH may have increased the ratio of large to small luteal cells in the CL (Rettmer et al., 1992). Nutrition can affect the response to a GnRH agonist, as response was optimal when beef heifers were supported on a high plane of nutrition (Leers-Sucheta et al., 1994).

Macmillan and Thatcher (1991) and Twariramungu et al. (1994) both reported an increase in the number of medium-sized follicles and atretic follicles after treatment with a GnRH agonist. Studies employing chronic GnRH agonist treatment showed a significant effect on dominant follicles of the next wave. Diameters of ovulatory follicles reached only to 7-9 mm due to suppression of pulsatile LH release (Gong et al., 1995). Pregnancy rates in beef heifers were improved by administering a GnRH agonist 11 to 14 days after insemination (Macmillan et al., 1985; Rettmer et al.,1992b). GnRH agonists can luteinize or induce ovulation of follicles; thus decreasing estrogen secretion. This decline in estrogen may prevent changes in uterine oxytocin receptor concentrations, delays luteolysis and allows maternal recognition of pregnancy to occur (Rettmer et al., 1992a).

Nutritional Effects on Reproduction

A common problem for the beef cow to overcome is a lack of energy intake to provide for maintenance, pregnancy, and lactation. Nutrition and suckling are two of the major factors which prevent resumption of normal estrous cycles during the postpartum period. By 15 days after calving, pituitary receptors for GnRH and estrogen are elevated (Nett et al., 1988). By Day 35 postpartum, the follicular populations are replenished and capable of ovulating (Dufour and Roy, 1985). The reestablishment of LH pulses is critical for a postpartum cow to return to cyclicity. Studies in the lactating rat have shown that serum LH was reduced and follicular maturation prevented due to high levels of serum prolactin or progesterone (Taya and Greenwald, 1982). Elevated levels of

prolactin may modify the return to cyclicity by reducing the responsiveness of the theca layer to LH (Weiss et al., 1981). Contrasting results exist for comparisons of energy restriction and progesterone concentrations. Caloric deficit can reduce progesterone secretion in lactating dairy cows (Villa-Godoy et al., 1988), non-lactating beef cows (Richards et al., 1989a) and lactating beef cows (Grimard et al., 1995). However, some studies detected no differences in progesterone concentrations of energy restricted beef cattle (Apgar et al., 1975; Spitzer et al., 1978; Schrick et al., 1990).

Body Condition Score and Body Weight

Body condition scoring is a critical instrument for cattle producers to monitor weight changes. Condition scores have been found to be correlated with reproductive performance (Lishman et al., 1979; Richards et al., 1986). Beef cows should calve at a BCS of 5-6 (on a scale of 1-9). Dairy cows should calve at a score of 3.5-4.0 (on a scale of 1-5). Dairy cows in poor body condition (1-2) had fewer antral follicles and poorer oocyte quality than cows with a BCS of 3-4 (Dominguez, 1995). Beef cattle in proper BCS had a higher proportion of healthy medium and large follicles (Ryan et al., 1994). Beef cattle losing weight before calving experience a delay returning to cyclicity. Every two pound decrease in weight extends the postpartum anestrous period (Dunn and Kaltenbach, 1980). Loss of one-half a body condition score or more delayed time to the first ovulation.

Percentage of body fat plays an important role in delaying cyclicity. Nonlactating cows and heifers losing weight have decreased luteal activity and fail to cycle (Richards

et al., 1989a). Beef cattle will stop cycling when their body fat content drops to 4.1 percent or less. Observed decreases in the frequency of LH pulses may be due to a lack of metabolic substrates available for the GnRH neurons in the hypothalamus. These animals have acute, decreased blood glucose levels. If this energy source is lacking, reproductive functions shut down in deference to more critical systems. Nonlactating cattle losing weight also have ovaries and corpora lutea which are reduced in weight (Rasby et al., 1991). Rasby and co-workers (1991) hypothesized that nutrients needed for maintenance of ovarian cell structure may be lacking.

Energy Restriction and Balance

Reduction in predicted energy balance can restrict the movement of follicles into the larger size categories (Lucy et al., 1991). Energy balance is a problem for high producing dairy and beef cattle. Maximum milk production usually occurs 2-4 weeks before maximum dry matter intake occurs. Nadir (maximum negative energy balance) occurs about 4 weeks after calving, with the first ovulation occurring 21 days after nadir. The dry period is a critical time for dairy cattle. Optimum condition prior to calving must be achieved so that the cow is prepared for the metabolic stress she is about to undergo. Growth of large follicles was shown to be delayed by lactation and suckling in beef cows (Williams, 1990). The early postpartum period of cattle is characterized by a low amplitude, low frequency secretion of LH.

Ovarian follicular maturational events are delayed in prepubertal beef heifers fed low energy diets due to a reduction in the number of LH pulses (Bergfield et al., 1994).

Studies performed by Imakawa and associates (1987) have shown that heifers maintained on low energy diets achieved puberty at a later age even though they had reached proper pubertal body weight. Primiparous cows are more sensitive to undernutrition than multiparous cows (Grimard et al., 1995). These animals are not producing as much milk during their first lactation, but have not yet reached their mature size (Senatore et al., 1996).

Insulin

Insulin stimulates granulosa cell mitosis. Insulin has been shown to induce expression of the c-fos and c-jun genes in the rat (Peluso et al.,1995), and these are key genes for initiating mitosis in granulosa cells. In the pig, insulin and FSH act synergistically to stimulate gap junction and microvilli formation, and enhance development of the mitochondria, golgi and endoplasmic reticulum (Amsterdam et al., 1988). Insulin has shown to be more mitogenic in granulosa cells than gonadotropin treatments (Peluso and Hirschel, 1987). The production of IGF-1 by cultured sheep follicles has been shown to be regulated by insulin (Wathes et al., 1995). Cows consuming a low energy diet often have lower levels of insulin (Schrick et al., 1992; Humblot and Grimard, 1996). Harrison and Randel (1986) showed that treatment with exogenous insulin increased ovulation rate in energy-restricted beef heifers. Insulin and IGF-1 have direct effects on ovarian follicles (Spicer et al., 1993) as these two hormones act together to stimulate estrogen production.

Effects of insulin reduction are not just seen at the follicular level. Low insulin

levels could act on the hypothalamus. Variations in blood glucose and insulin can affect the hypothalamic-hypophyseal-ovarian axis (Adashi et al., 1981). Glut-4 is an insulin induced glucose transporter in the hypothalamus. Since this area is where the GnRH neurons lie, reduced glucose transfer may affect the rate of GnRH secretion by these neurons.

Crude Protein Restriction and Excess

Beef cattle fed a diet lacking in crude protein prior to parturition have reduced pituitary contents of gonadotropin and do not respond well to GnRH (Nolan et al., 1988). Feeding excess crude protein to dairy cows can elevate plasma urea nitrogen. Increases in urea nitrogen have been correlated with poor fertility (Ferguson et al., 1988). Excess amounts of degradable intake protein can elevate concentrations of urea, ammonia and other nitrogenous compounds in body tissues (Ferguson and Chalupa, 1989). These substances can affect intermediary metabolism, corpus luteum function (Garwacki et al., 1979), cause toxic effects on oocytes (Umezaki and Fordney-Settlage, 1975) and early embryos, and reduce first service to conception rates (Canfield et al., 1990).

Increases in ammonia production could overload the detoxification capacity of the liver. Treacher and co-workers (1976) saw leakage of hepatic enzymes in high-producing dairy cattle consuming high protein diets. High tissue concentrations of ammonia can reduce the affects of macrophages and leucocytes. Detrimental effects of excess crude protein could possibly be due to an unfavorable uterine pH for embryo development (Elrod and Butler, 1993). Excess quantities of amino acids may have a negative affect on

reproduction via indirect effects on nutrient partitioning and energy balance (Ferguson and Chalupa, 1989).

3. Statement of the Problem

Various studies have attempted to identify traits of reproductively superior cows. Physiology of the hypothalamo-pituitary-ovarian axis affects many determinants of the reproductive capacity of the bovine female. Follicular growth depends on endocrine signals from the hypothalamus, pituitary and ovary. Studies by Fortune (1994), Spicer and Echternkamp (1986), Roche (1996) and Scarramuzzi and associates (1993) have related hormone concentrations to each stage of follicular development.

Histological studies by Erickson (1966, 1967), Erickson and associates (1976) and Monniaux and co-workers (1984) have demonstrated that the number of primary, growing and vesicular follicles is a highly variable trait in the bovine. A cow's chance for successful conception improves if she has a large population of follicles from which to develop and ovulate a single dominant follicle. Large quantities of follicles are not enough to ensure reproductive viability. Follicular quality is just as important as follicular quantity. Relationships between ovarian steroids and pituitary gonadotropins may prove to be useful indicators of bovine follicular populations and health. Large populations of primary and growing follicles may be beneficial for a high level of reproductive productivity. Rowland (1994) detected significant correlations of basal FSH and estradiol concentrations to follicular populations.

The degree to which hormonal, metabolic and nutritional variables are usefully related to ovarian structures has shown variable results. Identifying relationships between

these factors in the cyclic beef cow may help producers identify reproductively inferior animals so that they may be culled from their herd. The objective of this study was to relate histologically determined numbers of primary, growing and normal and atretic vesicular follicles with common reproductive, metabolic and nutritional parameters in the cyclic beef cow.

4. MATERIALS AND METHODS

Animals

Data were collected in a two-year study on forty-two cyclic Polled Hereford cattle. Twenty animals were evaluated in 1993 (**Year 1**) and twenty-two in 1994 (**Year 2**). The animal population consisted of 10 four-year old cows, 18 three-year old cows and 14 two-year old cows. Thirty-two cows were in various stages of lactation and ten animals were nonlactating. All animals were cycling and considered reproductively sound. Cows were maintained at the Tobacco Experiment Station in Greenville, Tennessee. Animals grazed fescue-based pastures, fed a soybean meal protein supplement and experienced an average daily gain of approximately 0.70 kg.

Treatment Procedure

In **Year One**, cows were at random stages of the estrous cycle. Cows were injected intramuscularly with 25 mg of prostaglandin $F_{2\alpha}$ (5 ml of Lutalyze; Upjohn Co., Kalamazoo, MI), and bled via jugular venipuncture (experimental Day 0). On Day 3 of the experiment, 10 μg (2 ml) of des-Gly 10 [D-Ala6]-Luteinizing Hormone Releasing Hormone Ethylamide (Sigma Diagnostics, catalog number L-4513FD, St. Louis, MO) were administered subcutaneously to each animal. This GnRH agonist is equivalent to the GnRH agonist "Buserelin" in potency. Jugular blood samples were collected at 0, 1.5, and 3 h post-injection to determine pituitary responsiveness. Cows received 25 mg

of prostaglandin $F_{2\alpha}$ on Day 11 and two blood samples separated by one hour were collected at the time of injection. Four days after the second $PGF_{2\alpha}$ injection (Day 15), 10 μg (2 ml) of the GnRH agonist were subcutaneously injected and jugular blood samples were collected three times at 0, 1.5, and 3 h after injection. Two days after GnRH treatment (Day 17), cows were bled twice at a one hour interval. Measurements of plasma progesterone were used to establish the quality of the early corpus luteum. Six days later (Day 23), two more jugular bleedings at one hour intervals were collected. Ovariectomy surgeries were performed at this time.

In experimental **Year 2**, 10 μg (2 ml) of the GnRH agonist were injected subcutaneously (experiment Day 0) and cows bled via jugular venipuncture 0, 2, and 4 h later. All animals were at random stages of their estrous cycle. On Day 7 of the experiment, 25 mg of prostaglandin $F_{2\alpha}$ (5 ml of Lutalyze) were injected intramuscularly. Jugular blood samples were obtained 0, 2 and 4 h later. Day of the estrous cycle was determined by concentrations of E_2 and P_4 after estrous synchronization. Cows were bled via jugular venipuncture twice at two hour intervals on Days 10, 11 and 12. Ten μg (2 ml) of GnRH were subcutaneously injected and jugular samples were collected at 0, 2 and 4 h later on Day 14. Cows were bled once and ovariectomized five days later (Day 19).

Histological Procedure

All cows were bilaterally ovariectomized via an incision made in the paralumber fossa of the left side under local and spinal anesthesia. Upon removal from the cow,

whole ovaries were fixed in Bouin's solution, which consists of acetic acid (100 ml), formalin (500 ml) and saturated Picric acid (1500 ml). Before fixation, ovarian width was measured (mm) and ovaries were sliced into quarters. These quarters were then fixed, washed in ethanol and toluene and embedded in paraffin in preparation for sectioning. Two 10 μm sections were prepared 100 μm apart from the uncut surfaces of the outer two segments of the ovary and from the center of the middle two ovarian segments.

Number and size of corpora lutea, and counts of primary, growing and vesicular follicles were determined by light microscopic examination. Primary follicles were classified by the presence of an oocyte surrounded by a single layer of granulosa cells. Growing follicles were identified by an oocyte with two or more complete layers of granulosa cells. To prevent spuriously high counts, primary and growing follicles were counted when at least a fraction of the oocyte was visible. Vesicular follicles were defined by the presence of an antrum and categorized as normal or atretic. Normal vesicular follicles were considered to have healthy granulosa layers, while follicles with absent granulosa layers or granulosa cells with at least ten percent of pycnotic cells were considered atretic. Diameter of healthy and atretic vesicular follicles was determined at the widest point of the follicle. Measurements were taken from the base of the granulosa layers. Diameter measurements allowed follicles to be classified by size into small (<3mm), medium (3-5mm) and large (>5mm) groups. Vesicular follicles were counted and measured in one slide from each quarter, unless a particular follicle was present in more than one quarter. An average was taken from the counts of primary and growing

follicles in the eight 10 μm sections from each ovary. This average was multiplied by the total number of 10 μm sections possible for that ovary.

A subset of eleven cows expressing a primary and secondary (GnRH stimulated) CL were randomly selected from both years. These animals were used to determine possible luteal type influence on measurements of follicular health, number and distance. Three cows were selected from Year 1, and 8 from Year 2. The ovarian section displaying the largest dimension of the CL was selected for analysis. All measurements were taken from the center of the CL. In terms of primary follicle location, a section roughly equivalent to 1/3 of the ovary was evaluated.

Radioimmunoassays

Follicle stimulating hormone (FSH) was measured by a procedure similar to one employed by Bolt and Rollins (1983). USDA-bFSH-1-2 provided by Dr. D. G. Bolt (USDA, Beltsville, Maryland) was used as the reference preparation. Assay sensitivity was 0.25 ng/ml with intra- and interassay coefficient of variation of < 7 % and <12%, respectively.

Concentrations of luteinizing hormone (LH) were determined by a procedure described by Brown and co-workers (1983). The reference preparation USDA-bLH-B-5 was donated by Dr. L. Reichert. Sensitivity of the assay was 31 pg/ml with intra- and interassay coefficients of variation of <7% and <10%, respectively.

A double-antibody assay technique described by Bolt and Rollins (1983) was used to determine concentrations of serum prolactin. Assay sensitivity was 0.05 ng/ml with

intra- and interassay coefficients of variation <5% and <10%, respectively.

Plasma insulin concentrations were determined with a solid-phase insulin radioimmunoassay kit (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA). Intra- and interassay coefficients of variation were <4% and <20%, respectively. Sensitivity of the assay was 0.21 ng/ml.

Progesterone concentrations were quantified using a modification of the procedure described by Jackson and co-workers (1989). Sensitivity of the P_4 assay was 0.10 ng/ml, with intra- and interassay coefficients of variation of <5% and <10%, respectively.

Concentrations of 17β-estradiol were determined by using a procedure described by Britt (1990). Assay sensitivity was 0.15 pg/ml with intra and interassay coefficients of variation of <8% and <15%, respectively.

Plasma androstenedione concentrations were determined with a solid phase androstenedione radioimmunoassay kit (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA). Sensitivity of the assay was 0.03 ng/ml.

Blood Urea Nitrogen

Blood urea nitrogen was determined by a colormetric procedure (Sigma Diagnostics, catalog number 535, St. Louis, MO). Urease hydrolyzed the combination of urea and diacetyl monoxime into ammonia. Ammonia concentrations were colormetrically determined at a spectrophotometer absorbance of 535 nm.

Statistical Analysis

Data collected from both years were analyzed separately, due to poor response to estrous synchronization in the second experimental year. All cows in Year 1 were in luteal phase (Day 9) when ovariectomized, however cows in Year 2 were on Day 5 (n=4), Day 9 (n=14) or Day 13 (n=4). Correlations and regressions were determined by the statistical package of SAS (SAS institute, 1985). Means, least square means, standard errors of means and standard deviations were determined by the General Linear Model (proc GLM) procedure of SAS. Animals were divided into low, medium and high groups for each analyzed variable. An attempt was made to place an even number of animals in each group, whenever possible.

Relationships between follicular populations and corpus luteum type were analyzed with a split-plot type design, employing the Mixed procedure (proc MIXED) of SAS. Wholeplot treatments consisted of a factorial with lactation status and cow age. Subplot treatments contained the corpus luteum type (primary and secondary).

5. RESULTS

Experimental Year One

Primary Follicles

Weaning weight (WWT; recorded at 7-9 months of age) of experimental cows separated into low ($\bar{x}$ =192.5 kg; n=5), medium ($\bar{x}$ =225 kg; n=9) and high ($\bar{x}$ =248.5 kg; n=5) groups tended to influence numbers of primary follicles (*P*=0.07). Ovaries removed from medium WWT cows contained more follicles than high group cows (220, 636 $\pm$ 41, 700 vs. 58, 138 $\pm$ 55, 947; *P*=0.03); however, number of primary follicles for medium cows did not differ from the low group (110, 794 $\pm$ 55, 947). Follicle numbers were not different when compared on the basis of age (199, 905 $\pm$ 77, 146), lactation status (199, 905 $\pm$ 64, 070) and days postpartum (178, 067 $\pm$ 78, 413).

Number of primary follicles correlated with prolactin (PRL) (r=0.49; *P*=0.06) in the lactating group. Linear regression of primary follicles and PRL concentrations provided the r-square value of 0.24 (*P*=0.06; scatter plot in appendix). Primary follicle counts for nonlactating cows correlated negatively with concentrations of follicle stimulating hormone (FSH; r= -0.92; *P*=0.02).

Growing Follicles

Number of growing follicles differed (*P*=0.04) when age and WWT were

evaluated without lactational status. Four-year old animals had fewer follicles (7, 576 ± 2, 613) than 3- (18, 351 ± 2, 003; *P*=0.006) and 2-year old cows (16, 757 ± 3, 783). Ovaries from low WWT cows contained fewer (9, 652 ± 2, 540) growing follicles than high (17, 858 ± 2, 773; *P*=0.05) and tended to have fewer follicles compared to medium cows (15, 891 ± 1, 915; *P*=0.07). Interactions among ages, lactational status and WWT tended (*P*=0.09) to influence populations of growing follicles. Follicle numbers and significance probabilities for age and WWT were similar to those mentioned above; however, status had no quantitative effect on growing follicle numbers (14, 721 ± 2, 634).

Growing follicle numbers differed (*P*=0.04) when blood urea nitrogen concentration interacted with lactational status. Cows with low BUN values ($\bar{x}$ =13.8 mg/dl; n=3) had increased growing follicle counts (21, 737 ± 8, 582) compared to high BUN group cows ($\bar{x}$ =23.5 mg/dl; n=5; 8,714 ± 2, 665; *P*=0.008) and tended to have more than medium BUN cows ($\bar{x}$ =17.8 mg/dl; n=12; 14, 381 ± 1, 920; *P*=0.07). Growing follicle populations did not differ based on days postpartum (13, 896 ± 3, 122) for experimental cows.

Correlations were detected between growing follicles, size of the primary corpus luteum (CL) (r=0.50; *P*=0.06) and PRL concentrations (r=0.54; *P*=0.04) in lactating cows. Primary CL size and growing follicle counts were analyzed by linear regression, and resulted in the R^2 value of 0.25. Linear regression of growing follicles and PRL concentration yielded an R^2 value of 0.29 (*P*=0.03; scatter plot in appendix). Nonlactating cows yielded positive correlations between growing follicles, WWT

(r=0.88; *P*=0.04) and estradiol-17β (E_2) concentrations (r=0.88; *P*=0.04), while growing follicles were inversely related to BUN values (r= -0.89; *P*=0.04).

Vesicular Follicles

Vesicular follicles differed (*P*=0.03) when age, lactational status and birth weight (BWT) of the cow were analyzed. Counts for 2-year old cows (147.0 ± 26.4) were different from 3-year olds (82.7 ± 13.0; *P*=0.03) and tended to be different from 4-year old cows (97.8 ± 13.7; *P*=0.09). Cows of higher BWT ($\bar{x}$ =40.5 kgs; n=2) had more follicles (158.4 ± 25.2) than medium ($\bar{x}$ =35 kgs; n=10; 82.6 ± 11.5; *P*=0.01) and low BWT cows ($\bar{x}$ =30 kgs; n=8; 158.4 ± 25.2; *P*=0.01). Lactational status and days postpartum had no influence on vesicular follicle quantities (Table 1.1).

