NUTRITIONAL REGULATION OF FETAL DEVELOPMENT AND PITUITARY, OVARIAN, AND THYROID FUNCTION IN BEEF COWS

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CHAPTER I

INTRODUCTION

Grasslands, that can not be tilled or harvested with conventional machinery, encompass vast areas of the United States. For this reason the beef cow plays an important role in agriculture. A cow can harvest grass from areas not suitable for farming and, via the rumen, convert low quality forages into energy and high quality protein.

The primary purpose of the beef cow is to produce a calf every 12 months. To maintain a yearly calving interval she must become pregnant by 85 days after calving. Because gestation length is fixed (about 282 days), the only stage of a cow's production cycle that can be altered is the length of the postpartum anestrous interval (time from calving to first estrus). Cows losing weight and body condition prior to calving have extended intervals from calving to first estrus (Dunn and Kaltenbach, 1980; Wettemann et al., 1982; Selk, 1986). Therefore, nutritional management before calving dictates the length of the postpartum anestrus interval.

About seventy percent of the cows exposed to fertile bulls wean a calf the subsequent year (Bellows et al.,

1979). Of the 30% of the cows that do not wean a calf, 17% fail to become pregnant during the breeding season (Bellows et al., 1979). Because of the narrow margin of profit in the cow-calf industry, cattlemen demand an increase in reproductive efficiency of beef cows; not to flood the market place with beef, but to produce as many pounds of calf with fewer cows. This would allow ranchers to free up land for other marketing options, such as retained ownership of calves, so as to increase potential profit margins.

Nutrition, body condition, and prepartum weight change influence reproductive efficiency of beef cows (Dunn and Kaltenbach, 1980; Wettemann et al., 1982). However, there is limited information on the influence of body condition, weight change, and nutrition on the concentration of hormones and metabolites in blood. Recent research indicates that varying the amount of supplemental protein prepartum influences the concentration of protein and glucose (Rasby et al., 1982) and estrone and progesterone in plasma (Mobley et al., 1983) of range cows.

An animal's energy reserve may influence the synthesis and(or) secretion of hormones. Animals in different body condition may differ in amounts of energy metabolites present in blood that are necessary for cellular functions. This may be the major cause of reproductive inefficiencies of cows in thin body condition. Therefore, we designed two experiments to determine the influence of body condition on reproduction. The first experiment used pregnant Hereford

cows slaughtered on day 260 of gestation to determine the influence of body condition on fetal and placental development. The second experiment used nonpregnant Hereford cows to investigate the influence of body condition on pituitary, thyroid, and ovarian function.

These experiments will aid in evaluating the influence of nutrient intake and body energy reserves on reproductive performance of beef cows. Cows losing weight and body energy reserves prepartum have extended intervals from calving to first estrus. Therefore, prepartum nutrition has a major impact on the number of cows exhibiting estrous cycles during the breeding season. Understanding hormonal changes that accompany reduced nutrient intake and changes in body energy reserves could result in feeding regimes that not only make efficient use of feed resources but increase cow productivity.

CHAPTER II

LITERATURE REVIEW

Mechanism(s) controlling the length of time from parturition to first estrus influences productivity and reproductive efficiency of farm animals. The objective of this section is to review factors affecting reproduction in farm animals. Understanding early embryonic development, energy demands of the fetus on the dam, how the dam accommodates nutrient demands of the fetus, and the influence of nutrition and body condition on reproductive function is essential in determining factors that affect reproductive efficiency.

Placentation In The Ewe, Cow, And Sow

After fertilization the zygote migrates from the oviduct to the uterus. As the zygote migrates to the uterus it undergoes cellular division forming a clump of cells referred to as the morula. After compaction, the morula differentiates into a hollow ball of cells and is referred to as a blastocyst with a fluid filled cavity called the blastocoel. The blastocyst consists of two cell layers, the inner endoderm and the outer ectoderm or trophoblast (Perry,

1981). The embryo forms from a thickened area of the trophoblast referred to as the embryonic disc, and the mesoderm is derived from the embryonic disc that expands between the extra-embryonic endoderm and trophectoderm. To this point, the blastocyst is surrounded by the zona pellucida, but once the zona is lost placenta, formation begins and ends when the fetal placenta comes into contact with the maternal epithelium for the exchange of nutrients to support fetal development.

The three germ layers devoted to placental formation include the endoderm, mesoderm, and trophectoderm (Perry, 1981). The amnion, the placental membrane closest to the fetus, consists of two layers, an inner ectoderm and outer mesoderm (splanchnopleure). The yolk sac and allantoic membrane consist of an inner endoderm and outer mesoderm. The chorion consist of an inner mesoderm and outer trophectoderm (somatopleure).

For this review, placentation is referred to when the trophoblast attaches to the uterine epithelium without eroding into the uterine stroma. Placentation occurs in the sow, cow, and ewe. Implantation is referred to when the trophoblast actively invades the maternal epithelium. Implantation occurs in rodents and women.