Vesicular follicles correlated with size of the secondary CL ($\bar{x}$ =5.2 mm; n=4; r= -0.62; *P*=0.01) in lactating cows. Nonlactating cows exhibited positive correlations between vesicular follicles, WWT (r=0.89; *P*=0.04), and E_2 concentrations (r=0.86; *P*=0.06) and a negative correlation with FSH concentrations (r= -0.87; *P*=0.05).

Age, lactational status and WWT influenced quantities of **small vesicular follicles** (*P*=0.05). Cows with medium WWT had 89.5 ± 10.7 small follicles, which tended to differ (*P*=0.08) from high (58.1 ± 16.2), but not low WWT (56.3 ± 15.9). Two-year old cows had more small vesicular follicles (102.6 ± 20.3) than 3- (50.9 ± 10.7; *P*=0.03) and 4-year olds (50.3 ± 14.0; *P*=0.05). No difference was detected between small follicle counts of lactating and nonlactating cows (67.9 ± 12.3).

Table 1.1. Least Square Means ± Standard Errors for Total Vesicular and Total Normal and Atretic Follicles for Two-, Three- and Four-Year Old Lactating and Nonlactating Cows Ovariectomized **Year 1**.

Variable	Normal	Atretic	Total
Age	43.7 ± 9.5	47.7 ± 10.1	91.4 ± 19.1
Status	43.8 ± 15.8	47.7 ± 8.4	91.4 ± 15.9
Days Postpartum	39.9 ± 9.5	43.3 ± 10.3	83.2 ± 19.4

Number of small vesicular follicles and size of the secondary CL (r= -0.61; *P*=0.01) correlated in lactating animals. Nonlactating cows exhibited a positive correlation between small follicles and WWT (r=0.93; *P*=0.02), while FSH (r= -0.83; *P*=0.07) and E_2 (r= -0.83; *P*=0.07) were inversely related to small follicles.

Medium vesicular follicle counts were influenced (*P*=0.02) by interactions among ages, lactational status and body weight prior to ovariectomy (OWT). Cows of high OWT ($\bar{x}$ =530 kgs; n=7) possessed more medium follicles (20.6 $\pm$ 4.4) than medium OWT ($\bar{x}$ =440 kgs; n=7; 7.4 $\pm$ 4.2; *P*=0.01) and did not differ from low ($\bar{x}$ =377.5 kgs, n=6; 12.8 $\pm$ 4.2). Ovaries of lactating cows had fewer medium follicles (7.0 $\pm$ 3.4) than nonlactating cows (20.2 $\pm$ 4.2; *P*=0.02), while follicle counts for the three ages were similar (13.5 $\pm$ 5.0).

Lactating cows with lower BUN values had more medium vesicular follicles (r= -0.70; *P*=0.003). Regression of medium vesicular follicles and BUN provides the R^2 of 0.49 (*P*=0.003; scatter plot in appendix). Nonlactating cows exhibited a negative correlation between medium follicles and FSH concentrations (r= -0.87; *P*=0.05), while a positive correlation exists between this follicular class and E_2 concentrations (r=0.87; *P*=0.05).

As was the scenario with medium vesicular follicles, age, status and OWT affected (*P*=0.04) populations of **large vesicular follicles.** Medium OWT cows had fewer (-0.1 $\pm$ 0.4) follicles than low (1.5 $\pm$ 0.4; *P*=0.02) or high (1.0 $\pm$ 0.4; *P*=0.02) OWT cows. Two-year old cows had (0.7 $\pm$ 0.7) follicles, which differed from 3- (1.1 $\pm$ 0.3; *P*=0.04) and 4-year old (2.0 $\pm$ 0.4; *P*=0.01). Lactation status had no measurable effect on counts

of large vesicular follicles (0.9 ± 0.4).

Large vesicular follicles exhibited a negative correlation with BUN (r= -0.57; *P*=0.02) for lactating cows. Linear regression of large vesicular follicles and BUN provided the R^2 value of 0.32 (*P*=0.02; scatter plot in appendix). Analyses of nonlactating cows yielded correlations between large vesicular follicles, age (r=0.82; *P*=0.09) and PRL concentrations (r=0.97; *P*=0.007), while large follicles were inversely related to yearling weight (YWT; r= -0.88; *P*=0.04).

Normal Vesicular Follicles

Lactational status, age and BWT of the cow influenced (*P*=0.03) quantities of **normal vesicular follicles.** Ovaries of high BWT cows contained more normal follicles (78.7 ± 12.1) than low (43.9 ± 7.6; *P*=0.01) and medium (38.1 ± 5.5; *P*=0.006) BWT cows. Two-year old cows had more normal follicles (73.0 ± 12.7) than 3-year old (40.2 ± 6.2; *P*=0.02), and tended to have more than 4-year old cows (47.5 ± 6.6; *P*=0.07). Normal follicle counts were unaffected by status or days postpartum (Table 1.1).

Size of the primary CL tended (*P*=0.06) to interact with age, lactational status and WWT to affect normal vesicular follicles. Cows with a medium CL ($\bar{x}$ =12.5 mm; n=8) had more normal vesicular follicles (50.8 ± 6.9) than large CL cows ($\bar{x}$ =15.3 mm; n=5; 24.2 ± 7.6; *P*=0.02), while follicle numbers for small CL cows (9.3 mm; n=6) (43.8 ± 10.5) did not differ. Two-year old cows had more normal follicles (56.3 ± 11.9) than 3-year olds (26.6 ± 6.6; *P*=0.03), while counts for 4-year olds did not differ

(35.9 ± 8.3). Lactational status (Table 1.1) and WWT (39.62 ± 8.5) had no effect on numbers of normal follicles.

Counts of normal vesicular follicles correlated with size of the secondary CL (r=-0.58; *P*=0.02) and basal PRL concentrations (r=0.50; *P*=0.06) in lactating cows. Linear regression analysis of normal vesicular follicles versus PRL revealed the r-square of 0.25 (*P*=0.06; scatter plots in appendix). Nonlactating cows exhibited positive correlations between normal follicles, WWT (r=0.96; *P*=0.01), and E_2 concentrations (r=0.89; *P*=0.04), while basal LH concentrations (r= -0.82; *P*=0.09) were inversely related to normal follicles.

Lactational status, age, WWT and size of the primary CL interacted to affect (*P*=0.03) quantities of **small normal vesicular follicles.** High WWT cows tended to have fewer follicles (27.9 ± 8.1) than medium cows (43.7± 4.6; *P*=0.08) while counts for low cows (33.3 ± 7.4) were not different. Cows with a large primary CL had fewer follicles (21.6 ± 6.0) than cows with medium CL (44.7 ± 5.4; *P*=0.01); however, follicle numbers for small CL cows (38.7 ± 8.3) did not differ. Two-year old cows had more small normal follicles (*P*=0.009) than 3-year olds (51.3 ± 9.4 vs. 20.4 ± 5.2); however, no difference was detected for lactational status (Table 1.2).

Small normal follicles exhibited a negative correlation with size of the secondary CL (r= -0.55; *P*=0.03) and a positive correlation with PRL concentrations (r=0.47; *P*=0.07) in lactating cows. Regression of small normal follicles and PRL provided the R^2 of 0.22 (*P*=0.07; scatter plot in appendix). Nonlactating cows exhibited positive

Table 1.2. Least Square Means ± Standard Errors for Small, Medium and Large Normal and Atretic Vesicular Follicles for Two-, Three- and Four-Year Old Lactating and Nonlactating Cows Ovariectomized **Year 1**.

Variable	Small	Medium	Large
Normal			
Age	38.5 ± 11.4	4.9 ± 2.3	0.4 ± 0.3
Status	38.5 ± 6.5	4.9 ± 2.0	0.4 ± 0.3
Days Postpartum	35.0 ± 7.7	4.6 ± 2.4	0.4 ± 0.3
Atretic			
Age	38.5 ± 8.0	8.7 ± 8.2	0.5 ± 0.5
Status	38.5 ± 6.6	8.7 ± 2.3	0.6 ± 0.4
Days Postpartum	35.4 ± 8.1	7.1 ± 2.8	0.6 ± 0.5

correlations between small follicles, WWT (r=0.98; *P*=0.002), and E_2 concentrations (r=0.84; *P*=0.07), while LH concentrations (r= -0.87; *P*=0.05) exhibited a negative correlation with small normal follicles.

Differences (*P*=0.04) were detected in quantities of **medium normal vesicular follicles** when lactational status, OWT, BUN and insulin concentrations interact. Cows with low BUN concentrations had more (9.3 $\pm$ 2.6) medium normal follicles than high cows (1.7 $\pm$ 2.1; *P*=0.04), while follicle numbers for medium BUN cows (4.4 $\pm$ 1.5) not different. High OWT cows had more follicles (7.8 $\pm$ 1.5) than medium OWT cows (2.3 $\pm$ 1.7; *P*=0.02), while medium follicle counts were similar for low OWT cows (5.3 $\pm$ 2.1). No differences between follicle counts were detected for either insulin concentrations (5.2 $\pm$ 1.8) or status (Table 1.2).

Negative correlations were distinguished between medium normal follicles and BUN concentrations (r= -0.64; *P*=0.01) in lactating beef cattle. When medium normal follicles and BUN were regressed, an R^2 of 0.41 resulted (*P*=0.01; scatter plot in appendix). Medium normal follicles correlated with WWT (r=0.82; *P*=0.08) and basal E_2 concentrations (r=0.93; *P*=0.02) in nonlactating cows.

Numbers of **large normal vesicular follicles** tended (*P*=0.09) to be influenced by OWT and progesterone (P_4). Cows with high concentrations of P_4 ($\bar{x}$ =6.0 ng/ml; n=7) had fewer large normal follicles (0.1 $\pm$ 0.2) than medium ($\bar{x}$ =4.3 ng/ml; n=8; 0.7 $\pm$ 0.2; *P*=0.04) and low P_4 cows ($\bar{x}$ =3.0 ng/ml; n=5; 0.8 $\pm$ 0.3; *P*=0.05). Animals with high OWT had more large normal follicles (1.0 $\pm$ 0.2) than cows with low (0.3 $\pm$ 0.2; *P*=0.03) or medium (0.3 $\pm$ 0.2; *P*=0.03) OWT.

Correlations were identified between large normal vesicular follicles and follicle classes described previously (Tables 1.3-1.8) in lactating and nonlactating cows. Nonlactating cows displayed a negative correlation between large normal vesicular follicles and BWT (r= -0.87; *P*=0.05).

Size of the **largest normal vesicular follicle** was affected by lactational status, age and LH concentrations (*P*=0.007). Cows with high basal LH concentrations ($\bar{x}$ =2.2 ng/ml; n=8) had smaller follicles (3.4 $\pm$ 0.7 mm) than cows with low LH ($\bar{x}$ =0.7 ng/ml; n=8; 6.1 $\pm$ 0.6 mm; *P*=0.003) and medium LH ($\bar{x}$ =0.9 ng/ml; n=4; 7.6 $\pm$ 0.8 mm; *P*=0.001). Lactating cows had larger follicles (7.1 $\pm$ 0.5 mm) than nonlactating cows (4.3 $\pm$ 0.7 mm; *P*=0.004). Ovaries from 3-year olds contained a larger normal follicle (6.8 $\pm$ 0.6 mm) than 4-year old cows (4.9 $\pm$ 0.6 mm; *P*=0.03), while follicle size was not different for 2-year old cows (5.3 $\pm$ 1.0 mm).

Lactating animals exhibited a correlation between the size of the largest normal follicle and large normal follicles (r=0.75; *P*=0.001), while no significant correlations were detected for this variable in nonlactating animals.

Atretic Vesicular Follicles

Lactational status and age tended (*P*=0.07) to influence quantities of **atretic vesicular follicles**. Lactating cows had fewer (35.2 $\pm$ 7.0) atretic follicles than nonlactating (60.2 $\pm$ 9.8; *P*=0.04) cows. No differences were detected among the least square means for all three age groups (Table 1.1).

Birth weight, age and status influenced (*P*=0.03) atretic follicle counts. High

Table 1.3. Correlation Coefficients Between Primary and Growing Follicles and Various Follicle Classes in Lactating Cows Ovariectomized **Year 1**.[a, b]

	TPF	TGF
TVF	0.65 (0.008)	0.17 (0.53)
TNVF	0.61 (0.01)	0.20 (0.48)
TAVF	0.68 (0.005)	0.13 (0.64)
SVF	0.67 (0.005)	0.15 (0.60)
SNVF	0.62 (0.01)	0.17 (0.54)
SAVF	0.71 (0.003)	0.11 (0.71)
MVF	0.14 (0.59)	0.21 (0.45)
MNVF	0.08 (0.78)	0.23 (0.41)
MAVF	0.13 (0.64)	0.05 (0.86)
LVF	0.02 (0.94)	0.02 (0.95)
LNVF	0.29 (0.28)	0.03 (0.92)
LAVF	-0.26 (0.34)	-0.11 (0.70)

a, parenthesis indicates p-value, b, TPF=total primary follicles, TGF= total growing follicles, TVF= total vesicular follicles, TNVF= total normal vesicular follicles, TAVF= total atretic vesicular follicles, SVF= small vesicular follicles, SNVF= small normal vesicular follicles, SAVF= small atretic vesicular follicles, MVF= medium vesicular follicles, MNVF= medium normal vesicular follicles, MAVF= medium atretic vesicular follicles, LVF= large vesicular follicles, LNVF= large normal vesicular follicles, LAVF= large atretic vesicular follicles.

Table 1.4. Correlation Coefficients Between Total Vesicular, Total Normal and Atretic Vesicular Follicles and Various Follicle Classes in Lactating Cows Ovariectomized **Year 1**.[a, b]

	TVF	TNVF	TAVF
TPF	0.65 (0.008)	0.61 (0.01)	0.68 (0.005)
TVF	N/A	0.98 (0.0001)	0.98 (0.0001)
TNVF	0.99 (0.0001)	N/A	0.94 (0.0001)
TAVF	0.98 (0.0001)	0.94 (0.0001)	N/A
SVF	0.99 (0.0001)	0.98 (0.0001)	0.97 (0.0001)
SNVF	0.97 (0.0001)	0.99 (0.0001)	0.93 (0.0001)
SAVF	0.97 (0.0001)	0.93 (0.0001)	0.99 (0.0001)
MVF	0.51 (0.05)	0.50 (0.05)	0.50 (0.05)
MNVF	0.36 (0.18)	0.36 (0.19)	0.35 (0.19)
MAVF	0.51 (0.05)	0.49 (0.06)	0.50 (0.05)
LVF	0.09 (0.75)	0.09 (0.76)	0.08 (0.76)
LNVF	0.26 (0.34)	0.23 (0.41)	0.31 (0.26)
LAVF	-0.16 (0.56)	-0.14 (0.62)	-0.19 (0.48)

a, parenthesis indicates p-value, b, TPF=total primary follicles, TGF= total growing follicles, TVF= total vesicular follicles, TNVF= total normal vesicular follicles, TAVF= total atretic vesicular follicles, SVF= small vesicular follicles, SNVF= small normal vesicular follicles, SAVF= small atretic vesicular follicles, MVF= medium vesicular follicles, MNVF= medium normal vesicular follicles, MAVF= medium atretic vesicular follicles, LVF= large vesicular follicles, LNVF= large normal vesicular follicles, LAVF= large atretic vesicular follicles.

Table 1.5. Correlation Coefficients Between Small Vesicular, Small Normal and Small Atretic Vesicular Follicles and Various Follicle Classes in Lactating Cows Ovariectomized **Year 1**.[a, b]

	SVF	SNVF	SAVF
TPF	0.67 (0.005)	0.62 (0.01)	0.71 (0.003)
TVF	0.99 (0.0001)	0.97 (0.0001)	0.97 (0.0001)
TNVF	0.98 (0.0001)	0.99 (0.0001)	0.93 (0.0001)
TAVF	0.97 (0.0001)	0.93 (0.0001)	0.99 (0.0001)
SVF	N/A	0.98 (0.0001)	0.98 (0.0001)
SNVF	0.98 (0.0001)	N/A	0.93 (0.0001)
SAVF	0.98 (0.0001)	0.93 (0.0001)	N/A
MVF	0.37 (0.17)	0.37 (0.18)	0.38 (0.16)
MNVF	0.22 (0.43)	0.21 (0.45)	0.23 (0.41)
MAVF	0.38 (0.15)	0.38 (0.16)	0.39 (0.14)
LVF	-0.04 (0.88)	-0.03 (0.91)	-0.03 (0.90)
LNVF	0.19 (0.49)	0.14 (0.60)	-0.08 (0.76)
LAVF	-0.25 (0.36)	-0.21 (0.46)	-0.28 (0.30)

a, parenthesis indicates p-value, b, TPF=total primary follicles, TGF= total growing follicles, TVF= total vesicular follicles, TNVF= total normal vesicular follicles, TAVF= total atretic vesicular follicles, SVF= small vesicular follicles, SNVF= small normal vesicular follicles, SAVF= small atretic vesicular follicles, MVF= medium vesicular follicles, MNVF= medium normal vesicular follicles, MAVF= medium atretic vesicular follicles, LVF= large vesicular follicles, LNVF= large normal vesicular follicles, LAVF= large atretic vesicular follicles.

Table 1.6. Correlation Coefficients Between Primary and Growing Follicles and Various Vesicular Follicle Classes in Nonlactating Cows Ovariectomized **Year 1**.[a, b]

	TPF	TGF
TVF	0.80 (0.10)	0.79 (0.11)
TNVF	0.66 (0.22)	0.91 (0.03)
TAVF	0.88 (0.05)	0.66 (0.23)
SVF	0.77 (0.12)	0.78 (0.11)
SNVF	0.68 (0.21)	0.87 (0.05)
SAVF	0.82 (0.08)	0.69 (0.20)
MVF	0.77 (0.12)	0.78 (0.11)
MNVF	0.60 (0.28)	0.91 (0.02)
MAVF	0.87 (0.05)	0.63 (0.25)
LVF	0.44 (0.45)	-0.55 (0.33)
LNVF	0.09 (0.88)	-0.43 (0.47)
LAVF	0.53 (0.35)	-0.54 (0.34)

a, parenthesis indicates p-value, b, TPF=total primary follicles, TGF= total growing follicles, TVF= total vesicular follicles, TNVF= total normal vesicular follicles, TAVF= total atretic vesicular follicles, SVF= small vesicular follicles, SNVF= small normal vesicular follicles, SAVF= small atretic vesicular follicles, MVF= medium vesicular follicles, MNVF= medium normal vesicular follicles, MAVF= medium atretic vesicular follicles, LVF= large vesicular follicles, LNVF= large normal vesicular follicles, LAVF= large atretic vesicular follicles.

Table 1.7. Correlation Coefficients Between Total Vesicular, Total Normal and Atretic Vesicular Follicles and Various Follicle Classes in Nonlactating Cows Ovariectomized **Year 1**.[a, b]

	TVF	TNVF	TAVF
TVF	N/A	0.96 (0.006)	0.98 (0.003)
TNVF	0.97 (0.006)	N/A	0.90 (0.03)
TAVF	0.98 (0.003)	0.90 (0.03)	N/A
SVF	0.99 (0.001)	0.97 (0.007)	0.96 (0.008)
SNVF	0.96 (0.009)	0.99 (0.001)	0.90 (0.03)
SAVF	0.98 (0.003)	0.92 (0.02)	0.99 (0.001)
MVF	0.95 (0.01)	0.91 (0.02)	0.94 (0.01)
MNVF	0.92 (0.02)	0.95 (0.01)	0.85 (0.06)
MAVF	0.92 (0.02)	0.84 (0.07)	0.95 (0.01)
LVF	-0.16 (0.79)	-0.32 (0.59)	-0.03 (0.96)
LNVF	-0.38 (0.52)	-0.40 (0.50)	-0.31 (0.55)
LAVF	-0.05 (0.93)	-0.25 (0.68)	0.11 (0.85)

a, parenthesis indicates p-value, b, TPF=total primary follicles, TGF= total growing follicles, TVF= total vesicular follicles, TNVF= total normal vesicular follicles, TAVF= total atretic vesicular follicles, SVF= small vesicular follicles, SNVF= small normal vesicular follicles, SAVF= small atretic vesicular follicles, MVF= medium vesicular follicles, MNVF= medium normal vesicular follicles, MAVF= medium atretic vesicular follicles, LVF= large vesicular follicles, LNVF= large normal vesicular follicles, LAVF= large atretic vesicular follicles.

Table 1.8. Correlation Coefficients Between Small Vesicular, Small Normal and Small Atretic Vesicular Follicles and Various Follicle Classes in Nonlactating Cows Ovariectomized **Year 1**.[a, b]

	SVF	SNVF	SAVF
SVF	N/A	0.98 (0.003)	0.99 (0.001)
SNVF	0.98 (0.003)	N/A	0.93 (0.02)
SAVF	0.99 (0.001)	0.93 (0.02)	N/A
MVF	0.89 (0.04)	0.86 (0.06)	0.89 (0.04)
MNVF	0.87 (0.05)	0.89 (0.04)	0.83 (0.08)
MAVF	0.86 (0.05)	0.79 (0.11)	0.89 (0.04)
LVF	-0.23 (0.71)	-0.33 (0.59)	-0.14 (0.82)
LNVF	-0.43 (0.47)	-0.39 (0.50)	-0.44 (0.46)
LAVF	-0.12 (0.85)	-0.26 (0.67)	-0.004 (0.99)

a, parenthesis indicates p-value, b, TPF=total primary follicles, TGF= total growing follicles, TVF= total vesicular follicles, TNVF= total normal vesicular follicles, TAVF= total atretic vesicular follicles, SVF= small vesicular follicles, SNVF= small normal vesicular follicles, SAVF= small atretic vesicular follicles, MVF= medium vesicular follicles, MNVF= medium normal vesicular follicles, MAVF= medium atretic vesicular follicles, LVF= large vesicular follicles, LNVF= large normal vesicular follicles, LAVF= large atretic vesicular follicles.

BWT cows had more follicles (79.8 ± 14.0) than medium (42.9 ± 8.8; *P*=0.03) and low BWT cows (44.4 ± 6.4; *P*=0.02). Two-year old animals had 74.3 ± 14.6 follicles, a value which tended to differ from 3- (42.5 ± 7.2; *P*=0.06), but not 4-year olds (50.3 ± 7.6). Quantities of atretic follicles tended to differ for nonlactating cows (65.2 ± 9.3) compared with lactating cows (46.2 ± 7.7; *P*=0.09).

Atretic vesicular follicles were inversely related to size of the secondary CL (r= -0.66; *P*=0.007) in lactating cows. Nonlactating cows exhibited a negative correlation between total atretic follicles and basal FSH concentrations (r= -0.95; *P*=0.01).

Weaning weight interacted with age and lactational status to influence (*P*=0.04) quantities of **small atretic vesicular follicles.** Cows of medium WWT tended to have more small atretic follicles (45.2 ± 5.7) than high (28.4 ± 8.6; *P*=0.08) and did not differ for low (27.6 ± 8.4) WWT cattle. Two-year olds had more small atretic follicles (51.8 ± 10.8) than 3-(25.2 ± 5.7; *P*=0.03) and 4-year old cows (24.3 ± 7.4; *P*=0.06). No differences were detected between least square means of lactating and nonlactating cows (Table 1.2).

Small atretic follicles correlated negatively with size of the secondary CL (r= -0.65; *P*=0.009) in lactating cows. Positive correlations for nonlactating cows were detected between small atretic follicles, WWT (r=0.87; *P*=0.05) and E_2 concentrations (r=0.81; *P*=0.09), while an inverse relationship was detected between FSH concentrations (r= -0.90; *P*=0.03) and this follicle class.

Age and lactational status interacted to affect (*P*=0.03) counts of **medium atretic follicles.** Lactating cows had fewer medium atretic follicles (3.5 ± 1.9) than nonlactating

cows (13.9 ± 2.7; *P*=0.004). Differences were not detected in number of medium atretic follicles for age (Table 1.2).

Lactating cows exhibited a negative correlation between medium atretic follicles and BUN (r= -0.68; *P*=0.005). Linear regression of medium atretic follicles and BUN yielded an R^2 value of 0.47 (p=0.005; scatter plot in appendix). Medium atretic follicles were inversely related to FSH concentrations (r= -0.95; p=0.01) in nonlactating cattle.

Interactions between age, status, OWT and P_4 concentrations affected numbers of **large atretic vesicular follicles** (*P*=0.01). Cows with high P_4 ($\bar{x}$ =6.0 ng/ml; n=7) tended to have more (0.7 ± 0.3) large atretic follicles than the medium P_4 group ($\bar{x}$ =4.3 ng/ml; n=8; -0.1 ± 0.3; *P*=0.06), while follicle numbers for low P_4 animals ($\bar{x}$ =3.0 ng/ml; n=5; 0.1 ± 0.5) did not differ. Low OWT cows had more large atretic follicles (1.3 ± 0.3) than medium (-0.6 ± 0.3; *P*=0.001) and high OWT cows (-0.1 ± 0.4; *P*=0.01). Two-year old cows had fewer large atretic follicles (-1.6 ± 0.6) than 3- (0.7 ± 0.2; *P*=0.006) and 4-year old cows (1.5 ± 0.3; *P*=0.0009). No differences were found between follicle counts based on lactational status (Table 1.2).

Lactating cows displayed significant, negative correlations between large atretic follicles, YWT (r = -0.54; *P*=0.03) and BUN (r= -0.46; *P*=0.08). Linear regression of large atretic follicles and BUN resulted in the R^2 of 0.21 (*P*=0.08; scatter plot in appendix). Regression of large atretic follicles and YWT yielded the r-square value of 0.29 (*P*=0.03; scatter plot in appendix). Large atretic follicles correlated negatively with YWT (r= -0.84; *P*=0.07) and positively with PRL concentrations (r=0.99; *P*=0.0006) in nonlactating cows.

Size of the Primary and Secondary Corpus Luteum

Peripheral progesterone concentrations were reflected by size of the primary CL (P=0.04). Cows with low concentrations of P_4 had a smaller CL (11.1 $\pm$ 1.0 mm) than medium animals (13.6 $\pm$ 0.7 mm; P=0.03) and tended to differ for high P_4 cows (13.4 $\pm$ 0.7 mm; P=0.06). Lactational status, while included in the model, had no apparent effect on size of the primary CL.

Correlations exist between size of the primary CL, total growing follicles (r=0.50; P=0.05) and basal P_4 concentrations (r=0.48; P=0.07) for lactating cows. Regression of total growing follicles and size of the primary CL resulted in a r-squared value of 0.25 (P=0.05; scatter plot in appendix). Size of the primary CL was inversely related to insulin concentrations (r= -0.90; P=0.03) in nonlactating cows.

No significant model could be identified to explain differences in size of the secondary corpus luteum, nor were any correlations for this variable identified in nonlactating cows. Lactating cows exhibited negative correlations between size of the secondary CL, vesicular follicles (r= -0.62; P=0.01), normal follicles (r= -0.58; P=0.02), atretic follicles (r= -0.66; P=0.007), small follicles (r= -0.61; P=0.01), small normal follicles (r= -0.55; P=0.03), small atretic follicles (r= -0.65; P=0.009), while a positive correlation was detected for basal P_4 concentration (r=0.52; P=0.04).

Hormonal Profiles

Basal concentrations of P_4 (P=0.03) and PRL (P=0.05) were affected by cow age and lactational status. Two-year old cows (6.35 $\pm$ 80.7 ng/ml; n=2) had higher P_4 values

than 3- (4.12 ± 0.4 ng/ml; n=8; *P*=0.01) but concentrations did not differ from 4-year old cows (5.01 ± 0.4 ng/ml; n=10). Progesterone concentrations were not different between lactating and nonlactating cows. Prolactin values differed between ages and lactational status. Plasma prolactin was reduced in 3-year old versus 2- and 4-year old cows (107.09 ± 39.27 ng/ml vs. 227.39 ± 42.06 ng/ml; *P*=0.04). Lactating cows had diminished peripheral prolactin concentrations compared to nonlactating cows (80.30 ± 36.61 ng/ml vs. 239.03 ± 51.09 ng/ml; *P*=0.01). No age or status differences were detected in any other analyzed hormonal variable.

Age, status and OWT interacted to affect PRL (*P*=0.05) and P_4 (*P*=0.04) concentrations. Prolactin and progesterone values for age and status were similar to those mentioned previously. Cows in the high OWT group tended to have higher prolactin concentrations (197.80 ± 51.59 ng/ml) than medium OWT (92.90 ± 49.65 ng/ml; *P*=0.08), while low OWT cows did not differ (178.15 ± 49.69 ng/ml). Cows with high OWT tended to have more progesterone (5.81 ± 0.50 ng/ml) than cows with medium OWT (4.83 ± 0.49 ng/ml; *P*=0.09), but did not differ for low OWT cows (4.94 ± 0.49 ng/ml).

Lactating cows exhibited correlations between insulin, PRL (r=0.81; *P*=0.0003) and FSH concentrations (r= -0.53; *P*=0.04). No correlations were found between insulin and any other analyzed hormone in nonlactating cows.

Follicle stimulating hormone concentrations in lactating cows were inversely related to insulin, basal E_2 concentrations (r= -0.45; *P*=0.09), and positively related to LH concentrations (r=0.48; *P*=0.07). Concentration of LH correlated only with FSH

concentrations (r=0.48; *P*=0.07) in lactating cows. No hormone correlations were identified for FSH, LH or PRL in nonlactating cows.

No correlations were found between P_4 and other hormones in lactating and nonlactating cows, while FSH and E_2 were inversely related in lactating cows. No correlations were found between E_2 and other hormones in nonlactating cows. Androstenedione concentrations did not differ for cow age or lactational status (0.1 ng/ml) and exhibited no correlations for any described variable.

Releasability of the Anterior Pituitary After GnRH Stimulation

Animals were grouped into high (FSH: 2.1-5.0 ng/ml, n=7; LH: 51-80 ng/ml, n=5), medium (FSH: 1.5-2.1 ng/ml, n=7; LH: 11-51 ng/ml, n=8) and low (FSH: 0-1.5, n=6; LH: 5-11 ng/ml, n=7) anterior pituitary groups. Fifty-five percent (11/20) remained constant in terms of high, medium and low gonadotropin concentrations released into the blood stream within one hour after the first GnRH treatment. Forty-five percent (9/20) were constant on the next treatment date, twelve days after the first. Between the two dates, thirty percent (6/20) were constant in terms of FSH release and thirty-five percent (7/20) were constant for LH release.

Considering that release of gonadotropins from the anterior pituitary influences follicular growth and development, release of gonadotropins on the last GnRH treatment date was compared to resulting follicular populations nine days later. FSH, LH, cow age, lactational status and the covariates E_2 and P_4 were included in a model to explain follicular populations. LH tended to influence large vesicular follicles (*P*=0.08), while

FSH (Table 1.9) influenced all small and medium normal and atretic vesicular follicle classes (P=0.03-P=0.07). Follicle numbers are divided corresponding to high ($\bar{x}$ FSH: 3.04 ng/ml; $\bar{x}$ LH: 30.65 ng/ml), medium ($\bar{x}$ FSH: 1.8 ng/ml; $\bar{x}$ LH: 17.45 ng/ml) and low ($\bar{x}$ FSH: 1.11 ng/ml; $\bar{x}$ LH: 11.54 ng/ml) FSH concentrations (Table 1.9).

Differences were detected in concentrations of PRL one and two hours after GnRH treatment based on age, status and OWT. Lactating cows had reduced amounts of PRL (124.70 $\pm$ 29.02 ng/ml) compared to nonlactating cows (269.16 $\pm$ 35.80 ng/ml; P=0.007) one hour after GnRH administration. High OWT had more PRL (235.78 $\pm$ 37.90 ng/ml) than medium OWT (133.81 $\pm$ 36.48 ng/ml; P=0.02), while low OWT cows did not differ (221.19 $\pm$ 36.51 ng/ml) one hour after treatment. Sampling performed two hours after GnRH administration illustrated age and OWT differences, but no differences were detected for status. Two-year old cows had less PRL (51.58 $\pm$ 42.68 ng/ml) than 3-(147.01 $\pm$ 18.54 ng/ml; P=0.05) and 4-(169.06 $\pm$ 23.78 ng/ml; P=0.04) year old cows. No differences among ages, status or OWT were detected in the plasma sample obtained before GnRH administration, nor were concentrations of any other hormone released after GnRH treatment altered by age, status or body weight.

When lactational status alone was considered, PRL concentration differed and E_2 concentrations tended to differ after GnRH treatment. One hour after GnRH administration, PRL concentration in lactating cows was lower (151.00 $\pm$ 22.40 ng/ml) than nonlactating cows (262.20 $\pm$ 38.80 ng/ml; P=0.02). Two hours after GnRH, amount of PRL in lactating cows was higher (167.93 $\pm$ 15.28 ng/ml) than nonlactating cows (104.60 $\pm$ 26.47 ng/ml; P=0.05). Differences were absent in the pre-

Table 1.9. Follicular Populations Corresponding to FSH Concentrations Released after GnRH Stimulation in **Year 1**.[a]

Follicle Class	Concentrations of FSH Low	Medium	High
TVF	123.5 ± 17.9	72.5 ± 14.2	63.8 ± 16.3
TNVF	60.4 ± 8.9	35.7 ± 7.0	28.7 ± 8.1
TAVF	63.4 ± 9.6	36.8 ± 7.6	35.1 ± 8.7
SVF	100.2 ± 14.6	63.1 ± 11.5	55.3 ± 13.3
SNVF	51.1 ± 7.2	32.6 ± 5.7	26.2 ± 6.6
MVF	22.4 ± 5.2	8.4 ± 4.1	7.8 ± 4.8
MAVF	13.5 ± 2.8	5.9 ± 2.2	5.6 ± 2.5
LRGSTNVF (mm)	4.5 ± 0.7	6.4 ± 0.6	7.2 ± 0.8

a, TVF= total vesicular follicles, TNVF= total normal vesicular follicles, TAVF= total atretic vesicular follicles, SVF= small vesicular follicles, SNVF= small normal vesicular follicles, MVF= medium vesicular follicles, MAVF= medium atretic vesicular follicles, LRGSTNVF= largest normal vesicular follicles.

GnRH sample. Lactating cows tended to have higher concentrations of E_2 (7.21 ± 1.10 ng/ml) than nonlactating cows (3.05 ± 1.83 ng/ml; *P*=0.06) one hour after GnRH administration. Two hours after GnRH treatment, lactating cows still tended to have more E_2 (6.69 ± 0.82 ng/ml) than nonlactating cows (3.56 ± 1.42 ng/ml; *P*=0.06). No differences were detected in E_2 concentrations between lactating and nonlactating cows in the pre-GnRH sample.

Body Weight and Cow Age Correlations

Birth weight in nonlactating cows related to E_2 (r=0.82; *P*=0.09) and inversely related to large normal follicles (r= -0.87; *P*=0.05). No correlations were found between BWT and any other analyzed variable for lactating cows. Weaning weight correlated with OWT (r=0.72; *P*=0.002) and age (r=0.57; *P*=0.02) in lactating cows. Nonlactating cows displayed negative correlations between WWT, BUN concentration (r=-0.81; *P* =0.09) and basal LH concentration (r= -0.90; *P*=0.03). Yearling weight in lactating cows did not correlate with any nonfollicular variable. For nonlactating cows, YWT was inversely related to PRL concentrations (r= -0.87; *P*=0.05).

Age correlated with days postpartum (r=0.46; *P*=0.08), WWT (r=0.57; *P*=0.02), and OWT (r=0.67; *P*=0.004) in lactating cows. Correlations for nonlactating cows transpired between age, OWT (r=0.90; *P*=0.03) and large vesicular follicles (r=0.82; *P*=0.09).

Experimental Year Two

Primary Follicles

Number of primary follicles were influenced (P=0.01) by age, lactational status, day of the estrous cycle at ovariectomy (Day), basal insulin concentrations and OWT. Cows with low insulin concentrations ($\bar{x}$ =0.23 ng/ml; n=5) had fewer primary follicles (123, 939 $\pm$ 47, 641) than high ($\bar{x}$ =0.52 ng/ml; n=8; 303, 995 $\pm$ 65, 759; P=0.04) and medium ($\bar{x}$ =0.38 ng/ml; n=8; 317, 663 $\pm$ 50, 067; P=0.02) insulin cows. Animals with moderate OWT ($\bar{x}$ =460 kgs; n=5) had more primary follicles (393, 271 $\pm$ 64, 437) than low ($\bar{x}$ =384 kgs; n=7; 131, 266 $\pm$ 53, 492; P=0.006) or high OWT cows ($\bar{x}$ =528 kgs; n=9; 221, 060 $\pm$ 48, 315; P=0.05). Cows ovariectomized on Day 9 of their cycle had fewer primary follicles (108, 525 $\pm$ 32, 659) than cows on Day 5 (251, 995 $\pm$ 59, 402; P=0.05) and Day 13 (385, 077 $\pm$ 71, 608; P=0.003). No differences in primary follicle numbers were evident for age (217, 248 $\pm$ 56, 008), lactational status (217, 248 $\pm$ 55, 698) or days postpartum (233, 045 $\pm$ 55, 168).

Correlations were absent between primary follicles and any other analyzed variable for nonlactating cows. Nonetheless, correlations were observed between primary follicles, birth weight (r=0.64; P=0.005) and other follicular classes (Table 2.1 thru 2.3) for lactating cows. Linear regression analysis of primary follicles and birth weight provided the r-square value of 0.41 (P=0.005; scatter plot in appendix).

Table 2.1. Correlation Coefficients Between Primary and Growing Follicles and Various Vesicular Follicle Classes in Lactating Cows Ovariectomized **Year 2**.[a, b]

	TPF	TGF
TVF	0.61 (0.009)	0.39 (0.12)
TNVF	0.70 (0.001)	0.40 (0.11)
TAVF	0.47 (0.05)	0.34 (0.18)
SVF	0.69 (0.002)	0.32 (0.20)
SNVF	0.73 (0.0008)	0.32 (0.21)
SAVF	0.52 (0.03)	0.27 (0.29)
MVF	0.23 (0.36)	0.46 (0.06)
MNVF	0.02 (0.93)	0.61 (0.008)
MAVF	0.26 (0.30)	0.37 (0.14)
LVF	-0.02 (0.93)	-0.09 (0.72)
LNVF	-0.19 (0.46)	0.07 (0.79)
LAVF	0.07 (0.79)	-0.10 (0.68)

a, parenthesis indicates p-value, b, TPF=total primary follicles, TGF= total growing follicles, TVF= total vesicular follicles, TNVF= total normal vesicular follicles, TAVF= total atretic vesicular follicles, SVF= small vesicular follicles, SNVF= small normal vesicular follicles, SAVF= small atretic vesicular follicles, MVF= medium vesicular follicles, MNVF= medium normal vesicular follicles, MAVF= medium atretic vesicular follicles, LVF= large vesicular follicles, LNVF= large normal vesicular follicles, LAVF= large atretic vesicular follicles.

Table 2.2. Correlation Coefficients Between Total Vesicular, Total Normal and Atretic Vesicular Follicles and Various Follicle Classes in Lactating Cows Ovariectomized **Year 2.**[a, b]

	TVF	TNVF	TAVF
TVF	N/A	0.93 (0.0001)	0.95 (0.0001)
TNVF	0.93 (0.0001)	N/A	0.77 (0.0003)
TAVF	0.95 (0.0001)	0.77 (0.0003)	N/A
SVF	0.97 (0.0001)	0.93 (0.0001)	0.90 (0.0001)
SNVF	0.93 (0.0001)	0.99 (0.0001)	0.76 (0.0004)
SAVF	0.87 (0.0001)	0.70 (0.001)	0.92 (0.0001)
MVF	0.81 (0.0001)	0.69 (0.002)	0.82 (0.0001)
MNVF	0.37 (0.14)	0.44 (0.07)	0.27 (0.29)
MAVF	0.83 (0.0001)	0.68 (0.002)	0.87 (0.0001)
LVF	-0.23 (0.36)	-0.28 (0.27)	-0.17 (0.50)
LNVF	-0.13 (0.61)	-0.23 (0.38)	-0.03 (0.88)
LAVF	-0.12 (0.63)	-0.12 (0.64)	-0.12 (0.64)

a, parenthesis indicates p-value, b, TPF=total primary follicles, TGF= total growing follicles, TVF= total vesicular follicles, TNVF= total normal vesicular follicles, TAVF= total atretic vesicular follicles, SVF= small vesicular follicles, SNVF= small normal vesicular follicles, SAVF= small atretic vesicular follicles, MVF= medium vesicular follicles, MNVF= medium normal vesicular follicles, MAVF= medium atretic vesicular follicles, LVF= large vesicular follicles, LNVF= large normal vesicular follicles, LAVF= large atretic vesicular follicles.