Before placentation can occur, the embryo must signal the maternal system of its presence in the uterus. The mechanism for normal luteolysis must be altered so that the corpus luteum of pregnancy is maintained.

Maintenance of Corpora Lutea Function in Early Pregnancy

Maternal recognition of pregnancy in the pig occurs on about day 12 or 13 of pregnancy (Ford et al., 1982) and the signal appears to be estradiol of blastocyst origin (Perry et al., 1973; 1976). It is postulated that estradiol causes the uterus to release $PGF_{2}\alpha$ in an exocrine fashion, into the uterine lumen, instead of via an endocrine fashion, into the uterine vasculature (Bazer et al., 1982). Release of $PGF_{2}\alpha$ in an exocrine fashion protects the corpus luteum from its luteolytic influence and the source of progesterone for pregnancy is maintained. Recent evidence indicates that a second release of estradiol from pig blastocysts between days 15 to 18 of pregnancy may be necessary to reinforce the first signal to insure maintenance of pregnancy (Geisert et al., 1986).

In the ewe, the corpus luteum is composed of two cell types, large and small luteal cells (Flint et al., 1982). Small luteal cells (12-22 um in diameter) possess receptors for luteinizing hormone (LH) and secrete progesterone in response to LH stimulation. Small luteal cells contain a small number of $PGF_{2}\alpha$ receptors. In contrast, large luteal cells (20-40 um in diameter) possess a small number of LH receptors but secrete large amounts of progesterone in the absence of LH stimulation. However, large luteal cells contain a large number of $PGF_{2}\alpha$ receptors. Maternal recognition of pregnancy in the ewe occurs between days 12 and 13 of gestation (Moore and Rowson, 1966), corresponding

to time when the corpus luteum is composed of large luteal cells with many $PGF_2\alpha$ receptors.

Demise of the corpus luteum in the ewe begins between days 13 and 14 of the estrous cycle. Recent evidence indicates that the pulsatile release of $PGF_2\alpha$ on days 13 and 14 of the estrous cycle in nonpregnant ewes is absent on day 14 in pregnant ewes (McCracken et al., 1984a; McCracken, 1984b). McCracken et al., (1984a) postulated that the demise of the corpus luteum in the ewe occurs by the following mechanism: 1. Concentrations of progesterone in plasma decreases and concentrations of estrogen in plasma increases toward the end of the estrous cycle. 2. Increasing estrogen during the estrous cycle stimulates the uterine endometrium to form oxytocin receptors. 3. Endogenous luteal oxytocin interacts with oxytocin receptors on the uterus stimulating $PGF_2\alpha$ secretion into the ipsilateral uterine vein, and $PGF_2\alpha$ begins to cause lysis of the ipsilateral corpus luteum. 4. Luteal oxytocin continues to be released upon $PGF_2\alpha$ stimulation, reinforcing the release of $PGF_2\alpha$ from the uterus.

To establish pregnancy in the ewe the luteolysin must be controlled. Sheep blastocysts secrete a low molecular weight glycoprotein (oTP1, MW 17,000 - 21,000) between day 12 and 21 of pregnancy (Godkin et al., 1982). Trophoblastin, a protein found in homogenates of sheep blastocysts that may be related or the same as oTP1, when infused into the uterus causes a prolonged maintenance of the corpus luteum (Martal

et al., 1979). However, the mechanism by which these proteins produced by the blastocyst maintains corpus luteum function remains elusive. Studies comparing the concentrations of $PGF_2\alpha$ in the uteroovarian vein of pregnant and nonpregnant ewes are contradictory (see review Bazer and First, 1983). However, recent evidence suggests that pulsatile release of oxytocin-neurophysin and $PGF_2\alpha$ (detected by measuring systemic concentrations of the prostaglandin metabolite (15-keto, 13-14 dihydro prostaglandin $F_2\alpha$ metabolite) is suppressed in pregnant compared to nonpregnant ewes (Moore et al., 1982). This suggests that the glycoprotein produced by the blastocyst may suppress oxytocin release by the corpus luteum that either reduces release of $PGF_2\alpha$ from the uterus or directly suppresses $PGF_2\alpha$ secretion from the uterine endometrium. However, the glycoprotein produced by the blastocyst may influence $PGF_2\alpha$ receptors on the ovarian luteal cells. The corpora lutea of pregnant ewes appeared less susceptible to $PGF_2\alpha$ than the corpus luteum of nonpregnant ewes (Pratt et al., 1977).

Maternal recognition of pregnancy in the cow occurs between days 14 and 16 of pregnancy. Cows that had embryos removed on days 17 or 19 of pregnancy exhibited longer interestrous intervals than cows with embryos removed on day 13 of pregnancy (Northy and French, 1980). This experiment also demonstrated the antiluteolytic effect of the bovine embryo. The day 16 to 24 bovine blastocyst produces a major

low molecular weight protein and a high molecular weight glycoprotein, one of which may signal the maternal system of its presence (Bartol et al., 1982). However, the day 16 to 17 bovine blastocyst can also convert androstenedione to estradiol (Eley et al., 1979). The involvement of estradiol in bovine maternal recognition scheme is not understood.