Table 2.3. Correlation Coefficients Between Small Vesicular, Small Normal and Small Atretic Vesicular Follicles and Various Follicle Classes in Lactating Cows Ovariectomized **Year 2**. [a, b]

	SVF	SNVF	SAVF
SVF	N/A	0.94 (0.0001)	0.71 (0.001)
SNVF	0.94 (0.0001)	N/A	0.90 (0.0001)
SAVF	0.90 (0.0001)	0.94 (0.0001)	N/A
MVF	0.65 (0.004)	0.64 (0.006)	0.56 (0.01)
MNVF	0.24 (0.35)	0.31 (0.21)	0.10 (0.70)
MAVF	0.69 (0.002)	0.65 (0.004)	0.62 (0.007)
LVF	-0.22 (0.39)	-0.20 (0.43)	-0.20 (0.43)
LNVF	-0.08 (0.74)	-0.27 (0.29)	0.17 (0.50)
LAVF	-0.14 (0.60)	-0.04 (0.88)	-0.24 (0.35)

a, parenthesis indicates p-value, b, TPF=total primary follicles, TGF= total growing follicles, TVF= total vesicular follicles, TNVF= total normal vesicular follicles, TAVF= total atretic vesicular follicles, SVF= small vesicular follicles, SNVF= small normal vesicular follicles, SAVF= small atretic vesicular follicles, MVF= medium vesicular follicles, MNVF= medium normal vesicular follicles, MAVF= medium atretic vesicular follicles, LVF= large vesicular follicles, LNVF= large normal vesicular follicles, LAVF= large atretic vesicular follicles.

Growing Follicles

Age, lactational status, Day and WWT influence (*P*=0.03) numbers of growing follicles located in the ovarian cortex. Medium WWT ($\bar{x}$ =210 kgs; n=7) animals had more growing follicles (12, 720 ± 1, 550) than low ($\bar{x}$ =186 kgs; n=7; 3, 792 ± 1, 976; *P*=0.002) and high WWT cows ($\bar{x}$ =238 kgs; n=7; 7, 686 ± 1, 809; *P*=0.03). Status tended (*P*=0.07) to affect growing follicle numbers, with lactating cows having more follicles (10, 161 ± 1, 240) than nonlactating cows (5, 971 ± 1, 956). Numbers of growing follicles did not differ for age (9, 104 ± 2, 035), Day (9, 104 ± 2, 478) and days postpartum (9, 419 ± 2, 237).

Lactating cows displayed correlations between growing follicles and WWT (r=0.58; *P*=0.01). Regression of growing follicles and weaning weight provided the R^2 value of 0.34 (*P*=0.01; scatter plot in appendix). Size of the largest normal vesicular follicle correlated negatively with growing follicles (r= -0.88; p=0.04) in nonlactating cows.

Vesicular Follicles

Ovarian content of **vesicular follicles** was strongly affected (*P*=0.008) by age, status, Day, OWT and FSH concentrations. Cows with high concentrations of FSH ($\bar{x}$ =1.2 ng/ml; n=6) had fewer vesicular follicles (58.6 ± 7.3) than low ($\bar{x}$ =0.45 ng/ml; n=4; 94.8 ± 10.0; *P*=0.01) and medium ($\bar{x}$ =0.85 ng/ml; n=11; 80.2 ± 7.3; *P*=0.01) FSH cows. Average OWT cows tended to have more vesicular follicles (94.4 ± 8.2) than high (66.9 ± 10.7; *P*=0.06) and low (72.3 ± 8.9; *P*=0.08) body weight cows. Two-year old

cows had fewer (51.3 ± 7.6) vesicular follicles than 3-year old cows (104.5 ± 8.6; *P*=0.001). Day 9 cows had fewer follicles (60.1 ± 6.6) than Day 13 cows (96.8 ± 10.8; *P*=0.01), while no difference was detected for cows ovariectomized on Day 5 (76.7 ± 9.1). Number of vesicular follicles did not differ for lactating and nonlactating cows (Table 2.4). Vesicular follicles correlated with age of the lactating cow (r=0.48; *P*=0.05), while nonlactating animals exhibited correlations only among follicle classes (Tables 2.5 thru 2.7).

Follicle stimulating hormone, body weight at ovariectomy, age, lactational status and Day influenced **small vesicular follicle** numbers (*P*=0.01). Fewer small follicles were found in cows with high basal FSH (43.2 ± 6.3), whereas ovaries from cows with low (71.4 ± 8.6; *P*=0.02) and moderate (64.0 ± 6.3; *P*=0.02) FSH concentrations contained more small follicles. Cows with average OWT tended to have more follicles than low OWT cattle (72.7 ± 7.0 vs. 51.9 ± 7.7; *P*=0.06), while high OWT (53.8 ± 9.2) and lactation status had no effect on number of small follicles (57.3 ± 7.7). Two-year old cows had fewer small vesicular follicles than 3-year old cows (40.0 ± 6.6 vs. 79.2 ± 7.4; *P*=0.004). Cows ovariectomized on Day 13 of their estrous cycle had more small follicles (80.0 ± 9.3) than Day 9 (44.9 ± 5.7; *P*=0.01) or Day 5 cows (53.6 ± 7.9; *P*=0.05).

Small vesicular follicles were inversely related to size of the secondary CL (r= -0.61; *P*=0.01) in lactating cows. Nonlactating cows displayed positive correlations between small follicles, WWT (r=0.93; *P*=0.02), E_2 concentration (r=0.83; *P*=0.07) and a negative correlation with basal FSH (r= -0.83; *P*=0.07).

Table 2.4. Least Square Means ± Standard Errors for Total Vesicular and Total Normal and Atretic Follicles for Two- and Three-Year Old Lactating and Nonlactating Cows Ovariectomized **Year 2**.

Variable	Normal	Atretic	Total
Age	33.1 ± 5.1	41.7 ± 4.5	75.0 ± 9.4
Status	33.1 ± 5.1	40.5 ± 4.5	75.0 ± 9.3
Day	33.1 ± 6.1	41.7 ± 5.5	75.0 ± 11.4
Days Postpartum	33.6 ± 5.3	42.6 ± 4.5	76.4 ± 9.5

Table 2.5. Correlation Coefficients Between Primary and Growing Follicles and Various Vesicular Follicle Classes in Nonlactating Cows Ovariectomized **Year 2**.[a, b]

	TPF	TGF
TVF	0.14 (0.82)	0.08 (0.88)
TNVF	0.32 (0.60)	-0.25 (0.68)
TAVF	-0.02 (0.97)	0.33 (0.58)
SVF	0.14 (0.81)	0.12 (0.84)
SNVF	0.22 (0.71)	0.004 (0.99)
SAVF	0.07 (0.91)	0.39 (0.51)
MVF	0.15 (0.81)	-0.09 (0.88)
MNVF	0.60 (0.28)	-0.37 (0.53)
MAVF	-0.02 (0.97)	0.01 (0.97)
LVF	-0.77 (0.12)	0.27 (0.66)
LNVF	0.70 (0.19)	-0.72 (0.16)
LAVF	-0.71 (0.17)	0.29 (0.64)

a, parenthesis indicates p-value, b, TPF=total primary follicles, TGF= total growing follicles, TVF= total vesicular follicles, TNVF= total normal vesicular follicles, TAVF= total atretic vesicular follicles, SVF= small vesicular follicles, SNVF= small normal vesicular follicles, SAVF= small atretic vesicular follicles, MVF= medium vesicular follicles, MNVF= medium normal vesicular follicles, MAVF= medium atretic vesicular follicles, LVF= large vesicular follicles, LNVF= large normal vesicular follicles, LAVF= large atretic vesicular follicles.

Table 2.6. Correlation Coefficients Between Total Vesicular, Total Normal and Atretic Vesicular Follicles and Various Follicle Classes in Nonlactating Cows Ovariectomized **Year 2**.[a, b]

	TVF	TNVF	TAVF
TVF	N/A	0.91 (0.03)	0.96 (0.01)
TNVF	0.91 (0.03)	N/A	0.75 (0.14)
TAVF	0.96 (0.01)	0.75 (0.14)	N/A
SVF	0.98 (0.003)	0.92 (0.02)	0.92 (0.02)
SNVF	0.78 (0.11)	0.87 (0.05)	0.64 (0.24)
SAVF	0.92 (0.02)	0.72 (0.16)	0.97 (0.006)
MVF	0.69 (0.19)	0.52 (0.36)	0.72 (0.16)
MNVF	0.22 (0.71)	0.12 (0.83)	0.24 (0.69)
MAVF	0.77 (0.12)	0.59 (0.29)	0.80 (0.10)
LVF	-0.55 (0.33)	-0.48 (0.40)	-0.52 (0.36)
LNVF	-0.50 (0.39)	-0.32 (0.59)	-0.59 (0.29)
LAVF	-0.23 (0.71)	-0.12 (0.83)	-0.26 (0.67)

a, parenthesis indicates p-value, b, TPF=total primary follicles, TGF= total growing follicles, TVF= total vesicular follicles, TNVF= total normal vesicular follicles, TAVF= total atretic vesicular follicles, SVF= small vesicular follicles, SNVF= small normal vesicular follicles, SAVF= small atretic vesicular follicles, MVF= medium vesicular follicles, MNVF= medium normal vesicular follicles, MAVF= medium atretic vesicular follicles, LVF= large vesicular follicles, LNVF= large normal vesicular follicles, LAVF= large atretic vesicular follicles.

Table 2.7. Correlation Coefficients Between Small Vesicular, Small Normal and Small Atretic Vesicular Follicles and Various Follicle Classes in Nonlactating Cows Ovariectomized **Year 2**.[a, b]

	SVF	SNVF	SAVF
SVF	N/A	0.88 (0.04)	0.93 (0.02)
SNVF	0.88 (0.04)	N/A	0.72 (0.17)
SAVF	0.92 (0.02)	0.72 (0.17)	N/A
MVF	0.54 (0.35)	0.11 (0.85)	0.58 (0.30)
MNVF	0.07 (0.90)	-0.24 (0.69)	0.18 (0.76)
MAVF	0.63 (0.25)	0.22 (0.71)	0.65 (0.23)
LVF	-0.49 (0.40)	-0.28 (0.65)	-0.56 (0.32)
LNVF	-0.56 (0.32)	-0.51 (0.38)	-0.58 (0.30)
LAVF	-0.12 (0.84)	0.13 (0.83)	-0.26 (0.66)

a, parenthesis indicates p-value, b, TPF=total primary follicles, TGF= total growing follicles, TVF= total vesicular follicles, TNVF= total normal vesicular follicles, TAVF= total atretic vesicular follicles, SVF= small vesicular follicles, SNVF= small normal vesicular follicles, SAVF= small atretic vesicular follicles, MVF= medium vesicular follicles, MNVF= medium normal vesicular follicles, MAVF= medium atretic vesicular follicles, LVF= large vesicular follicles, LNVF= large normal vesicular follicles, LAVF= large atretic vesicular follicles.

Quantities of **medium vesicular follicles** were influenced (P=0.05) by age, status and Day. Two-year old cows had fewer medium follicles than 3-year olds (10.7 $\pm$ 2.1 vs. 19.5 $\pm$ 3.0; P=0.01), while status had no observable effect on medium follicles (15.1 $\pm$ 2.5). Follicle counts were higher for cows ovariectomized on Day 5 than those from ovaries removed on Day 9 (20.1 $\pm$ 3.5 vs. 12.2 $\pm$ 2.0; P=0.05); however, Day 13 follicle numbers were not different (13.0 $\pm$ 3.7).

Adding OWT and basal FSH concentration to the afore mentioned model also affected medium follicle numbers (P=0.004). Low FSH cows (20.9 $\pm$ 2.6) had more medium follicles than high (13.4 $\pm$ 1.9; P=0.04) and medium (12.9 $\pm$ 1.9; P=0.02) animals. Cows with higher OWT had fewer medium follicles (10.3 $\pm$ 2.7) than animals with moderate weight (19.7 $\pm$ 2.1; P=0.01), while low OWT cows were not different (17.2 $\pm$ 2.3). Follicle differences on Days 5 and 9 were similar to those listed above. No status differences were observed (15.7 $\pm$ 1.8), but age influenced follicle counts (2-yr; 8.9 $\pm$ 2.0 vs. 3-yr; 22.6 $\pm$ 2.2; P=0.001).

Lactating cows exhibited a positive correlation for medium follicles and age (r=0.47; P=0.05) and tended to negatively correlation with Day (r= -0.45; P=0.07). No correlations were detected for lactating cows.

No likely model manifested itself for **large vesicular follicles**. Correlations were observed between large vesicular follicles and follicular variables presented previously for lactating and nonlactating cows (Tables 2.1-2.3; 2.5-2.7).

Normal Vesicular Follicles

Age, lactational status, Day, OWT and basal FSH concentrations affected number of **normal vesicular follicles** (P=0.02). High FSH cows had fewer normal follicles (23.5 ± 4.0) than low (45.9 ± 5.6; P=0.008) and moderate (36.3 ± 4.1; P=0.03) FSH animals. Cows with low OWT tended to have fewer follicles (30.1 ± 5.0) than medium OWT cows (43.4 ± 4.5; P=0.06), while these least square means were not different from high OWT cows (32.3 ± 5.9). Fewer normal follicles were located in ovaries removed from cows on Day 9 (26.4 ± 3.7) than on Day 13 (45.3 ± 6.0; P=0.02), while normal follicles were not different on Day 5 (Table 2.4) . Two-year old cows had fewer follicles (26.8 ± 4.2) than 3-year old cows (43.7 ± 4.8; P=0.03). No difference was detected in normal follicle counts for lactating and nonlactating cows (Table 2.4). Total normal follicles correlated with other follicular classes (Tables 2.1-2.3; 2.5-2.7) in lactating and nonlactating cows.

Quantities of **small normal vesicular follicles** were influenced by age, lactational status, Day, OWT and FSH concentration (P=0.007). Cows with high FSH concentrations exhibited reduced counts of small normal follicles (20.1 ± 3.4) compared to low (43.1 ± 4.7; P=0.002) and medium (35.1 ± 3.5; P=0.006) FSH animals. Diminished OWT cattle had fewer small normal follicles compared to cows of moderate OWT (27.2 ± 4.2 vs. 40.6 ± 3.9; P=0.03). Animals ovariectomized on Day 13 had a greater quantity of small normal follicles (44.5 ± 5.1) than on Day 9 (23.9 ± 3.1; P=0.006) or Day 5 (30.1 ± 4.3; P=0.05). Two-year old animals had reduced follicle counts (24.6 ± 3.6) in contrast to 3-year old cows (41.0 ± 4.1; P=0.01). Lactation status

did not influence counts of small normal follicles (Table 2.8)

Small normal follicles negatively correlated with FSH concentrations (r= -0.42; *P*=0.09) in lactating cows, while nonlactating animals exhibited a positive correlation between small normal follicles and size of the secondary CL (r=0.98; *P*=0.004).

Age, lactation status, Day and size of the primary CL interacted to influence **medium normal vesicular follicle** tallies (*P*=0.05). Cows with a medium CL ($\bar{x}$ =12.5 mm; n=10) had more medium normal follicles (3.2 $\pm$ 0.6) than small ($\bar{x}$ =7.0 mm; n=6; 0.9 $\pm$ 0.8; *P*=0.03) or large ($\bar{x}$ =17.0 mm; n=6; 1.1 $\pm$ 0.7; *P*=0.01) sized CL. Nonlactating cows displayed a negative correlation between medium normal follicles and birth weight (r= -0.88; p=0.04), while lactating cows exhibited correlations between medium normal follicles and other follicular classes (Tables 2.1-2.3).

Counts of **large normal vesicular follicles** were influenced by age, status and size of the primary CL (*P*=0.05). Corpus luteum size was the most influential, with a small CL associated with the least large normal follicles (-0.2 $\pm$ 0.2) compared to large (0.7 $\pm$ 0.2; *P*=0.01) and medium (0.5 $\pm$ 0.2; *P*=0.02) CL cows. Large normal follicles did not differ for age, status and Day (Table 2.8).

Correlation analysis found positive relationships between large normal follicles, days postpartum (r=0.52; *P*=0.03), size of the primary (r=0.55; *P*=0.02) and secondary (r=0.46; *P*=0.06) CL, FSH (r=0.48; *P*=0.04) and P_4 concentrations (r=0.42; *P*=0.09), and an inverse relationship with E_2 (r= -0.43; *P*=0.08) in lactating cows.

Table 2.8. Least Square Means ± Standard Errors for Small, Medium and Large Normal and Atretic Vesicular Follicles for Two- and Three-Year Old Lactating and Nonlactating Cows Ovariectomized **Year 2**.

Variable	Small	Medium	Large
Normal			
Age	31.1 ± 5.2	2.2 ± 0.7	0.4 ± 0.3
Status	31.1 ± 5.1	2.1 ± 0.7	0.4 ± 0.3
Day	31.1 ± 6.2	2.1 ± 0.8	0.4 ± 0.3
Days Postpartum	31.0 ± 5.4	2.3 ± 0.8	0.4 ± 0.3
Atretic			
Age	26.7 ± 3.2	13.0 ± 2.2	2.0 ± 0.6
Status	26.6 ± 3.1	13.0 ± 2.2	2.0 ± 0.6
Day	26.6 ± 3.8	13.0 ± 2.6	2.0 ± 0.7
Days Postpartum	27.5 ± 3.1	13.0 ± 2.3	2.0 ± 0.5

Regression of large normal follicles and basal FSH yielded the R^2 value of 0.23 (P=0.04; scatter plot in appendix). Linear regression of large normal vesicular follicles and size of the primary CL resulted in an R^2 value of 0.30 (P=0.02; scatter plot in appendix). Nonlactating cows displayed correlations between large normal follicles, basal PRL (r=0.94; P=0.01) and E_2 (r=0.99; P=0.001).

Age, lactational status, Day, OWT, insulin and LH concentrations interacted and tended to affect the **size of the largest normal vesicular follicle** (P=0.06). Size of the normal follicle was increased when insulin concentrations were low (8.9 $\pm$ 1.2 mm) compared with medium (1.5 $\pm$ 1.1 mm; P=0.004) and high concentrations (4.7 $\pm$ 1.6 mm; P=0.01). High concentrations of LH ($\bar{x}$ =2.2 ng/ml; n=8) resulted in a larger normal follicle (7.2 $\pm$ 1.4 mm) than cows with medium ($\bar{x}$ =0.9 ng/ml, n=4; 3.4 $\pm$ 0.7 mm; P=0.05) LH concentrations. Cows with low OWT had the smallest normal follicle (2.5 $\pm$ 1.0 mm) compared with medium (7.0 $\pm$ 1.6 mm; P=0.03) and high OWT animals (5.5 $\pm$ 1.0 mm; P=0.06). Three-year old cows had a larger normal follicle (7.6 $\pm$ 1.4 mm) than 2-year old cows (2.4 $\pm$ 0.8 mm; P=0.01). Status and Day differences were not apparent for size of the largest normal vesicular follicle (4.2 $\pm$ 1.0 mm).

Lactating cows displayed correlations between normal follicle size, large normal and large atretic follicles. Nonlactating animals exhibited negative correlations between size of the largest normal follicle, primary CL size (r= -0.84; P=0.07) and basal P_4 concentrations (r= -0.90; P=0.03). Linear regression of normal follicle size and progesterone yielded the R^2 value of 0.81 (P=0.03; scatter plot in appendix).

Atretic Vesicular Follicles

Age, lactational status and Day influenced (P=0.01) numbers of **atretic vesicular follicles.** Three-year old cows had more atretic follicles (52.7 ± 5.3) than 2-year old cows (30.7 ± 3.7; P=0.001), whereas counts for Day and status were not different (Table 2.4).

Including basal FSH concentrations and OWT with status, age and Day also impacted atretic follicle numbers (P=0.003). High FSH cows had fewer atretic follicles (35.0 ± 3.7) than low (48.8 ± 5.1; P=0.05), however follicle number did not differ for cows with medium FSH concentrations (43.4 ± 3.7). Cows with higher OWT had fewer atretic follicles (34.7 ± 5.4) than medium (50.9 ± 4.2; P=0.03), but did not differ from low OWT cows (41.7 ± 4.5). Two-year old cows had fewer atretic follicles (24.5 ± 3.9) than 3-year old cows (60.3 ± 4.4; P=0.0002). Day 13 cows (51.4 ± 5.5) had more atretic follicles than those on Day 9 (33.5 ± 3.3; P=0.02), while counts of atretic follicles did not differ for Day 5 or lactational status (Table 2.4).

Atretic follicles correlated with age (r=0.67; P=0.003), WWT (r=0.44; p=0.07) and OWT (r=0.50; P=0.04) for lactating cows. Linear regression of atretic follicles and OWT yielded the r-square value of 0.25 (P=0.04; scatter plot in appendix). Nonlactating cows exhibited correlations among atretic follicles and other follicular classes (Tables 2.5 thru 2.7).

Small atretic vesicular follicles were influenced by age, status and Day (P=0.02). Ovaries from two-year old cows contained fewer small atretic follicles (19.7 ± 2.6) than ovaries from 3-year old cows (33.6 ± 3.7; P=0.003). Status and Day provided no noticeable effect on small atretic follicular populations (Table 2.8).

Correlations were detected between small atretic follicles, age (r=0.67; *P*=0.003), WWT (r=0.43; *P*=0.08) and OWT (r=0.51; *P*=0.03) in lactating cows. Linear regression of small atretic follicles and body weight at ovary removal generated the R^2 value of 0.26 (*P*=0.03; scatter plot in appendix). Nonlactating cows exhibited correlations between small atretic follicles and other follicular classes (Tables 2.6 thru 2.8).