$PGF_2\alpha$ appears to be the luteolytic agent in the cow. Passive immunization of cows against $PGF_2\alpha$ prolongs the estrous cycle (Fairclough et al., 1981), and injecting $PGF_2\alpha$ shortens the life span of the corpus luteum (Lauderdale, 1972). However, it appears that follicular development occurring around day 15 of the estrous cycle regulates the regression of the corpus luteum. Luteolysis occurred earlier in heifers treated with E_2-17 β on day 13 of the estrous cycle compared to control heifers (Knickerbocker et al., 1984). It appears that the presence of bovine conceptus alters $PGF_2\alpha$ response to E_2-17β. Pregnant heifers exhibited a reduced PGFM response when injected with E_2-17β compared to nonpregnant heifers (Thatcher et al., 1985). The antiluteolytic product of the bovine embryo may influence estrogen induced oxytocin receptors on the uterus similar to that which occurs in the ewe. Oxytocin (Shams, 1983) and neurophysin (Shams et al., 1985) are produced and secreted by the CL into venous drainage. The antiluteolytic product may influence oxytocin and neurophysin production by the corpus luteum or directly effect $PGF_2\alpha$ production by the uterus to maintain CL function for pregnancy.

Maternal Immunological Changes to Support Pregnancy

Once the blastocyst enters the uterus, the maternal immune system must be altered so that the blastocyst is not rejected. The immune response of T and B cells may be blocked by either antigens shed by the trophoblast, antibody-antigen complexes produced during pregnancy, or suppressor T lymphocytes that are produced during pregnancy (Beer and Billingham, 1979). In addition, uterine secretions of the cow (Roberts, 1977), sow (Murray et al., 1978), and ewe (Segerson, 1981) contain a immunosuppressive factor(s).

Placental hormones such as human chorionic gonadotropin (hCG) and pregnant mare serum gonadotropin (PMSG) may play a role in suppressing the immune system of the dam. Glycoproteins produced by the blastocyst may coat the conceptus and prevent the maternal lymphocytes from recognizing antigens produced by the conceptus (Masters et al., 1983). Hormones of maternal origin may modify the immune system of the dam so that the fetoplacental unit can be tolerated by the dam. The concentration of progesterone in lymph of sheep appears to be 10 to 1000 fold greater than in the peripheral circulation between days 15 and 45 of gestation (Staples et al., 1982). These data suggest that leukocytes in the lymph are exposed to high concentrations of progesterone possibly causing an immunoprotective function. Prostaglandins (F and E series) produced by the endometrium and conceptus may play a role as immunoprotective agents (Godwin and Webb, 1980).

Another immunological barrier may be formed when migrating binucleate cells of the chorion fuse with maternal epithelial cells of the endometrium to form multinucleated giant cells (Wooding et al., 1984). This syncytium represents a fetomaternal hybrid tissue that may allow the fetoplacental unit to exist as a foreign tissue in the uterus.

Placentation in the Sow

The embryo cannot survive an entire pregnancy without attaching to the uterine endometrial epithelial surface. Attachment of the fetal membranes to the maternal endometrium provides an avenue for the transport of nutrients and waste products between the conceptus and the maternal blood system.

Attachment of the fetal placental membranes of the pig blastocyst to the maternal uterine epithelium of the sow is considered noninvasive. However, if the pig blastocyst is transplanted to an ectopic site outside the uterine lumen, it will implant into the tissue in an invasive manner (Samuel, 1971; Samuel and Perry, 1972). Plasminogen activator, produced by the trophectoderm, stimulates invasive growth of the blastocyst (Ossowski et al., 1973). The endometrium of the pig secretes a progesterone induced protease inhibitor that protects the uterus from plasminogen activator (Fazleabas et al., 1982).

Placentation in the sow occurs in three phases; apposition, adhesion, and attachment. Apposition begins

about day 12 and ends about day 18 of pregnancy, adhesion begins about day 13 and ends about day 20 of pregnancy, and attachment begins about day 16 and ends about day 24 of pregnancy (King et al., 1982).

Day 11 pig blastocysts elongate into a filamentous form (Geisert et al., 1982) and become trapped in the folds of the uterus without overlapping one another (Anderson et al., 1978). Each blastocyst occupies a specific portion of the uterine lumen. Myometrial contractions aid in the spacing process but diminish as apposition begins; therefore, the blastocyst can no longer move freely within the uterine lumen. On about day 14 there is a slight dilation in the area of the embryonic disc of the blastocyst to insure closer apposition to the endometrium, then adhesion begins in this area (King et al., 1982). After dilation is completed in the disc area, the allantois begins to develop and fluid accumulates which causes the cavity to distend, and the area of contact between the fetal membranes and the maternal epithelium increases. This process initiates adhesion (King et al., 1982). On day 19 of gestation, the mesodermal lining of the allantois begins to make contact with the chorion. The blood vessels of the mesoderm penetrate into the chorion and by day 30 of gestation the chorion is heavily vascularized by allantoic blood vessels (Wislocky and Dempsey, 1946).

Ultrastructural features of the uterine lumen examined on day 11 of pregnancy, indicate that the low columnar

epithelium has flattened apical surfaces sparsely covered with microvilli (King et al., 1982). From apposition to attachment, the maternal epithelial cells change from flat columnar cells with no rounded protuberances to tall columnar cells with rounded protuberances (King et al., 1982). Placentation occurs when the microvilli of the trophectoderm interdigitate with the projections of the tall columnar cells of the uterine epithelium. The chorionic microvilli never penetrate the basal lamina of the uterus; therefore, attachment is considered noninvasive. Attachment is complete by day 24 of gestation.

Areolae, modified regions of the chorion that are in close contact with openings of the uterine glands, appear by about day 17 of gestation (King et al., 1982). The areolae become larger as pregnancy progresses. Exchange of readily diffusible substances occurs at the sides and tips of the areolae and larger substances are transported at the base (Friess et al., 1980). Gaseous exchange occurs in interareolea areas, a place where the vascular bed of the chorion is in close proximity to the maternal capillaries (Perry, 1981).

Placentation in the Cow

Placentation in the cow and ewe occurs in three distinct but overlapping phases; apposition phase, adhesion phase, and attachment phase (King et al., 1982). The final phase brings about the formation of a placentome, (interdigitation of the maternal caruncle and fetal

cotyledon) an area where nutrients and gaseous exchange between the maternal and fetal system occurs.

After hatching through the zona pellucida, the blastocyst orients itself in preparing for attachment. Trophoblastic cells of the day 10 to 16 bovine conceptus are covered with a network of microvilli that may be involved in absorbing extracellular products prior to attachment (Guillomot and Guay, 1982). Apposition of the trophoblast to the uterine epithelium begins between days 17 and 18 of pregnancy (King et al, 1981). During the apposition phase, the height of uterine epithelial cell is reduced, possibly in response to the presences of the trophoblast (King et al., 1981). Cytoplasmic projections that appear on the uterine cell surface between days 12 and 21 of pregnancy may be involved with either an apocrine or merocrine type secretion (King et al., 1981; Guillomot and Guay, 1982).

Adhesion of the chorion to the caruncle can be detected as early as day 20 of gestation (King et al. 1980). Microvilli are not present on all trophoblastic cells, but only those cells possessing microvilli form cotyledons. By day 24 of gestation, microvilli on the trophoblast begin to interdigitate with the maternal epithelium, and by day 27 of gestation, definite placentomes are formed. The caruncular surfaces on the uterine lumen appear smooth between days 20 and 29 of gestation (King et al., 1980). Also at this time there is intercotyledonary and intercaruncular attachment (King et al., 1981). The trophoblast overlying the uterine

gland openings attach to the uterine lumen and may be involved in absorbing products produced by the uterine glands (Guillomot and Guay, 1982). Between days 30 and 35 of gestation, villi and crypts form in between the cells of the trophoblast and the maternal caruncles protrude and become ridged. Microvilli, villi, and crypts interdigitate the caruncular ridges to form the placentomes.

Binucleate cells, derived from unicellular cells at the basement membrane of the chorion, begin to migrate toward the maternal epithelium between days 17 and 19 of gestation. Between days 20 and 29 of gestation, binucleate cells derived in the region of the embryo come into contact with the maternal epithelium (Greenstein et al., 1958; King et al., 1980; Wathes and Wooding, 1980; Wooding, 1984). As the binucleate cell migrates toward the maternal epithelium, there is an increase in the number of granules inside the cell. It is hypothesized that these granules contain placental lactogen (Flint et al., 1979).

The chorion maintains close association with the maternal epithelium beginning about day 20 of gestation until parturition. Migrating binucleate cells of the chorion fuse with the epithelial cell of the uterus to form multinucleated giant cells (King et al., 1981; Wooding, 1984). By day 24 of gestation, multinucleated giant cells account for about 50% of the uterine epithelial area (Wathes and Wooding, 1980). Binucleate cell migration continues throughout gestation in the cow, but the formation of

multinucleated cell ceases on about day 28 of pregnancy.

The formation of multinucleated cells is considered a type of syncytium (King et al., 1981; Wooding, 1984). This syncytium reduces the distance between the fetal and maternal capillary supplies; therefore, products of fetal origin can be delivered to the maternal system by the binucleate cell.

Bovine Fetal Development

Data pertaining to growth of fetal membranes and accumulation of fetal fluids are sparse. Most researchers restrict themselves to studies of total fluid volumes and total membrane weights; therefore, only limited data are available on volumes of fluid in individual membranes and weights of individual membranes of the conceptus.

Bovine fetal weight in utero can be best described by a fourth order regression equation (Winter et al., 1941; Eley et al., 1978). By about day 200 of gestation, the fetus is only 25 to 30 percent of it's predicted weight at parturition (Ferrell et al., 1976). Therefore, 75 to 80 percent of fetal weight gain occurs during the last 80 to 85 days of gestation. This illustrates the rapid weight gain that occurs during the last trimester of pregnancy. The rapid growth also indicates the increased nutrient requirement of the dam to support fetal growth during the last trimester of pregnancy.