Age and Day interacted to influence numbers of **medium atretic vesicular follicles** (*P*=0.02). Two-year old cows had fewer medium atretic follicles (8.6 ± 1.6) than 3-year old cows (16.6 ± 2.4; *P*=0.009). Cows ovariectomized on Day 5 tended to have more follicles (16.3 ± 2.8) compared to ovaries removed on Day 9 (10.3 ± 1.5; *P*=0.08).

Adding FSH concentrations and OWT to the above variables also impacted medium atretic follicular counts (*P*=0.001). Cows with low concentrations of FSH had more atretic medium follicles (17.9 ± 2.0) than cows with medium (11.9 ± 1.4; *P*=0.02) or high (10.9 ± 1.4; *P*=0.01) FSH concentrations. Cows of higher OWT had fewer medium atretic follicles (8.8 ± 2.1) than medium OWT cows (17.5 ± 1.6; *P*=0.006), and tended to have fewer follicles than cows of low OWT (14.4 ± 1.7; *P*=0.08). Two-year old cows had fewer follicles than 3-year old cows (7.0 ± 1.5 vs. 20.1 ± 1.7; *P*=0.0002), and Day 5 cows had an increased number of follicles (16.6 ± 1.8) in contrast to cows ovariectomized on Day 9 (10.8 ± 1.3; *P*=0.01). As was the case for the three variable model, lactational status had no discernable effect on medium atretic follicle counts (Table 2.8).

Lactating cows exhibited a positive correlation between medium atretic follicles

and age (r=0.52; *P*=0.03),while Day (r= -0.43; *P*=0.08) was inversely related. No correlations for any nonfollicular variable were detected for nonlactating animals.

Inventories of **large atretic vesicular follicles** were explained by an interaction of Day, OWT, basal P_4 concentration and size of the primary CL (*P*=0.007). Animals in the medium P_4 group ($\bar{x}$ =3.8 ng/ml; n=10) had more large atretic follicles (3.7 $\pm$ 0.4) than low ($\bar{x}$ =2.5 ng/ml; n=7; 1.4 $\pm$ 0.5; *P*=0.004) and high P_4 cows ($\bar{x}$ =6.6 ng/ml; n=4; 2.0 $\pm$ 0.5; *P*=0.01). In terms of CL size, cows with a small-diameter primary CL had more large atretic follicles (4.3 $\pm$ 0.6) than a medium- (0.8 $\pm$ 0.4; *P*=0.0004) or large-diameter (1.9 $\pm$ 0.5; *P*=0.01) primary CL. Cattle with higher OWT had more large atretic follicles (3.3 $\pm$ 0.5) than low (1.7 $\pm$ 0.5; *P*=0.04) or medium OWT cows (2.1 $\pm$ 0.4; *P*=0.03). Ovaries removed from Day 9 cows had less atretic follicles (1.4 $\pm$ 0.3) than Day 5 (2.9 $\pm$ 0.6; *P*=0.02), and tended to have fewer atretic follicles than Day 13 cows (2.8 $\pm$ 0.6; *P*=0.08).

Large atretic follicles tended to correlate negatively with days postpartum (r= -0.45; *P*=0.06) in the lactating group. Nonlactating cows displayed correlations between large vesicular follicles other follicular classes (Tables 2.6 thru 2.8).

Size of the Primary and Secondary Corpus Luteum

Age, lactational status, Day and P_4 concentration can define size of the primary corpus luteum (*P*=0.0004). Cows with high progesterone had a larger CL ($\bar{x}$ =16.3 $\pm$ 1.2 mm) compared to cattle with low P_4 concentrations ($\bar{x}$ =7.8 $\pm$ 1.1 mm; *P*=0.0001). Status, Day and age did not affect CL size.

Lactating cows yielded correlations between CL size, large normal follicles (r=0.55; *P*=0.02) and P_4 concentrations (r=0.53; *P*=0.02). Nonlactating cows exhibited positive correlations between corpus luteum size, P_4 concentrations (r=0.90; *P*=0.03), FSH concentrations (r=0.81; *P*=0.09), WWT (r=0.90; *P*=0.03) and YWT (r=0.82; *P*=0.09). Negative correlations tended to occur between primary CL size, size of the largest normal follicle (r= -0.84; *P*=0.07) and BUN values (r= -0.82; *P*=0.08).

Size of the secondary CL could not be explained by combinations of any variable analyzed. Large normal follicles (r=0.46; *P*=0.06) tended to correlate positively with size of the secondary CL in lactating cows. Nonlactating cows demonstrated correlations between size of the secondary CL, small follicles (r=0.93; *P*=0.02) and small normal follicles (r=0.98; *P*=0.004).

Hormonal Profiles

Differences were detected in concentrations of insulin, BUN, LH and E_2 based on age of the cow, lactational status and/or Day. LH concentrations were the only hormone which tended to differ (*P*=0.08) when age, status and Day were considered. Lactating cows had more LH (1.00 $\pm$ 0.06 ng/ml) than nonlactating cows (0.71 $\pm$ 0.09 ng/ml; *P*=0.01), while no differences were detected between LH concentrations for age and Day.

When age, lactation status and Day were considered, LH (*P*=0.03) and BUN (*P*=0.05) concentrations differed, while E_2 tended to differ (*P*=0.07). More LH was detected in lactating cows compared to nonlactating cows (1.00 $\pm$ 0.05 ng/ml vs 0.71 $\pm$ 0.09 ng/ml; *P*=0.008), while Day differences were absent. BUN values did not

differ based on status (26.88 ± 1.19 mg/dl); however, Day 13 cows had higher quantities of BUN (29.99 ± 1.5 mg/dl) than those on Day 9 (25.99 ± 0.89 mg/dl; *P*=0.02) and Day 5 (24.06 ± 1.50 mg/dl; *P*=0.01). Plasma E_2 varied according to Day, but not status. Cows ovariectomized on Day 5 had higher E_2 concentrations (8.95 ± 1.27 pg/ml) than Day 9 (5.79 ± 0.74 pg/ml; *P*=0.03) and Day 13 cows (5.24 ± 1.27 pg/ml; *P*=0.04).

Differences were observed in concentrations of BUN (*P*=0.02) and tended to be observed for E_2 (*P*=0.08) and insulin (*P*=0.09) when Day alone was analyzed. Day 13 cows had higher BUN concentrations (29.88 ± 1.42 mg/dl) than Day 5 (23.95 ± 1.42 mg/dl; *P*=0.008) and Day 9 cows (25.86 ± 0.76 mg/dl; *P*=0.02). Estradiol values were higher in Day 5 cows (8.50 ± 1.27 pg/ml) than Day 9 (5.27 ± 0.68 pg/ml; *P*=0.03) and Day 13 cows (4.79 ± 1.27 pg/ml; *P*=0.05). Insulin concentrations were higher on Day 9 (0.38 ± 0.03 ng/ml) than Day 13 (0.25 ± 0.06 ng/ml; *P*=0.05), while concentrations on Day 5 did not differ from the other two days.

When OWT was compared to hormone concentrations at ovariectomy, differences were detected in FSH (*P*=0.03) and insulin (*P*=0.05) concentrations. High OWT cows had higher concentrations of FSH (1.16 ± 0.10 ng/ml) than low (0.82 ± 0.11ng/ml; *P*=0.03) and medium (0.82 ± 0.09 ng/ml; *P*=0.01). Insulin concentrations were higher in heavier cows (0.42 ± 0.04 ng/ml) compared to medium (0.28 ± 0.04 ng/ml; *P*=0.02) and low (0.31 ± 0.05 ng/ml; *P*=0.09) OWT cows.

Insulin correlated positively with E_2 (r=0.55; *P*=0.02) in lactating cows, and with P_4 (r=0.97; *P*=0.006), and negatively to BUN (r= -0.83; *P*=0.07) in nonlactating cows. Blood urea nitrogen was inversely related to P_4 (r= -0.48; *P*=0.04) in lactating and

nonlactating cows (r= -0.83; *P*=0.08). No correlations were detected between FSH, LH and any other hormone variable in lactating and nonlactating cows. Prolactin correlated with E_2 (r=0.89; *P*=0.04) in nonlactating, but no correlations were yielded for lactating cows. Androstenedione did not differ between ages, lactational status or Day (0.1 ng/ml), and did not show any significant correlations with any other measured variable.

Releasability of the Anterior Pituitary After GnRH Stimulation

All cows were treated with GnRH thirteen days apart (May 13, 26) to determine the quantity of gonadotropins (LH, FSH) released. Cows were grouped into high (LH: 30-76 ng/ml, n=7; FSH: 1.4-3.5 ng/ml, n=7), medium (LH: 17-30 ng/ml, n=8; FSH: 0.85-1.4 ng/ml, n=9) and low (LH: 5-17 ng/ml, n=7; FSH: 0.5-0.85 ng/ml, n=6) groups based on quantities of FSH and LH released. Forty-five percent (10/22) of cows released either high, medium or low quantities of FSH and LH after first GnRH stimulation. Fifty-five percent (12/22) treated thirteen days later were in the same LH and/or FSH group. Thirty-two percent (7/22) were in the same FSH group after the second treatment as they were on the first, and forty-one percent (9/22) were in the same LH group on both treatment groups.

Age, status, Day, FSH, LH and the covariates, E_2 and P_4 were useful in predicting effects on the follicle populations observed five days later at ovariectomy. Day of the cycle at treatment (Day 0, 4, 8) must be included in the model due to the response, late response, or lack of response to synchronization prior to GnRH treatment. Differences in total vesicular follicles (*P*=0.006), total normal follicles (*P*=0.006), total atretic follicles

(P=0.01), small follicles (P=0.006), medium follicles (P=0.08), large follicles (P=0.08), small normal follicles (P=0.01), medium normal follicles (P=0.08) and small atretic follicles (P=0.01) were associated with high ($\bar{x}$ FSH: 2.25 ng/ml; $\bar{x}$ LH: 47.47), medium ($\bar{x}$ FSH: 1.5 ng/ml; $\bar{x}$ LH: 43.66) and low ($\bar{x}$ FSH: 1.11 ng/ml; $\bar{x}$ LH: 31.2 ng/ml) FSH concentrations (Table 2.9).

When hormone concentrations after GnRH administration (1 and 2 hours later) were compared to age, status, Day and OWT, slight differences were found in FSH and E_2. Pre-GnRH concentrations of FSH (P=0.09) and concentrations two hours after GnRH (P=0.002) tended to be affected by the afore mentioned variables. High OWT cows had more FSH (0.72 $\pm$ 0.05 ng/ml) than low (0.55 $\pm$ 0.06 ng/ml; P=0.05) and medium (0.56 $\pm$ 0.05 ng/ml; P=0.03). Lactational status, age and Day displayed no appreciable effects on FSH concentrations. Samples obtained two hours after GnRH administration exhibited changes due to status, Day and OWT. Lactating cows had reduced quantities of FSH (1.83 $\pm$ 0.20 ng/ml) compared to nonlactating cows (2.92 $\pm$ 0.33 ng/ml; P=0.008). Cows treated while on Day 8 had more FSH (3.13 $\pm$ 0.44 ng/ml) than cows on Day 4 (1.97 $\pm$ 0.22 ng/ml; P=0.03) or on Day 0 (2.02 $\pm$ 0.38 ng/ml). Cows with lower OWT tended to release more FSH (3.01 $\pm$ 0.36 ng/ml) than medium (2.08 $\pm$ 0.34 ng/ml; P=0.06) and high (2.04 $\pm$ 0.32 ng/ml; P=0.07). No age effects were observed for FSH concentrations. Estradiol concentrations two hours after GnRH tended to be higher (P=0.06) for cows ovariectomized on Day 0 (15.26 $\pm$ 2.48 pg/ml) than those on Day 4 (8.32 $\pm$ 1.41 pg/ml; P=0.02). Age, status and OWT were not a factor for E_2 concentrations.

Table 2.9. Follicular Populations Corresponding to FSH Concentrations Released After GnRH Stimulation in **Year 2**.[a]

	Concentrations of FSH		
Follicle Class	Low	Medium	High
TVF	82.4 ± 13.4	88.8 ± 7.6	41.2 ± 10.4
TNVF	36.3 ± 7.4	41.0 ± 4.2	14.9 ± 5.7
TAVF	45.7 ± 7.1	47.5 ± 4.0	26.4 ± 5.5
SVF	60.0 ± 11.1	70.0 ± 6.3	29.4 ± 8.6
SNVF	31.5 ± 7.8	39.9 ± 4.4	13.9 ± 6.1
SAVF	28.6 ± 4.9	30.9 ± 2.7	15.8 ± 3.8
MVF	21.0 ± 4.7	15.8 ± 2.7	9.1 ± 3.6
MNVF	4.4 ± 1.2	1.7 ± 0.7	1.2 ± 1.0
LVF	0.9 ± 0.8	2.6 ± 0.5	3.0 ± 0.6

a, TVF= total vesicular follicles, TNVF= total normal vesicular follicles, TAVF= total atretic vesicular follicles, SVF= small vesicular follicles, SNVF= small normal vesicular follicles, SAVF= small atretic vesicular follicles, MVF= medium vesicular follicles, MNVF= medium normal vesicular follicles, LVF= large vesicular follicles.

Removal of OWT from consideration yielded differences in E_2 (P=0.01) and P_4 (P=0.05) concentrations. Two-hours after GnRH treatment, E_2 levels were highest for cows on Day 0 of their cycle (15.81 $\pm$ 2.21 pg/ml) compared to Day 4 (8.49 $\pm$ 1.29 pg/ml; P=0.009) and Day 8 (9.55 $\pm$ 2.40 pg/ml; P=0.05). Status and age differences were not present for E_2 concentrations. Progesterone concentrations one hour after GnRH treatment were higher for cows on Day 0 (2.82 $\pm$ 0.43 ng/ml) than those on Day 4 (1.77 $\pm$ 0.25 ng/ml; P=0.04). Lactating cows tended to have less P_4 (1.80 $\pm$ 0.24 ng/ml) than nonlactating cows (2.59 $\pm$ 0.40 ng/ml; P=0.07). Two-hours post-GnRH treatment, P_4 was higher for cows on Day 0 (3.58 $\pm$ 0.53 ng/ml) than those on Day 4 (1.86 $\pm$ 0.31 ng/ml; P=0.01) and Day 8 (1.98 $\pm$ 0.58 ng/ml; P=0.04). No age or status differences were observed for this sampling time.

Body Weight

Birth weight correlated with YWT (r=0.47; P=0.05) in lactating cows, while PRL was inversely related (r= -0.88; P=0.04) to BWT in nonlactating cows. Weaning weight correlated with YWT (r=0.83; P=0.0001), OWT (r=0.62; P=0.008) and insulin (r=0.41; P=0.09) in lactating cows. Nonlactating cows exhibited correlations between WWT, YWT (r=0.97; P=0.007) and FSH (r=0.83; P=0.08).

Correlations were illuminated between YWT, insulin (r=0.55; P=0.02), OWT (r=0.80; P=0.0001), WWT (r=0.83; P=0.0001) and BWT (r=0.47; P=0.05) in lactating cows. Nonlactating cows exhibited correlations between YWT, FSH (r=0.90; P=0.04) and WWT (r=0.97; P=0.007). Body weight at ovariectomy correlated with insulin

(r=0.55; *P*=0.02), FSH (r=0.55; *P*=0.02), E_2 (r=0.44; *P*=0.07) and YWT (r=0.80; *P*=0.0001) in lactating cows. Nonlactating cows displayed a positive correlation between body weight and FSH (r=0.87; *P*=0.05).

Age of the Cow and Day of the Cycle at Ovariectomy

Age of lactating cows correlated with FSH (r=0.42; *P*=0.09), insulin (r=0.50; *P*=0.04), OWT (r=0.79, *P*=0.0002) and YWT (r=0.45; *P*=0.06). Age correlated negatively with basal LH (r= -0.95; *P*=0.01) in the nonlactating group. Lactating cows yielded correlations between Day and BUN (r=0.66; *P*=0.003), while Day exhibited a negative correlation with PRL (r= -0.88; *P*=0.05) for nonlactating cows.

Does Corpus Luteum Type Influence Ovarian Follicle Populations?

Corpus luteum type may exert an influence on ovarian follicle populations and health. To examine possible relationships between the corpus luteum and follicle type, a subset of eleven cows were randomly selected. These cows were taken from **Year 1** (n=3) and **Year 2** (n=8). Cows were lactating (n=4) and nonlactating (n=7), two- (n=4), three- (n=5) and four-years (n=2) of age.

Primary corpus lutea (CL) averaged 12.4 $\pm$ 1.6 mm, while the secondary were 7.3 $\pm$ 1.6 mm in size (*P*=0.01). Luteal type did not affect the number of primary follicles in the section of the ovary containing the CL (approximately 1/3 of the ovary). This number was affected by age (*P*=0.03) alone, as 2-year old cows had 8.9 $\pm$ 1.9 primary follicles, 3-year olds had 11.9 $\pm$ 1.9 primary follicles and 4-year old cows had 1.0 $\pm$ 3.0.

Distance of the closest primary follicle to the CL was influenced by CL type (P=0.04). The closest primary follicle was 4.7 ± 1.3 mm to the secondary CL, while the closest primary follicle to the primary CL was 8.8 ± 1.3 mm. Total numbers of primary follicles for the tissue section were influenced by both CL type (P=0.03) and age (P=0.01). The section with the secondary CL contained 53.9 ± 7.3 primary follicles and the primary CL section included 36.6 ± 7.3 follicles. Sections for the 2- and 3-year old cows contained 64.8 ± 8.6 and 63.7 ± 8.6 primary follicles, respectively. Four-year old cows had 7.3 ± 13.8 follicles for the section.

Luteal type only influenced the total number of growing follicles for the tissue section containing the CL (P=0.02). Growing follicles were greater for the secondary CL (2.8 ± 0.6) than for the primary CL (1.2 ± 1.0). Values for total growing follicles around the CL and distance of the closest growing follicle did not differ.

Total vesicular follicles were not influenced by the CL type, while total normal vesicular follicles tended to differ between the CL types (P=0.06). The number of normal vesicular follicles near the primary CL was 2.5 ± 0.4 and 1.6 ± 0.4 near the secondary CL. Interactions between CL class and age also influenced numbers of normal follicles (P=0.009). Numbers of normal follicles near the primary CL in two-year old cows were 2.8 ± 0.6, and 0.5 ± 0.6 follicles were associated with the secondary CL. This pattern was similar for 4-year old cows with normal follicle numbers being greater near the primary (2.4 ± 0.9) than the secondary CL (1.1 ± 0.9). Three-year old animals had more normal follicles near their secondary CL (3.2 ± 0.6) than their primary CL (2.3 ± 0.6). The distance of the closest normal vesicular follicle to the primary or secondary follicle was

not different.

Total atretic vesicular follicles were not influenced by CL type; however the distance of the closest atretic follicle varied according to CL type (P=0.03). The closest atretic follicle to the primary CL was 8.4 ± 1.6 mm, and the closest atretic follicle to the secondary CL was 4.1 ± 1.6 mm. Size of the closest atretic vesicular follicle was influenced both by CL type (P=0.04) and an interaction between CL type and age (P=0.03). Size of the closest atretic follicle was larger around the primary CL (4.1 ± 0.7 mm) than the secondary CL (2.0 ± 0.7 mm). The largest atretic follicle (5.4 ± 1.5 mm) was observed near the primary CL in 4-year old cows. Size of the atretic follicle near the secondary CL in these 4-year old cows was -0.5 ± 1.5 mm. Three-year old cows had a 3.7 mm ± 0.9 mm atretic follicle closest to their primary CL, while the size of the follicle near the secondary CL was 2.0 ± 0.9 mm. For 2-year old animals, size of the atretic follicle near the secondary CL was larger (4.4 ± 0.9 mm) than the follicle closest to the primary CL (3.2 ± 0.9 mm).

Numbers of small, medium and large vesicular follicles were not influenced by CL type. Small, normal vesicular follicles were not influenced by CL type alone, but interaction with age (P=0.006) affected the quantity of these small normal follicles. Two-year old cows had more follicles near their primary (2.8 ± 0.6) than secondary CL (0.5 ± 0.6). This pattern held for the 4-year old animals, which had 2.3 ± 0.9 small normal follicles surrounding their primary CL, and 1.2 ± 0.9 follicles around the secondary CL. More small normal follicles were located near the secondary CL (3.0 ± 0.6), than the primary CL (1.8 ± 0.6) in 3-year old cows.

CL type tended (P=0.09) to influence the number of medium-sized normal follicles. Primary CL had 0.2 $\pm$ 0.1 medium normal follicles versus 0.04 $\pm$ 0.1 follicles near the secondary CL. Numbers of large, normal follicles were unaffected by CL type. Small and large atretic, vesicular follicles were not affected by CL type, age or lactational status. Medium atretic follicles tended (P=0.09) to be influenced by an interaction between lactational status and CL type. More medium atretic follicles were found near the primary CL in lactating (0.6 $\pm$ 0.3) and nonlactating cows (1.5 $\pm$ 0.5). Lactating cows had 0.2 $\pm$ 0.3 medium, atretic follicles near their secondary CL versus 1.3 $\pm$ 0.5 follicles for nonlactating cows.