Growth rate of the fetus is greatest between days 80

and 220 of gestation, then decreases from day 222 of gestation to parturition (Rattray et al., 1974; Ferrell et al., 1976; McKeown et al., 1976; Eley et al., 1978). This indicates that growth rate of the fetus in late gestation exceeds the ability of the maternal and placental unit to supply nutrients for rapid growth.

Growth of the fetal membranes between days 40 and 100 of gestation is best described by a second order regression equation (Eley et al., 1978). Development of the fetal membranes precedes fetal development (Ferrell et al., 1976; Eley et al., 1978). In addition, uterine development precedes both fetal membrane and fetal development (Ferrell et al., 1976). This suggests that fetal development depends on prior development of the uterus and fetal membranes that supply nutrients to the fetus.

Total volume of fluids accumulating in the bovine extraembryonic membranes between days 80 and 280 of gestation are best described by a third order regression equation (Authur, 1969; Eley et al., 1978; 1979). However, after separating total volume of fluids into allantoic and amniotic fluids, allantoic fluid volume accumulating in the extraembryonic membranes during the first 100 days of gestation is best described by a linear regression equation and amniotic fluid volume accumulating in extraembryonic membranes during the first 100 days of gestation is best described by a fourth order regression equation (Eley et al., 1978). Allantoic fluid accumulates at a faster rate

during the first 60 days of gestation; then, allantoic and amniotic fluids accumulate at about the same rate until 100 days of gestation. In the pig (Knight et al., 1977), there are two major times in gestation that allantoic fluid volume increases. The increase between days 20 and 30 of gestation and appears to be associated with the expansion of the chorioallantoic membrane and establishment of the initial contact between the membranes and endometrium. Rate of increase in allantoic fluid volume is correlated (r=.90) with the concentrations of estrone sulfate in allantoic fluid (Knight et al., 1977). Estrogens can alter permeability of cells to water (Szego and Sloan, 1961) and change electrolyte movement that may influence fluid accumulating in the allantoic sac (Goldstein et al., 1980).

Bazer et al., (1981) proposed that the uterine arterial progesterone to estrogen ratio influences electrolytes and the amount of fluid accumulating in the porcine allantoic sac. A wide progesterone to estrogen ratio (concentration of estrogen greater than progesterone) causes fluid to accumulate in the allantoic sac, and as the progesterone and estrogen ratio narrows, fluid ceases to accumulate in the allantoic sac. During a time when the progesterone to estrogen ratio is small, Na^+ is actively pumped out of the fetal system into the maternal system; therefore, a membrane potential is established between the maternal and fetal blood supplies. As the progesterone and estrogen ratio increases, Na^+ begins to flow into the fetal blood supply

and Na^+ causes movement of water and glucose along with it, causing an increase in Na^+, water, and glucose in the fetal blood supply.

Porcine amniotic fluid volume appears not to be influenced by estrogen or progesterone; therefore, the mechanism by which fluid accumulates in the allantoic and amniotic cavities are under different controls (Bazer et al., 1981). However, in rodents, prolactin may have a role in accumulating water in the amniotic and allantoic cavities (Holt and Perks, 1975; Manku et al., 1975). Less water accumulates in the allantoic cavity in the absence of prolactin; whereas, prolactin caused water to be retained in the amniotic cavity.

Hormones, Nutrients, and Constituents in Fetal Fluids

The amniotic and allantoic fluids have interested scientists for many years. It was first thought that the allantoic fluid originated from the mesonephros and served as a reservoir for fetal waste. These functions are not supported by research. The mesonephros does not produce water but redistributes the water available to it (Bazer and First, 1983). In addition, the allantoic fluid contains a pool of nutrients and electrolytes that can be redistributed according to needs of the fetus (Bazer et al., 1981). The amniotic fluid bathes the fetus, aids in protecting the fetus, but also allows the fetus to develop in a liquid environment independent of the forces of gravity (Bazer et al., 1981).

The principle conjugated estrogen in bovine amniotic and allantoic fluids is estrone sulfate (Robertson and King, 1975; 1979; Eley et al., 1979) and estrone is the major unconjugated estrogen (Robertson and King, 1975; 1979; Eley et al., 1979).

Concentrations of estrone sulfate in bovine placental fluids increase between days 33 and 140 of gestation (Robertson and King, 1975; 1979; Eley et al., 1979) and, although several orders of magnitude less, concentrations of estrone parallel those of estrone sulfate. From day 140 to day 180 of gestation, concentrations of estrone sulfate in placental fluids decrease, then increase until about day 210 of gestation, followed by a decrease until parturition. Concentrations of estradiol in placental fluids increase until about day 50 of gestation (Eley et al., 1979); then concentrations of estradiol in extraembryonic membranes are variable until parturition (Robertson and King, 1979). However, estradiol concentrations are greater in the amniotic than in allantoic fluid from day 97 to day 270 of gestation (Robertson and King, 1979).

Between days 33 and 111 of gestation, concentrations of estrone sulfate are greater in amniotic than allantoic fluid; whereas, concentrations of unconjugated estrogens are greater in the allantoic fluid (Eley et al., 1979). This suggests that the fetus has the enzymes, in the liver or kidney, to conjugate free estrone and a mechanism to pool conjugated estrogen in the amniotic fluid. The bovine

placenta contains sulfatransferase activity (Adams and Rosen, 1965; Holcenberg and Low, 1974). In addition, sulfates are synthesized in the bovine ovary by gonadotropin-stimulated enzymes similar to those found in the adrenal and uterus (Sturm and Hannappel, 1979). After estrogens are conjugated by the uterus or conceptus, they are available for transfer to the maternal circulation in a form to minimize estrogenic activity. Unconjugated estrogens may alter blood flow or placental fluid movement, and therefore, influence the amount of nutrients available to the fetus.

Prostaglandin $F_2\alpha$ ($PGF_2\alpha$) is undetectable in maternal peripheral plasma throughout pregnancy until the time of parturition (Eley et al., 1979). However, $PGF_2\alpha$, from the uterus, accumulates in fetal fluids throughout gestation (Eley et al., 1979).

Progesterone in maternal plasma is elevated between days 40 and 70 of gestation (Donaldson et al., 1970; Wettemann and Hafs, 1973), then remains constant until concentrations decrease at parturition (Mobley et al., 1983). Progesterone in fetal fluids increase as fetal fluid volumes increase (Eley et al., 1979). This suggests that progesterone is sequestered in the fetal fluids.

Concentration of estrone and estrone sulfate in maternal blood are difficult to detect until about day 70 of gestation (Robertson and King, 1979). However, large quantities of estrone and estrone sulfate appear in

placental fluids as early as day 27 of gestation (Eley et al., 1979; Robertson and King, 1979). This indicates that these estrogens are retained in placental fluids until a threshold capacity is reached or the maternal system eliminates estrogens faster than they are produced.

Placental fluids have a nutritive role in all domestic animals. Allantoic fluid is a storehouse of nutrients that are at the disposal of the developing fetus. Fetal fluids contain glucose, fructose, protein, and electrolytes that are of major importance to the fetus (Goldstein et al. 1980; Bazer et al., 1981). Ions such as sodium and potassium may have a role in fluid transfer between the maternal and fetal systems (Goldstein et al., 1980).

In the pig, glucose and fructose accumulate in the allantoic fluid between days 21 and 40 of gestation (Bazer et al., 1981). This corresponds to times when allantoic fluid volume increases. Therefore, water and glucose may accumulate in allantoic fluid by the same mechanism. The progesterone to estrogen ratio and Na^+/K^+ ATPase activity influence fluid movement into the allantoic cavity and may be the mechanism that moves glucose into the allantoic fluid (Bazer et al., 1981). However, in the cow, there is a greater concentration of glucose in the maternal vasculature than in the fetal vasculature (Ferrell et al., 1983). This concentration gradient between the maternal and fetal blood supplies may promote movement of glucose from maternal fluids to fetal fluids by facilitative diffusion (Munroe et

al., 1983).

Glucose in the ovine fetal system is converted to fructose by the chorioallantois (Alexander et al., 1955). Fructose is then sequestered in the allantoic fluid and is not transferred to the maternal circulation.

Protein accumulates in bovine allantoic fluid as pregnancy progresses (Eley et al., 1979). Protein may be produced by extraembryonic membranes or maternal proteins may be transferred to the fetal compartment with the movement of water.

Regulation Of Uterine Blood Flow

Studies designed to determine oxygen consumed by the uterine complex indicated that steroids may influence uterine blood flow. Estrogen can cause blood vessels to dilate and progesterone can cause blood vessel to constrict (Ford, 1985). These early experiments suggested a complex mechanism that controls uterine blood flow that involved steroids, neurotransmitters, and receptors. Recent evidence indicates that the fetus and/or the uterus influences the amount of blood that reaches the uterus about the time of maternal recognition of pregnancy and throughout pregnancy.

Estrogen and Progesterone

Infusing conjugated estrogens into pregnant ewes results in increased uterine blood flow (Greiss and Marston, 1985). Changes in blood flow did not occur until after the first hour of infusion, and the increase in conductance,

indicating a decrease in resistance, reflected uterine blood flow changes. In addition, exogenous estrogen caused uterine blood vessels to dilate and blood flow to increase.

Blood flow to the uterus varies between animals and stage of the estrous cycle. Uterine blood flow in the ewe is reduced when the ovary contains a corpus luteum and greatest when the ovary contains a follicle (Huckabee et al., 1968).

Estradiol or estrone administered either intramuscularly or intravenously to ewes caused an increase in uterine blood flow (Huckabee et al., 1970). In addition, injecting estradiol 17-β into one uterine artery caused an increase in blood flow in the artery on the side of the injection (Killam et al., 1973). This illustrates a local effect of estrogens on uterine blood flow.