6. DISCUSSION

Results confirm previous data from histological studies of bovine ovarian tissue (Erickson, 1967; Erickson et al., 1976). As has been shown, follicle populations in the bovine are highly variable and dependent on multiple factors (Monniaux et al., 1984; Wise, 1987). Neither year responded in a uniform nor predictable manner, confirming the previous statement. While genetic heritability may determine follicle quantity at conception, management and environmental factors seem to determine how many follicles actually develop and eventually ovulate. High, significant correlations (0.44-0.99) were observed between most follicle classes in both years, indicating solid, predictable relationships among members of different follicle size and health classes. Primary and growing follicles correlated more closely with small and to a certain extent medium vesicular follicles, while medium and large vesicular follicles exhibited strong correlations between their size classes. Counts of total vesicular follicles exhibited the highest correlations with small and medium follicle classes, and did not correlate with any of the large follicle classes.

Body Weight Influence on Follicle Populations

GnRH treatment has been shown to reduce ovarian weight and stimulate oocyte maturation. Body weight of the cows influenced follicle populations in both years, but more so in **Year 2**. This was somewhat surprising as weights in **Year 1** were lower than

weights of cattle ovariectomized in **Year 2**. It must be reiterated that frame size and genetic background of these cows are unknown. Weight differences may be due simply to larger frame sizes or late-maturing cows. The range was 335 kgs to 562 kgs, with an average of 440.3 kgs in **Year 1**, while the range in **Year 2** cows was 348 kgs to 567 kgs, and an average of 463.9 kgs. These **Year 2** animals were 20 kgs heavier on average than **Year 1** cows, but most all of the experimental cows were gaining weight at the time of ovariectomy. Average daily gain was estimated to be approximately 0.70 kgs. Body weight measurements were taken eleven days prior to ovariectomy in **Year 2**, but the last available weight was collected twenty-eight days before ovary removal in **Year 1** cows. This late weigh date may have had a slight influence on reported results. Certain metabolic indicators (insulin and BUN concentrations) seem to indicate a greater nutritional stress on **Year 2** animals. Insulin concentrations were lower in **Year 2** than **Year 1** animals. This is important, as insulin concentrations are lower in fasting or restricted animals (Bassett, 1974; Richards et al., 1989b). BUN concentrations were higher in **Year 2** cows. High BUN concentrations indicate inadequate usage or excessive intake of consumed protein, and loss of body weight often follows (Kaim et al., 1983; Canfield et al., 1990). Even though these animals were gaining weight, these are animals may still be in negative energy balance. Cattle gaining weight while they are negative energy balance may improve their energy balance somewhat. However, they are still experiencing the negative effects of past body weight loss.

High body weight cows in **Year 1** had more medium vesicular, medium normal and large normal follicles, while low body weight cows had more large vesicular

follicles. This last number may reflect a larger number of atretic follicles for this weight class. These results generally agree with previous studies examining relationships between fertility, body condition, energy balance and follicle numbers in dairy and beef cattle (Wiltbank et al., 1962; Butler and Smith, 1989; Lucy et al., 1991; Dominguez, 1995; Grimard et al., 1995). Cows in better body condition had more healthy, vesicular follicles, indicating possibilities of advanced selection and recruitment capabilities for dominant follicles compared to cows in negative energy balance.

While body condition scores of these cows can only be estimated (4.0-5.0), animals in lower body condition are often in negative energy balance for longer periods of time. Ovariectomized cattle with lower body energy reserves often have lower concentrations of LH and IGF-1 (Richards et al., 1991). Beam and Butler (1997) found those dairy cattle which ovulated their first dominant follicle after being supplemented with lipids, had IGF-I concentrations 40% higher the first two weeks postpartum compared to cows that had several waves of nonovulatory follicles. Animals with superior quantities of IGF-I may achieve their first ovulation sooner during postpartum anestrus. Suppressed LH concentrations may lead to smaller follicles in low energy cows, as any dominant follicle in these cows may not receive LH concentrations necessary to facilitate its growth to ovulatory size. Beef cattle must reach lowest energy balance nadir by 31 days postpartum so they can be rebred by 41 days postpartum (Beef NRC, 1996). This allows for 10 days to recover from this lowest negative energy balance, resume cyclicity and ovulate their dominant follicle (Butler et al., 1981). Insulin concentrations begin to increase at this time, which in turn increases LH receptor number

(Amsterdam et al., 1988), critical for development of the ovulatory follicle. While beef cattle do not have the great nutrient and energy demands of lactation to contend with, they are nursing a calf. Suckling of a calf often hinders the recovery of the hypothalamo-hypophyseal-ovarian axis (Wiltbank and Cook, 1958; Casida et al., 1968; Williams and Griffith, 1995) and the release of gonadotropins critical for follicular maturation and ovulation.

Follicle numbers and diameters are often reduced early in the postpartum period. Average days postpartum for Year 1 cows was 72.2 days with a range of 35-96 days. Year 2 cows averaged 99 days postpartum and had a range of 62-154 days. Since the experimental cows were all later than 35 days postpartum, small numbers of large and medium follicles are probably not due to the influence of suckling and lactation. Weiss and co-workers (1981) found fewer 4-6 mm follicles in cows less than 35 days postpartum. Reduced follicle size is not due to a deficiency of receptors for FSH and LH by nine weeks postpartum in lower body condition cows (Rhind et al., 1992). Anterior pituitary receptors for GnRH are elevated by Day 15 postpartum, and concentration of LH in the anterior pituitary is restored by Day 30 (Nett et al., 1988). This indicates that the physiological mechanisms for ovulation are in place by 30-35 days postpartum and successful ovulation should take place unless environmental factors interfere.

Cows in **Year 2** cows responded somewhat differently. Medium body weight cows had more follicles than low or high body weight grouped animals. One possible explanation for this finding is the number of animals per group. Nine cows were included in the high group, five in the medium group and seven in the low group. As five is a

fairly low number, this may have influenced these results. These five cows may just have had higher follicle counts compared to the remainder of the experimental cows, since follicle numbers are so variable in the bovine. Considering this information, it is still safe to conclude that higher body weight animals had more follicles. While medium cows may have had the highest counts, follicle numbers for high weight cows consistently surpassed counts of low body weight cows. Medium body weight cows had the highest number of primary follicles, total vesicular follicles, total normal follicles, total atretic follicles, small follicles, medium follicles, small normal follicles, medium atretic follicles and size of the largest normal follicle. One exception was large atretic follicles, where high body weight cows had more of these follicles than the medium weight group. Again, these results may relate back to a lack of information on genetic histories of the ovariectomized cattle. When total atretic vesicular follicles and body weight were regressed, an r-square value of 0.25 resulted. This indicates a low, but significant cause and effect relationship between body weight and follicle numbers. The fact that the value is low indicates the influence of additional variables are at work.

FSH, LH and Follicle Numbers

Size of the largest normal vesicular follicle was affected in **Year 1** cows only. High LH cows had smaller follicles than low and medium LH concentration cows. Another name for this classification of follicles could be large, estrogen active follicles, as these are the follicles that should be destined to ovulate. All cows ovariectomized during this year were on Day 9 of their estrous cycle, and by this point in time, should

have developed LH receptors on follicular granulosa cells (Bodensteiner et al., 1996).

LH should not have been inhibitory to follicle growth at this stage, unless the 2.2 ng/ml average in the high LH group may have been inhibitory to follicular growth. Receptors for LH may have been down regulated or defective and non-functional. Some sort of a physiological threshold may exist, and concentrations greater than 1 or 1.5 ng/ml, as seen in our results may influence an intraovarian control system and inhibit growth, although data is lacking in support of this idea. Picton and McNeilly (1991) elucidated a threshold theory for FSH, where stimulation of FSH above a critical threshold level stimulated follicular growth. Inskeep and associates (1988) detected more LH receptors on follicles removed from postpartum cows after nine days of norgestomet treatment. Norgestomet elevates LH concentrations after six days of treatment, and subsequent estrogen production. These results seem to disagree with a threshold theory for LH. Luteinizing hormone may be acting on an intraovarian growth factor, or may stimulate prorenin production by the theca cells (Mukhopadhyay and Brunswig-Spickenheier, 1996). Prorenin is high in atretic follicles, and may inhibit granulosa cell proliferation.

Lactational status was also important in this model, with lactating cows having larger follicles than nonlactating cows. These results contrast with studies performed by Grimard and associates (1995), which found reduced follicle size, and lower LH pulse frequency in lactating beef cows on low energy diets. Additional studies with lactating animals (Rutter and Randel, 1984; Schrick et al., 1990; Wright et al., 1992) have found no effect of energy restriction on LH pulse amplitude or frequency. Reduced size of the

largest normal vesicular follicle in nonlactating cows may be due to a decrease in LH; however, lactating animals would be expected to have reduced quantities of LH. Animal numbers must be taken into consideration as we have 15 lactating cows compared to 5 nonlactating cows. Five cows may not really be enough animals to detect a true difference. LH concentrations from one blood sample can be difficult to interpret real physiological importance. Pulsatility is most important and it is difficult to speculate pulse amplitude and frequency from one sample. Increased LH pulsatility maintains the first wave dominant follicle (Roche, 1996). While it is tempting to imply that cows with high basal LH have high LH pulsatility, it is not accurate.

No FSH differences were detected in the first year; however, FSH interacted with body weight to influence various follicle populations. Cows with low FSH concentrations had more vesicular follicles in all affected follicle classes. Cows with high body weights also had higher concentrations of FSH (above 1 ng/ml) compared to medium and low body weight cows (below 1 ng/ml). Vizcarra and associates (1997) have found that FSH is not a limiting factor for ovarian activity in nutritionally deprived cattle, and any observed changes may be due to differences in release or transcription of the FSH gene. High FSH concentrations appear to be inhibitory to the growth of follicles in most size classes. These results may be due to cycleday, as cows in **Year 2** were ovariectomized on Day 9 (n=14), Day 13 (n=4) or Day 5 (n=4) of their cycle. Day 10-12 is often considered a selection period, and should be preceded by an increase in FSH (Sunderland et al., 1994).

FSH concentrations peak 1-2 days before selection of the dominant follicle in

heifers, then concentrations begin to drop when differentiation of this follicle occurs (Adams et al., 1992a). This rise in FSH should be supporting follicular growth, not inhibiting it. Follicular growth is dependent on FSH in the early stages of follicular growth, and the number of small follicles that develop depends on the level of FSH exposure (Campbell et al., 1995). The dominant follicle should be increasing in size and preparing to secrete estrogen and inhibin in order to suppress FSH concentrations and regress the subpopulation of follicles. High concentrations of FSH appear to be inhibitory to follicular growth at this point in time, perhaps indicating that selection and differentiation of the dominant follicles have already occurred. Scaramuzzi and co-workers (1993) defined a model for follicle selection based on gonadotropin responsiveness. Perhaps the ovulatory follicle is at work, suppressing FSH concentrations to a level critical for its' survival (< 1.0 ng/ml).

Differences were detected in day of the estrous cycle, with cows on Day 13 having more follicles (vesicular, small and medium vesicular, normal vesicular, small normal and atretic vesicular follicles) than cows ovariectomized on Day 9. Loss of dominance (first wave) and recruitment of a new cohort of follicles (second wave) may be occurring at this time of the estrous cycle (Roche, 1996). This is a likely explanation since smaller follicle populations instead of large follicles are affected at this time.

We must not forget the role that body weight plays in this picture. FSH seems to be associated with body weight. The picture becomes murkier when we consider that higher body weight cows had more FSH, more FSH was associated with fewer follicles and medium and high body weight cows had more follicles than lower body weight cows.

Heavier cows may have larger, more metabolically active ovaries, with larger follicles secreting more estrogen and inhibin to feedback and suppress FSH levels. Perhaps the low body weight cows are susceptible to additional metabolic stressors, and vesicular follicle populations are deprived of substrates needed for growth. Studies in the ovine (McNeilly et al., 1987; Xu et al., 1989) have shown increased ovarian follicular activity as liveweight of the animal increases. Late gestation nutrient restriction inhibits growth of the liver, while bone and the CNS are less affected (Bell and Schoknect, 1991). Growth of the ovary follows a similar allometric growth pattern and may react in a similar fashion as the fetal liver. Weight of the anterior pituitary does not change in accordance with body weight changes (Rasby et al., 1991). Boukhliq and associates (1996) studied associations between liveweight increases of FSH and pituitary responsiveness in ovariectomized sheep. Results from this study indicated that FSH concentrations were normal in heavier ewes, and that pituitary responsiveness (receptor number) to estrogen and inhibin may be reduced in these animals. Similar mechanisms may be operating in this case. Cows with increased peripheral FSH concentrations may be experiencing increasing inhibin production by the dominant follicle, and this inhibin may be acting to inhibit growth and development of follicle populations. Most animals (18/22) that had medium to high FSH levels (0.7 ng/ml-1.4 ng/ml) had high to medium levels of estrogen (4.3 ng/ml-16.15 ng/ml). This may indicate that more estrogen, and possibly inhibin, are being increasingly produced by the dominant follicle in these cattle.

Insulin-like growth factors and their associated binding proteins play key roles in folliculogenesis. Numerous studies have shown the gonadotropin potentiating effects of

these substances (Adashi, 1992; Spicer et al., 1993; Monget and Monniaux, 1995). High follicular fluid levels of IGF binding protein-3 can inhibit aromatase activity and the ability of FSH to bind to its receptors *in vitro* (Shimasaki et al., 1990). FSH actions may be dependent on the bioavailability of IGF-1 (Adashi et al., 1992). Richards and associates (1991) found that energy restricted cows had decreased peripheral levels of LH and IGF-1, but it was unknown whether intraovarian IGF-1 was decreased. IGF-I levels are high in ovarian tissue from many species, including the rat, where ovarian IGF-I gene expression is third compared to uterine and liver expression (Adashi et al., 1992). Results from a study performed by Spicer and associates (1992) showed that plasma IGF-1 decreases associated with short term fasting do not significantly influence intraovarian IGF-I levels. If this holds true for IGF-I, similar results may be expected for insulin and the various other paracrine/autocrine acting growth factors in the ovary. Even though systemic concentrations of insulin were lower in **Year 2** cows, any sort of negative energy balance they may have experienced may only have systemic and not intraovarian effects. Body weight, even though associated with more follicles, may not be as important as energy status on an individual basis (Humblot and Grimard, 1996).

Insulin, Body Weight and Follicle Populations

Insulin concentrations were higher for cows ovariectomized **Year 1** (range: 0.35-1.45, ave: 0.75 ng/ml) than cows in **Year 2** (range: 0.15-0.59, ave: 0.34 ng/ml). These data indicate a metabolic stress on **Year 2** animals, as fasting or nutritional stress causes reduced insulin levels (Bassett, 1974; Schrick et al., 1992). This is often the result of a

decrease in glucose concentrations, and thus a decrease of important metabolic substrates for amino acid uptake and metabolism, glucose uptake and steroid biosynthesis (Olefsky, 1976; Harrison and Randel, 1986). Insulin concentrations interacted with body weight, age, status and Day to affect number of primary follicles and size of the largest normal vesicular follicle in **Year 2**, while insulin concentrations tended to affect medium atretic vesicular follicles for **Year 1** cows.

Insulin concentrations without considering body weight tended to influence the primary follicle populations of **Year 2** cows. This is important because it shows that the effect may not just be mediated through body weight, it may be a direct ovarian effect. The most pronounced effects were seen in low insulin cows, as they had the lowest primary follicle counts. Medium and high insulin cows had similar follicle numbers indicating low levels may be detrimental, but a threshold can be reached where insulin concentration itself no longer plays as large a role in follicular growth.

While IGF is normally more important in endocrine-mediated tissue functions and insulin actions more peripheral, low intraovarian insulin may affect either primordial or primary follicle activation. Since intraovarian IGF's modify and amplify gonadotropins (Adashi et al., 1992), insulin probably has a similar role. High insulin concentrations may cross react with receptors for IGF-I (Spicer and Echternkamp, 1995). Wandji and co-workers (1996) hypothesized from *in vitro* studies that the ovarian stroma may contain substances that inhibit primordial follicle activation. Depriving ovarian tissue of insulin interferes with glucose and amino acid uptake and metabolism, and may not allow sufficient substrate availability for growth. Insulin is mitogenic (Peluso and Hirschel,

1987), stimulates granulosa cell mitosis (Peluso et al., 1995) and may be involved in the proliferation of granulosa cell layers which allow a primary follicle to survive atresia and continue on to complete later stages of growth.

Size of the largest normal follicle was higher in cows with lower insulin concentrations than medium and high-insulin cows. Differences between body weight means were not significant, but body weight must be included in the model to reach some sort of significance. These results disagree with a study performed by Simpson and associates (1994) where insulin treatment in a superovulation protocol increased the diameter of large follicles (11.4 vs 10.6 mm). Glucose levels were lower in insulin treated cows as well. Follicle sizes were determined via transrectal ultrasound, and while this method is adequate for measuring follicle size and growth it may not be a precise indicator of granulosa cell health. Many of these experimental cows exhibited large follicles, but many of them were atretic, as could be the case in this cited study. Cows with lower insulin levels may have higher circulating glucose levels or a reduced clearance rate, and this additional substrate available for growth of the follicle may explain why the size of the largest normal follicle was higher in low insulin cows. Lower insulin may be advantageous at certain points of the cycle, as this may eliminate premature follicular responsiveness to LH at a critical point of follicular growth. Insulin infusion in the ewe was shown to decrease glucose concentration, and consequently LH pulse frequency (Downing and Scaramuzzi, 1997). Another possibility is that low insulin concentrations may be associated with high LH pulse frequency, thus supporting higher growth rates of dominant follicles, providing that LH receptors are fully functional in

these follicles.

Body weight, nutrition status and insulin interact at the hypothalamic and pituitary levels. Insulin receptors have been identified in the anterior pituitary (Adashi et al., 1981) and hypothalamus (Landau et al., 1983). Downing and Scaramuzzi (1997) infused insulin into luteal phase, cycling ewes and detected an inhibitory response on LH secretion. Thus, indicating that insulin may influence hypothalamic and pitutary secretion. Insulin may be influencing neuropeptide Y (NPY), a neuropeptide of pancreatic origin that is found in the central nervous system. NPY fibers are located in the paraventricular nucleus of the hypothalamus (Morley, 1987) and NPY acts to stimulate food intake. Animals that have a limited nutrient intake have been shown to express increased levels of NPY (Sahu et al., 1988) and increased NPY is associated with decreased LH concentrations. Keisler and Lucy (1996) hypothesized that low insulin may allow NPY to increase, thus altering food intake and shifting nutrients away from reproductive tissues and towards tissues of higher metabolic demand. Van den Hurk and associates (1997) have found NPY nerves grow outward during primary follicle formation, indicating a possible role for his neuropeptide. This is in contrast to work by Morimoto and co-workers (1981) in which it was determined that neural control of follicular development did not occur at the primordial or preantral stage.

When insulin concentrations on different cycledays were contrasted (5, 9 and 13), cows on Day 9 had increased insulin concentrations compared to cows on Day 13. Schrick and associates (1990) found a decrease in insulin concentrations in energy restricted cows as the estrous cycle progressed (Day 11-Day 18). This decrease was

postulated to suppress glucose uptake in ovarian tissues for steroid production. Follicle counts were increased on Day 13 compared to Day 9, but 53 % (51.4/96.8) of these follicles were atretic. This may indicate that lower insulin and thus lower substrate availability for the developing granulosa cells, affects follicular health.

Insulin correlated with E_2 and P_4 concentrations in lactating animals. Campbell and associates (1995) have exhibited *in vivo* and *in vitro* evidence that insulin and IGF-I interact to affect estrogen production in the sheep. Considering this in terms of substrate availability, more insulin allows for more glucose and more amino acids available as substrates for hormone production. Since insulin increases granulosa cell mitosis (Peluso et al., 1995), increased estrogen should also be produced from these proliferating cells.

Interaction Between LH, Insulin, Body Weight and Follicles

Higher insulin levels resulted in more primary and total atretic follicles, while high LH concentrations were associated with fewer follicles. Results from this model reiterate points made previously about interactions between insulin, LH, body weight and glucose availability. Fasted animals may have lower glucose levels, but enough glucose could be available to support GnRH neurons, adequate LH secretion and follicular growth (Keisler and Lucy, 1996). High LH may be inhibitory to growth of small and medium vesicular follicles which have not expressed LH receptors during the switch from FSH to LH dependency. One of the first signs of atresia is the failure to develop LH receptors (Roche, 1996).