Treatment of ovariectomized nonpregnant ewes with progesterone did not effect uterine blood flow (Caton et al., 1974). However, if ewes received estrogen and were then treated with progesterone, they then had a decrease in uterine blood flow. This illustrates that progesterone can act as an antagonist to estrogen or can inhibit the response of estrogen depending on the sequence that the hormones are administered. In addition, nonpregnant ewes monitored on day 14 or 15 of the estrous cycle had reduced blood flow to the uterus compared to pregnant ewes on the same days of pregnancy (Ford et al., 1979c). The strong negative correlation ($r = -.71$) between progesterone and uterine blood flow is evidence that at the uterine level estrogen

acts to increase blood flow during the estrous cycle and progesterone acts to decrease blood flow during the luteal phase of the estrous cycle.

Studies of endothelial cell morphology of uterine arteries indicate that arteries ipsilateral to the ovary bearing the corpus luteum or corpus albican possess fewer endothelial cells that extend into the artery lumen compared to cells from arteries on the contralateral side (Ford et al., 1980). The typical morphology of the endothelial cells indicate that cells in the arteries ipsilateral to the ovary containing the corpus luteum appear to be flattened resulting in a large area of contact with the internal lumen of the vessel. Endothelial cells from the contralateral artery appeared to extend into the lumen a considerable distance resulting in a reduced area of contact with the internal lumen of the vessel. In addition, ovariectomized ewes treated with estradiol tended to have increased numbers of endothelial cells that extend into the lumen of the uterine artery (Ford et al., 1980). Likewise, progesterone or progesterone followed by estrogen decreased the number of cells that extended into the artery lumen. These data suggest that progesterone has a local effect on cell morphology of the uterine artery.

Arterial lumen diameter and thickness of the tunica layer did not differ for arteries ipsilateral or contralateral to the corpus luteum (Ford et al., 1977b). However, recent evidence suggests that estrogens change

uterine arterial tone (Ford, 1985). Uterine tone is markedly depressed as concentrations of estrogen become greater causing the vessel to become distended and flaccid. Tone is a function of the amount of Ca^{++} available to the contractile proteins (Bolton, 1979). Calcium is derived from extracellular sources and its availability to the cell is regulated by the opening and closing of Ca^{++} channels in the vessel membranes responding to changes in surface membrane potentials. Therefore, estrogens may alter Ca^{++} channels to reduce blood vessel tone and promote vasodilation.

Prostaglandins

When uterine arteries were removed from nonpregnant ewes on day 0, 3, 6, or 10 of the estrous cycle, only arteries that were ipsilateral to the corpus luteum on day 10 of the cycle had increased vasoconstriction when perfused with $PGF_2\alpha$ followed by norepinephrine (Ford et al., 1977b). Regardless of the stage of the estrous cycle, arteries ipsilateral to the corpus luteum that were perfused with $PGF_2\alpha$ followed by nerve stimulation had increased smooth muscle contraction compared to uterine arteries contralateral to the corpus luteum (Ford et al., 1977a; 1976). In addition, ipsilateral uterine arteries, removed on day 10 of the estrous cycle and perfused with $PGF_2\alpha$ followed by norepinephrine and electrical stimulation had increased vessel constriction compared to ipsilateral arteries from day 0, 3, and 6 of the cycle of nonpregnant ewes (Ford et al., 1977a). This indicates an association between the

length of time that the artery is exposed to progesterone and the response of the artery to $PGF_2\alpha$ and nerve stimulation. It also indicates that progesterone produced by the corpus luteum has a local effect on vascular smooth muscle. The difference in contractility between the ipsilateral and contralateral uterine arteries may involve progesterone's local effect on the artery that influences the action of $PGF_2\alpha$ and norepinephrine.

It has been proposed that progesterone and estrogen act together with $PGF_2\alpha$ to regulate vasoactivity of the uterine artery by either regulating the number and/or activity of α-adrenergic receptors, the amount of neurotransmitter released, or a combination of the two (Ford, et al., 1977a). During the follicular phase of the estrous cycle, the uterine artery is exposed to estrogens that cause a decrease in release of norepinephrine (McKercher et al., 1973), possibly depleting the number of α-adrenergic receptors and results in vasodilation. During the luteal phase, α-adrenergic receptors may proliferate when the uterine artery is exposed to progesterone, and increasing amounts of $PGF_2\alpha$ may activate the release of norepinephrine that binds to the α-adrenergic receptors causing the vascular smooth muscle to constrict facilitating vasoconstriction.

Fetal Products

Greiss and Anderson (1970) observed a transient increase in blood flow in the gravid uterus on days 13 to 15 of pregnancy in the ewe. Ford et al., (1979b) observed that

the pattern of blood flow to the uterus of pregnant and nonpregnant cows was similar until day 14 postmating; however, between days 14 and 18 of pregnancy there was a 2 to 3 fold increase in blood flow to the gravid uterus compared to the nongravid uterus. Ford and Christenson (1979a) observed that the pattern of blood flow to the uteri of sows in early pregnancy was similar to the sow's previous cycle until day 11 after mating. After day 11 of pregnancy, uterine blood flow increased 3 to 4 fold with no increase in blood flow during this period in the nonpregnant sow. These data suggest that the presence of the ovine, porcine, and bovine conceptus exerts a local effect on uterine blood flow.