Blood Urea Nitrogen

Measurements of blood urea nitrogen (BUN) concentration are often a reflection of nitrogen balance in an animal (Howard et al., 1987; Ferguson et al., 1988; Canfield et al., 1990). Urea is synthesized in increased amounts when protein or amino acids are catabolized. Amino groups are removed from amino acids and converted to urea via oxidative deamination. Increased BUN values often reflect an animal with poor efficiency of protein deposition and weight loss (Canfield et al., 1990). Weight loss probably occurs due to the high ATP cost of oxidizing amino acids and converting ammonia to urea. High BUN levels usually reflect excessive quantities of highly soluble protein in the diet.

Blood urea nitrogen readings for **Year 1** averaged 18.90 mg/dl with a range of 12.88 to 26.34 mg/dl, and **Year 2** values averaged 26.25 mg/dl and ranged from 19.08 to 32.01 mg/dl. Cows in our study were fed a soybean meal protein supplement, and these BUN values may reflect that component of the diet. Soybean meal is highly soluble in the rumen and microbes may not be able to convert all of the available urea nitrogen into microbial protein unless enough fermentable carbohydrate is included to increase the microbial protein requirement. If large quantities of urea are present, the animal will convert this substance to ammonia and carbon dioxide. Ammonia present in high amounts can be toxic to mammalian cells, as evidenced in a study by Umezaki and associates (1975) where urea was employed as a spermicide.

While blood urea nitrogen affected numerous follicle classes in **Year 1**, concentrations in **Year 2** did not influence any of the follicle populations. It is unusual,

but may be due to the fact that all animals had very high readings (above 18 mg/dl) and any BUN effect may have been masked by the other metabolic influences (body weight, insulin). Blood urea nitrogen exhibited a high, negative correlation with size of the primary CL, progesterone and insulin concentrations. High urea levels may affect luteal formation due to possible toxic effects on luteal cells, as urea in high quantities may be toxic to mammalian cells (Dasgupta et al., 1970). Day 13 cows had higher BUN concentrations compared to Day 9 and Day 5 cows. Increased BUN may be occurring late in the estrous cycle due to an increase in amino acid oxidation. These animals may be mobilizing protein in an attempt to acquire glucose for ovarian substrates.

Cows ovariectomized in **Year 1** had a wider range and larger difference between their high and low BUN values on the day of ovary removal. Follicle counts decreased in the afore mentioned follicle classes as BUN concentrations increased. Higher amounts of degradable protein decrease uterine pH during in luteal phase dairy heifers (Elrod and Butler, 1993) and subsequent declines in fertility occur. Urea inhibits binding of LH to ovarian granulosa receptors *in vitro* (Canfield et al., 1990) and may act *in vivo* as well. Alterations in blood pH level could make conditions unsuitable for follicular growth. Follicles may not be able to survive and grow in a more acidic environment. Follicles that do survive may have damaged or nonfunctional oocytes due to any urea that crosses the granulosa layer into the follicular fluid. Landau and associates (1996) found that ewes fed a soybean meal versus a corn gluten meal/ground corn grain mixture had fewer follicles and that these follicles matured earlier.

Producers must determine an adequate protein supplementation level for their

cattle. Protein supplements are often costly, and as has been shown, too much protein is not only excreted, it can be detrimental to fertility. Nolan and associates (1988) have shown that diets deficient in crude protein may not respond optimally to GnRH or may exhibit reductions in pituitary gonadotropin levels. Optimizing nitrogen to energy ratios is critical to increasing cattle productivity.

Birth Weight and Weaning Weight

Associations were detected between certain follicles populations, birth weight and weaning weight (7-9 mo.) of our experimental cows, not their calves. Numerous studies have examined relationships between these variables and other reproductive parameters, but compared weights have been for calves and not the cows themselves. More relationships between these variables and follicle classes were observed in **Year 1** than **Year 2**, and this is probably due to interference of other metabolic conditions in the second year.

Populations of ovarian follicles between medium and low BWT cows were similar, and a reason for this may be numbers of animals in each group. Ten cows were placed in the medium BWT group, 8 in the low and only 2 in the high BWT group. Lack of animals in this group may lead to some of the significance observed. Weaning weight influenced primary, growing, small, small normal and small atretic vesicular follicles. Follicle counts for medium and high weaning weights were similar, but significantly higher from cows with low weaning weights for total primary and growing follicles.

Previous studies examining relationships between these variables are lacking.

Associations do seem likely due to the fact that they exist in varying degrees in both years. Not as many relationships were observed in **Year 2**, but considering various confounding factors this is not surprising. As was stated before, animal numbers may be a problem for birth weight differences observed in **Year 1**. Only two cows were in the high birth weight group and it is difficult to make conclusions on data from two cows. The second year did show a fairly high cause and effect relationship between primary follicles and birth weight. This birth weight measurement should be free of outside influence, because only in-utero, placental transport or genetic factors would be likely to affect this weight measurement. Perhaps the Polled Hereford is genetically endowed with higher primary follicle numbers and this may be reflected by higher birth weight calves. Studies relating these variables with consistent results are lacking. It is difficult to justify suggesting producers start to select cows that produce larger calves (40-50 kgs). Even though resulting animals may have a greater follicular pool, increased management problems occur (dystocia) with larger birth weight calves.

No prior studies have been located in the literature comparing weaning weight and ovarian follicle populations. Generally, medium weaning weight cows had more follicles than high and low weaning weight cows in **Year 2**, and **Year 1** medium and high weaning weights had similar, but higher follicle counts than low weaning weight cows.

It is important to note that these follicle counts have been taken at 2, 3 and sometimes 4 years of age and plenty of management factors interact to affect follicle populations between this age and 7-9 months. What is of importance is that the bovine has a set maximum number of follicles determined shortly after conception. She

continues to lose follicles throughout her life, not gain them. Using weaning weights, while not perfect, may be a simple predictor of reproductive capacity in the replacement heifer.

These data also have implications for those advocating growth promoting substances in replacement heifers. Implants, by causing increased growth, may actually increase the rate of turnover in ovarian follicle stockpiles. Growth promoting substances improve average daily gain while maintaining or slightly increasing feed intake and improving feed efficiency (Galbraith, 1980). Lipid deposition decreases while protein and lean tissue accretion increases after treatment with these substances (Galbraith, 1980). Trenbolone acetate is one such anabolic agent that when used in beef cows reduces carcass fat and stimulates lean tissue deposition. Somatotropin works in a similar fashion, involving the partitioning of nutrients for increased production or gain. Small follicles tend to be increased in lactating dairy cows treated with bST (Lucy et al., 1992). It is tempting to speculate that while improving production, bST may be increasing the depletion of follicle reserves in these cows. B-adrenergic agents are also used as growth promoting substances in ruminants. Beta-adrenergic agents are similar to the catecholamines (dopamine, norepinephrine and epinephrine) in their actions. These substances increase growth rates by influencing lipid, carbohydrate and protein metabolism. Partitioning of nutrients and increased growth promotion by these various substances may cause an increase in ovarian follicle turnover.

Progesterone and Size of the Primary and Secondary Corpus Luteum

These results indicate that counts of large follicle classes can be explained by size of the primary corpus luteum, progesterone secretion, body weight, age, status and cycleday . Medium sized CL's seemed to be associated with increased follicle counts compared to large and small, though large CL's had more follicles than small CL's. This may be due simply to ovarian surface area, or more likely, the pattern of blood flow to the CL (Usmani et al., 1985). The corpus luteum requires considerable blood flow, and follicles in the vicinity of this structure may find it beneficial to grow, and absorb nutrients that may leak from the capillaries. Bruce and Moor (1976) studied blood flow to the CL and follicles in sheep and determined that blood flow to the follicles (normal and atretic) and CL were both very high, varying slightly with cycleday. Fortune and associates (1988) reported that ovarian blood flow is highest during the luteal phase and that progesterone levels correlate with ovarian blood flow. Body weight or weight loss may influence progesterone concentrations. Hamudikuwanda and associates (1996) have determined that adipose tissue is a reservoir of progesterone and that lypolysis may serve to release this hormone into the blood stream. If this is true, high progesterone readings from cows mobilizing tissue may not be reflective of a healthy, functioning CL, but simply a loss of adipose tissue.

Observing a progesterone association with these follicles is promising, as progesterone concentrations may be able to reflect certain follicle populations. However, several variables interact to affect follicles and not just the progesterone concentrations. High progesterone concentrations are often associated with atretic follicles. More large

follicle classes were affected than small follicles, indicating the CL may speed up follicle growth or differentiation. Size of the largest normal vesicular follicle exhibited a high, negative correlation with primary corpus luteum size. This disagrees with a study by Ginther and associates (1989a) which found no effect of the CL on suppressing follicle growth. Elevated progesterone causes infrequent LH pulses during the luteal phase. Progesterone priming of LH is important for follicular selection and development (Garcia-Winder et al., 1987), as norgestomet treatment produced a dominant follicle that secreted more estrogen than nontreated cows. Subsequent production of a CL with a regular life-span may involve this selected follicle. Progesterone concentrations taken from the peripheral circulation may be misleading, particularly during the luteal phase. High progesterone values are most likely reflective of a large CL, not large quantities of atretic follicles (Wise, 1987).

No discernable models were apparent that determined size of the secondary corpus luteum; however, negative correlations were observed between this accessory corpus luteum and numerous follicle populations in both **Years 1** and **2**.

Secondary corpus luteum formation appears to exert a negative affect on follicle growth. **Year 1** results must be interpreted with caution, as only 5 of 20 cows exhibited a secondary CL. This may be too small an animal population to make accurate inferences. If the secondary CL is indeed inhibitory to small follicle growth, it may be due to the effects of recent tissue remodeling or secretion of increased TNF-α by luteal macrophages or the dominant follicle (Terranova, 1997). TNF-α has been shown to inhibit FSH induced granulosal estrogen production in the cow, and this may play a role

in reducing the population of small follicles. Some follicle classes exhibited a positive correlation with the secondary CL, and this may be due to the effects of additional nutrient-rich blood flow to the ovary.

Prolactin and Follicle Populations

No significant model was observed to explain differences in follicle number or health; however, significant correlations and regressions were identified for prolactin and several follicle populations. While regression values were fairly low, they are statistically significant. Values for nonlactating cows should be interpreted cautiously due to low animal number (n=5). While binding sites for prolactin are present on the plasma membrane of the bovine ovary (Poindexter et al., 1979), Beavers and associates (1988) determined that there are no specific binding sites for prolactin in theca and granulosa cell layers of the follicle. This probably precludes any direct effect of prolactin on follicle growth or differentiation. Prolactin may act to modify follicle populations (Weiss et al., 1981). Addition of prolactin to cultured granulosa cells acted to suppress response to gonadotropins. Recently, it has been postulated that extrapituitary prolactin may be mitogenic or act as a cytokine (Ben-Jonathan et al., 1996). Prolactin may act as a co-activator of immune cells. Prolactin may not have a direct effect on follicle population or health, but evidence exists for a modulatory role.

One point to consider in reference to prolactin concentrations is that many of these animals exhibited endocrine signs of tall fescue toxicosis. Ten out of twenty cows in **Year 1** had prolactin levels below 100 ng/ml, and 17/20 had prolactin concentrations

below 150 ng/ml. **Year 2** cows had a more pronounced effect, with 14/22 below 100 ng/ml and 20/22 below 150 ng/ml. Prolactin levels are considerably higher in summer (166 ng/ml) than in the winter months (60 ng/ml) in cattle not experiencing any inhibitory effects on prolactin levels (Benoit et al., 1987) and in cows affected by endophyte (Thompson and Stuedemann, 1993). In the bovine, prolactin levels are highest during the luteal phase (Dieleman et al., 1986). McKenzie (1993) observed reduced numbers of primary and vesicular follicles; however, Seals and associates (1996) detected no difference in follicular waves for control heifers versus those ingesting endophyte-infected fescue. With renewed interest in the extrapituitary effect of prolactin, tall fescue infected animals may be undergoing more cellular stress than previously thought.

Cow Age, Status and Cycleday

Age, status and cycleday all played roles in determining follicle quantities. In **Year 1**, cows had decreased numbers of follicles as they aged. This may be due to the fact that the 2-year old first calf heifers are still partitioning energy toward growth, not reproduction. Follicular development may be impaired by any negative energy balance they are experiencing.

Cattle, like humans and other mammals experience a reduction in follicular stock piles as they age (Erickson, 1976). Approaching reproductive senesance often accelerates loss of human follicular populations (Richardson et al., 1987). Germ-cell numbers decline as chronological age increases which may be reflected in a reduction in fertility (Talbert, 1968). Older animals, may still have viable oocytes, but often follicular

abnormalities or impaired hypothalamic function are more apparent (Erickson, 1967). Corpus luteum size decreases in aging mice (Harmon and Talbert, 1970) and this may play a role in poor follicular function.

Lactating cows had fewer total atretic, medium and medium atretic follicles than nonlactating cows in **Year 1**. This may indicate that metabolic demands associated with milk production and/or suckling may reduce follicle populations in the ovary. Suckling suppresses LH concentrations (Carruthers and Hafs, 1980), acting predominantly at the hypothalamic level and this in turn may exert an inhibitory affect on follicular growth. Pituitary stores of LH are replenished by Day 30 of the postpartum period (Nett, 1988), but the amount of GnRH acting on the pituitary is not sufficient to restore LH pulsatility. Medium follicles classes may be more susceptible to a lack of gonadotropins, elevated postpartum estradiol (Williams, 1990) or opioid tone, all of which probably play a role in regulation of postpartum follicular growth.

Cycleday impacted follicle numbers for cattle ovariectomized in **Year 2**. Cows ovariectomized on Day 9 generally had fewer follicles than cows ovariectomized on Day 13 or 5. This probably is related to the size and secretory ability of the primary CL. Corpus luteum size and development is at its greatest by Day 9. An early study on follicular populations (Rajakoski, 1960) found an inhibition of follicle growth when CL size was maximal, and that most large follicles measured approximately 5 mm. Rajakoski (1960) found fewer large follicles during Days 8-11 of the cycle in heifers, but did see at least one large follicle late in the cycle. However, while this study involved ovaries taken from animals on each day of the cycle, very low animal numbers were

employed (1-3 for each day). Cycleday correlated only with prolactin concentration and size of the largest normal vesicular follicle.

Primary Versus Secondary Corpus Luteum

Various studies have been performed in the ewe to determine if the presence of a corpus luteum has an effect on follicle number or size. Dailey and associates (1982) found no discernable relationship between the CL and diameter of the closest follicle or distance of the largest follicle. Schrick and co-workers (1993) determined that the CL had no affect on the quantity of follicles ≥ 4 mm or average diameter of the large follicles on that ovary. These previous laproscopic and ultrasound studies, while complete, are somewhat limited in that they only consider antral follicles and can not adequately judge follicular cell health. Our purpose was to evaluate all classes of follicles in the tissue section where size of the primary and secondary CL's were maximal and compare follicle populations surrounding each luteal structure.

CL type influenced distance of the closest primary follicle, total numbers of primary follicles for the tissue section, number of growing follicles for the tissue section, total normal follicles, distance of the closest atretic follicle to the CL, size of the closest atretic follicle, small normal, medium normal and medium atretic follicles. Small normal follicles were affected by an interaction between age and CL type, and medium atretic follicles were influenced by a status and CL type interaction.

One of the most striking observations is the lack of primary and growing follicles located in the tissue on the periphery of the corpus luteum, particularly the primary CL.

In most cases, plenty of tissue is available for primary follicle growth and occasionally one or two primary follicles are observed. Tissue surrounding the CL ends up consisting of about 1/3 of the ovary, which is a considerable amount of tissue to be left unpopulated. Due to the large amount of blood flow to the CL and surrounding ovarian stroma, and the lack of vascularity to primordial and primary follicles, one tends to speculate that this area of tissue could be advantageous to growth. Nutrients and other substances may leak from the CL. This may explain why this area is relatively unpopulated. An inhibitory substance or growth factor may be seeping out from the CL. Extensive tissue remodeling and cellular signaling occurs in the dynamic extracellular matrix (Getzenberg et al., 1990). Tissue inhibitors of metalloproteinases 1 and 2 (TIMP) are involved in this tissue restructuring. TIMP-1 expression is maximal in early luteal phase, while TIMP-2 is maximal during the mid-luteal phase (Smith et al., 1996). These molecules could be inhibiting growth or causing atresia of follicles in this area of the ovary. TNF-α is also involved in tissue restructuring (macrophages), and has previously been shown to be inhibitory towards small follicle populations. Luck and associates (1995) have shown that type I and IV collagens are involved in tissue remodeling as well. Numerous hydoxylases are associated with this tissue turnover, and may influence follicular atresia.

More normal vesicular follicles were observed near the primary CL than the secondary CL. This may be a result of the tissue remodeling, as atretic follicles were located closer to the secondary CL than the primary CL. Enzymes or nitric oxide released during tissue remodeling may damage adjacent follicles. Primary CL's had larger atretic follicles near them than did the secondary CL (4.1 vs 2.0 mm). More

medium-sized follicles were located near the primary CL than the secondary CL. We tend to think that the secondary CL would be demanding more of the blood supply, and that it would be more advantageous for a follicle to be closer to that structure. Apparently, the extracellular matrix has not yet recovered and is still exerting an inhibitory affect.

In summary, this study confirms previous conclusions that follicle populations in the bovine ovary are extremely variable and influenced by a myriad of factors. Genetics may determine follicle numbers that a cow is endowed with, but how she is managed throughout her reproductive life will influence how quickly her follicular pool is diminished. Nutrition and body weight play important roles along with endocrinology in predicting fertility. Weaning weight may be a useful production tool, as it has been shown to predict primary, growing and small follicular populations. Negative energy balance may interact with FSH concentrations to influence follicle numbers. The corpus luteum may be playing a key role in the regulation of primary follicle populations. Serious implications may exist if the primary CL is indeed inhibiting primary follicle growth, or speeding up ablation of primary follicles from the ovarian pool. Superovulation may be limiting the reproductive lifespan of cattle. If indeed the CL does increase follicular loss, the cow may become a feasible model for the study of human senescence and infertility. Since numerous factors influence ovarian architecture, it is difficult to pinpoint one true indication of follicular growth and development. Hormone concentrations alone do not conclusively reflect numbers of follicles available; however, they must be included in a complex and tortuous picture of ovarian physiology.

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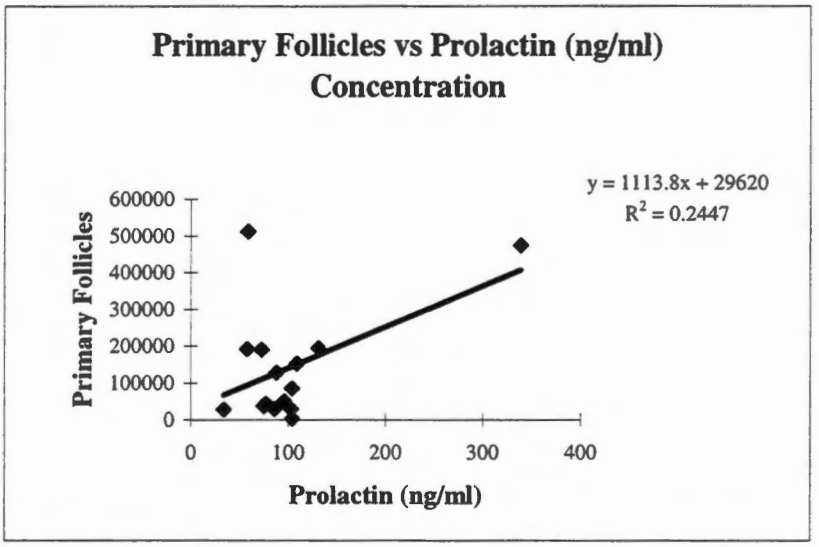
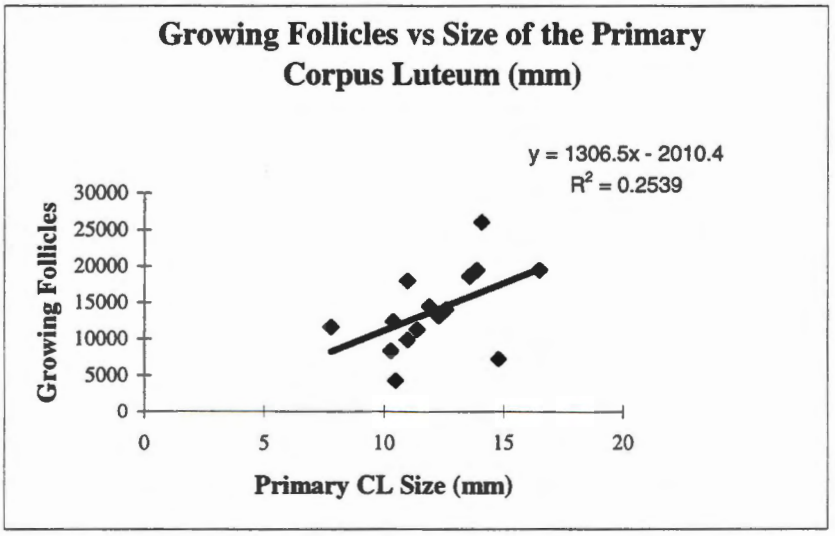
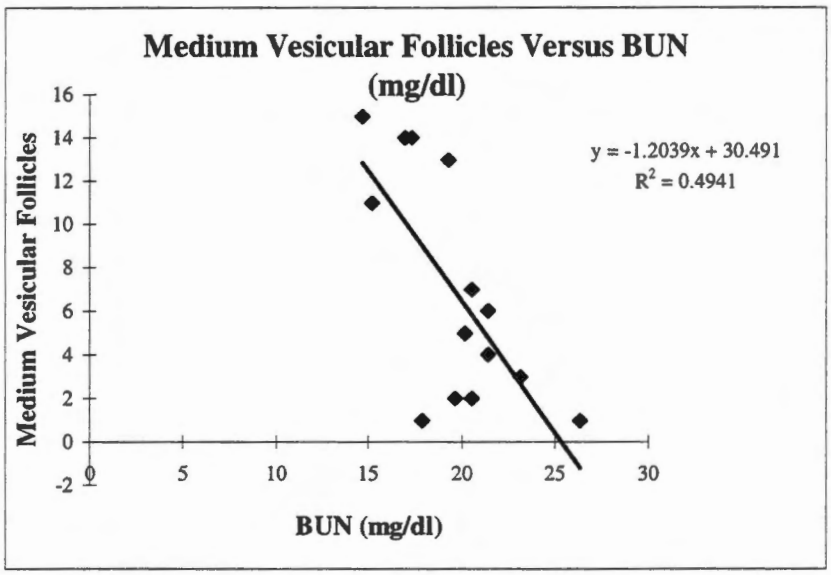
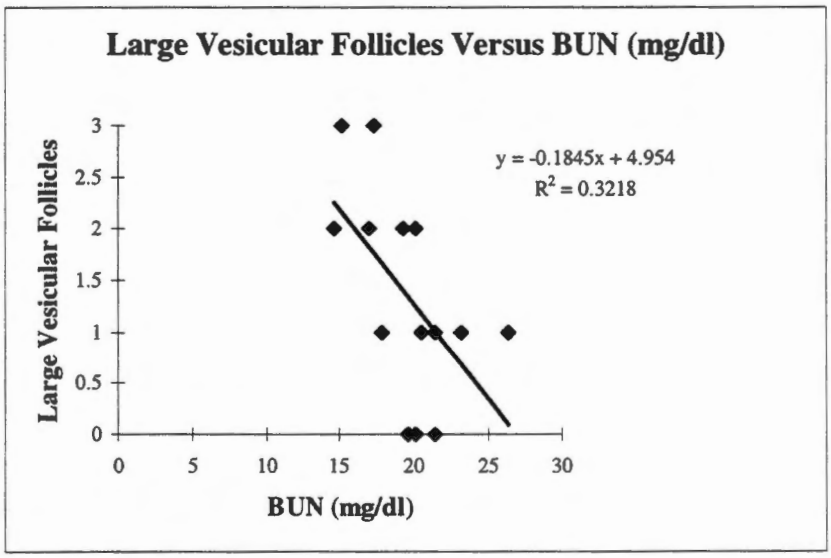
APPENDIX

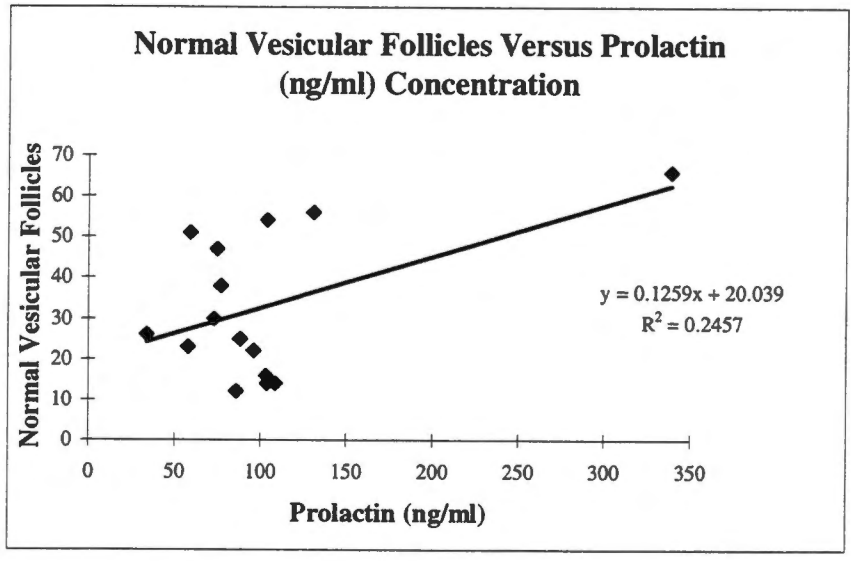
Regression Scatter Plots for Lactating Cows Ovariectomized **Year 1**

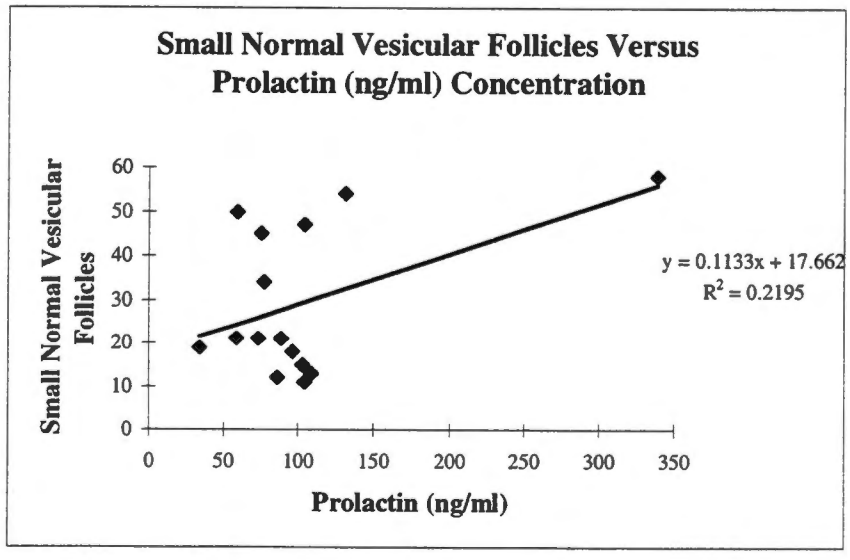


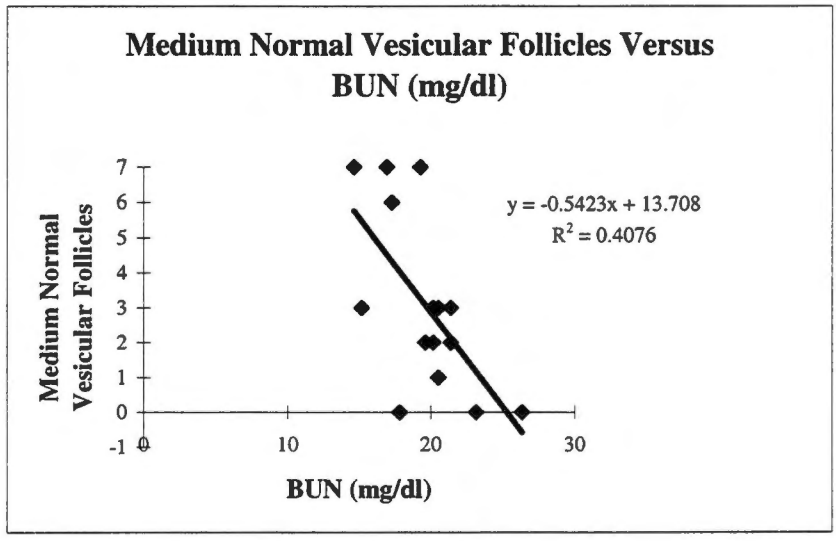


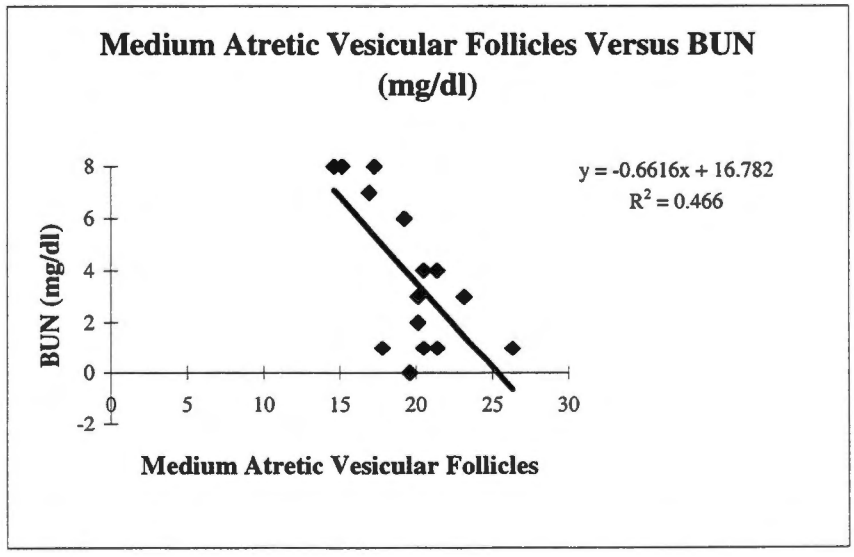

Medium Vesicular Follicles Versus BUN (mg/dl)
Medium Vesicular Follicles
BUN (mg/dl)
y = -1.2039x + 30.491
R2 = 0.4941
16
14
12
10
8
6
4
2
0
-2
0
5
10
15
20
25
30


Large Vesicular Follicles Versus BUN (mg/dl)
Large Vesicular Follicles
BUN (mg/dl)
y = -0.1845x + 4.954
R2 = 0.3218
3
2.5
2
1.5
1
0.5
0
0
5
10
15
20
25
30

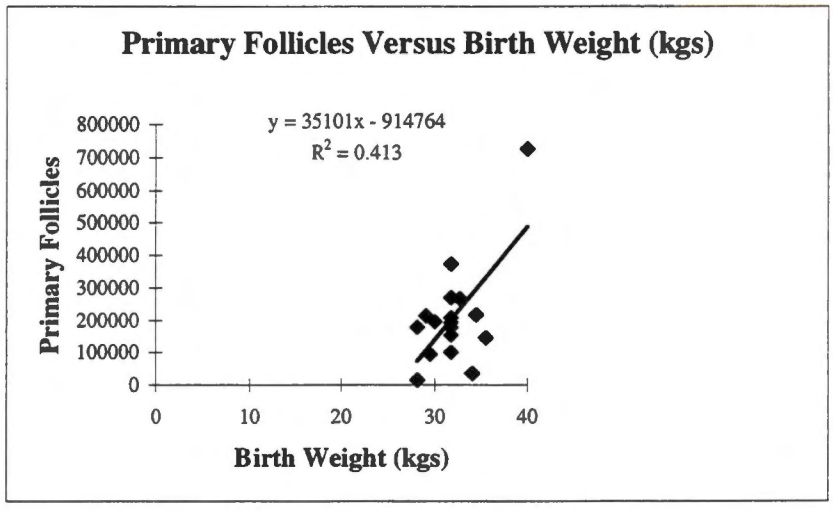

Normal Vesicular Follicles Versus Prolactin (ng/ml) Concentration
Normal Vesicular Follicles
70
60
50
40
30
20
10
0
0
50
100
150
200
250
300
350
Prolactin (ng/ml)
y = 0.1259x + 20.039
R2 = 0.2457

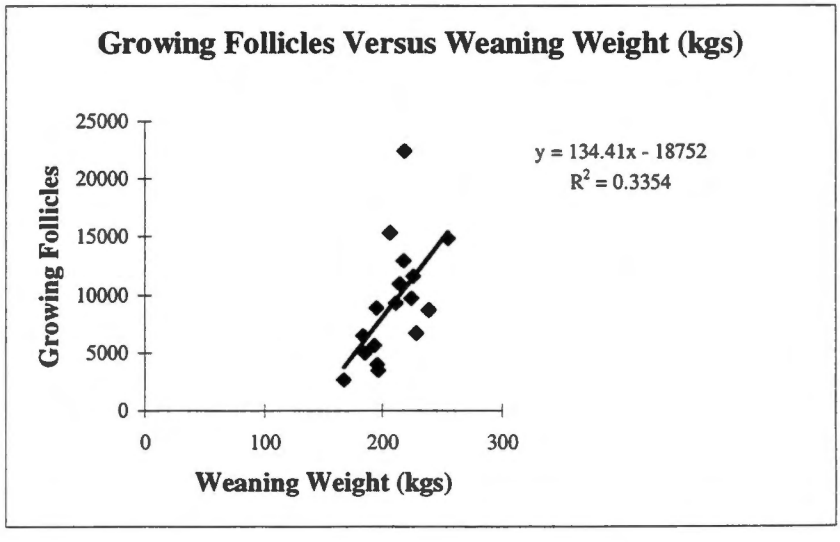

Small Normal Vesicular Follicles Versus Prolactin (ng/ml) Concentration
Small Normal Vesicular Follicles
60
50
40
30
20
10
0
0
50
100
150
200
250
300
350
Prolactin (ng/ml)
y = 0.1133x + 17.662
R2 = 0.2195

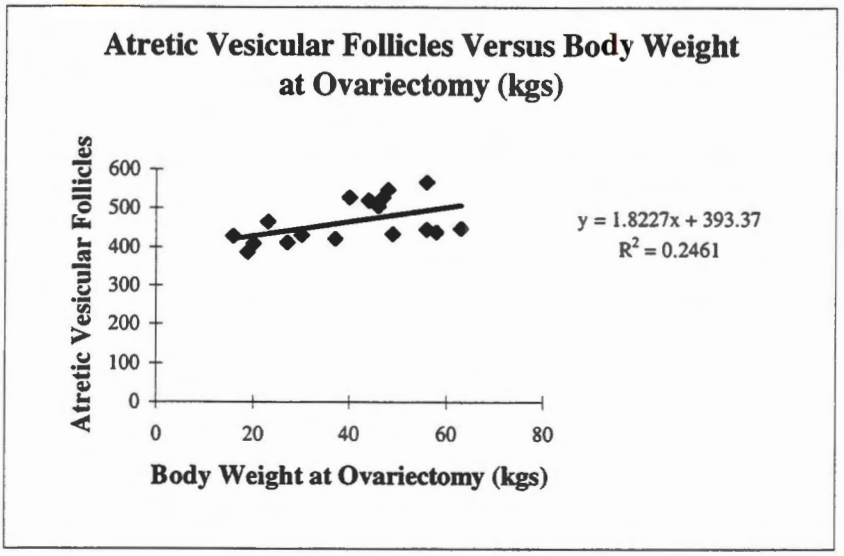

Medium Normal Vesicular Follicles Versus BUN (mg/dl)
Medium Normal Vesicular Follicles
BUN (mg/dl)
y = -0.5423x + 13.708
R2 = 0.4076
7
6
5
4
3
2
1
0
-1
0
10
20
30

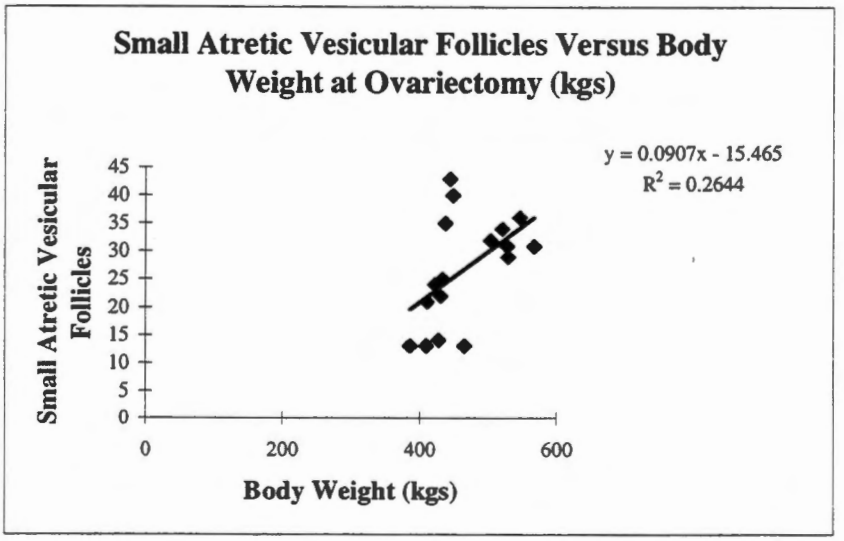

Medium Atretic Vesicular Follicles Versus BUN (mg/dl)
BUN (mg/dl)
Medium Atretic Vesicular Follicles
y = -0.6616x + 16.782
R2 = 0.466
8
6
4
2
0
-2
0
5
10
15
20
25
30

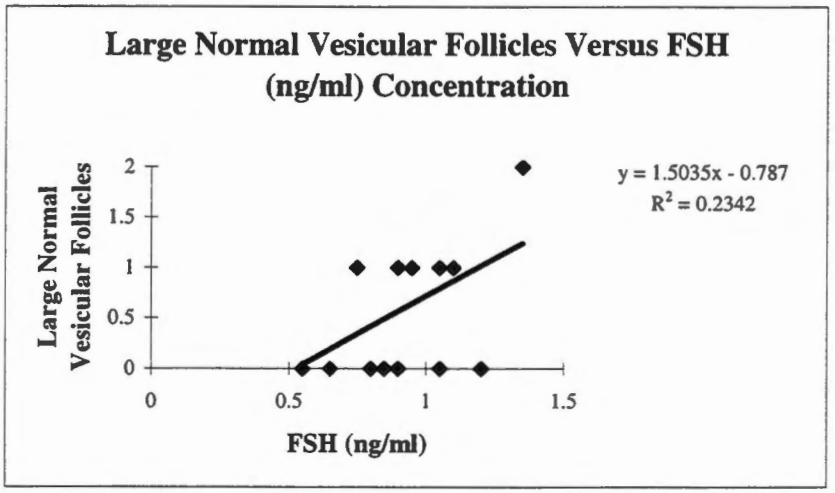
Regression Scatter Plots for Lactating Cows Ovariectomized **Year 2**

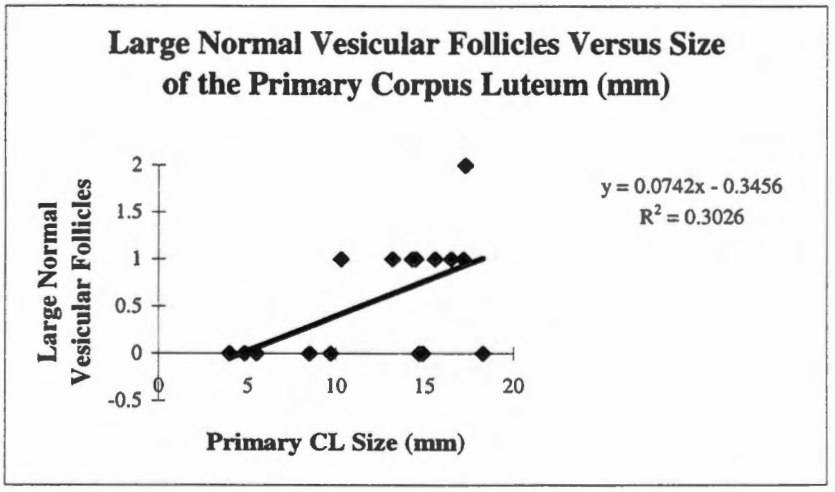





Atretic Vesicular Follicles Versus Body Weight at Ovariectomy (kgs)
Atretic Vesicular Follicles
600
500
400
300
200
100
0
0
20
40
60
80
Body Weight at Ovariectomy (kgs)
y = 1.8227x + 393.37
R2 = 0.2461


Small Atretic Vesicular Follicles Versus Body Weight at Ovariectomy (kgs)
Small Atretic Vesicular Follicles
45
40
35
30
25
20
15
10
5
0
0
200
400
600
Body Weight (kgs)
y = 0.0907x - 15.465
R2 = 0.2644


Large Normal Vesicular Follicles Versus FSH (ng/ml) Concentration
Large Normal Vesicular Follicles
2
1.5
1
0.5
0
0
0.5
1
1.5
FSH (ng/ml)
y = 1.5035x - 0.787
R2 = 0.2342


Large Normal Vesicular Follicles Versus Size of the Primary Corpus Luteum (mm)
Large Normal Vesicular Follicles
2
1.5
1
0.5
0
-0.5
0
5
10
15
20
Primary CL Size (mm)
y = 0.0742x - 0.3456
R2 = 0.3026

VITA

Heather L. McLane was born in Auburn, New York on March 18, 1972. She attended catholic and public schools in the Auburn area and graduated from Auburn High School in June, 1990. In August of that year she entered Cornell University and graduated with a Bachelor of Science degree in Animal Science in May of 1994. She then entered The University of Tennessee, Knoxville in August, 1994 and officially received her Master's degree in August of 1997.

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