Uterine arteries removed on day 15 of the estrous cycle of ewes and infused with uterine flushings from day 15 pregnant ewes contracted less after $PGF_{2\alpha}$ treatment than did arteries that had not been infused with uterine flushings (Ford et al 1976). In addition, uterine arteries removed from heifers on day 16 postestrus and infused with day 16 uterine flushings followed by electrical stimulation constricted less after $PGF_{2\alpha}$ than did uterine arteries not infused with uterine flushings (Ford, 1979b). This decrease in constriction was maintained through subsequent perfusion with $PGF_{2\alpha}$. These results suggest that the conceptus and/or pregnant uterus regulate uterine blood flow. The action may be mediated by altering the ability of $PGF_{2\alpha}$ to release norepinephrine or by modifying the α-adrenergic receptors.

However, in the pig, the day 13 conceptus can synthesize catechol estrogens from free estrogens in vitro, and catechol estrogens are found in flushings from day 13 pregnant pigs (Ford, 1985). Catechol estrogens reduce Ca^{++} uptake by the uterine artery but do not influence uptake of Ca^{++} by the mesenteric artery (Ford, 1985). These data indicate that catechol estrogens may increase uterine blood flow by influencing Ca^{++} uptake causing changes in the tone of the uterine artery.

Nutrient Partitioning For Fetal Development

The mechanism by which different tissues use nutrients may influence food intake (Baile, 1971). Nutrients are used for body maintenance, body growth, and for establishing body reserves such as energy stores (lipids), glucose reserves (glycogen), and amino acid reserves (labile protein). During pregnancy, a substantial portion of the nutrients ingested by the female support fetal and mammary gland growth. Growth of the fetus or mammary gland is of no direct advantage to the dam, but demands metabolic changes in the dam to support the needs of these tissues.

Nutrient partitioning involves two types of regulation, homeostasis and homeorhesis (Bauman and Currie, 1980). Homeostatic control involves maintenance of a constant internal environment. Homeorhesis involves changes in metabolism to insure a uniform flow of nutrients to support a physiological state. Nutrient requirements in late

gestation are about 75% greater for pregnant than for nonpregnant animals of the same weight. However, the pregnant animal is able to support the rapid fetal growth the occurs in late gestation by changes that occur in the maternal metabolism.

The efficiency of use of metabolizable energy is low during pregnancy in sheep (Graham, 1964; Rattray et al., 1974b) and cattle (Moe and Tyrrell, 1972). The inefficient use of metabolizable energy in pregnant animals appears to be associated with the sizable cost to maintain placental, fetal, uterine, and mammary tissues (Rattray et al., 1974b).

Fetal membranes and the fetus grow rapidly during late gestation (Winters et al., 1941; Eley et al., 1979) and by about day 210 of gestation have only attained 30 to 40% of the predicted weight at parturition (Ferrell et al., 1976). Fetal organs develop early in pregnancy (Winters et al., 1941); therefore, much of increase in weight that occurs in late gestation is a result of an increase in tissue mass.

To understand the interactions between the maternal and fetal systems, it is necessary to understand the specific nutrients used during pregnancy. The uptake of oxygen by the umbilical vein of sheep, goat, and cattle averages 7 to 9 ml/min per kg of fetal weight (Battaglia and Meschia, 1978). Because oxidative metabolism primarily uses carbohydrates and amino acids, it is estimated that in the pregnant ewe about 56 kcal/day per kg of fetus is consumed for maintenance type processes of the fetus, and during late

gestation, about 32 kcal/day per kg of fetus of gross energy accumulates in the conceptus. For cattle in late gestation, 2.3 Mcal/day is consumed in oxidative metabolism and 1 Mcal/day accumulates in the fetus (Battaglia and Meschia, 1978). These data indicate that because oxidative metabolism uses carbohydrates and amino acids, the cost of maintaining a fetus is expensive to the dam.

Hyperthyroidism of the ruminant fetus (Thornburn and Hopkins, 1973) appears to establish a thermal gradient between the fetus and the dam (Abrams et al., 1969). Fetal hyperthermia is achieved at a considerable cost to the dam because the metabolic substrates are glucose, amino acids, and lactate.

Chronic underfeeding of the dam results in retarded growth of the fetus because fetal substrates are derived from the dam (Everitt, 1964). Data on nutrient requirements of the bovine fetus are limited; however, there are data on nutrients used in metabolism of the fetal lamb (Battaglia and Meschia, 1978). The fetal lamb consumes about 8 gm/day per kg of fetal weight of carbon of which 40% is retained and the remainder leaves as CO_2 and urea carbon. Glucose comprises about 50 to 70% of the total substrates oxidized by the fetal lamb, of which 20 to 25% of the glucose comes from the conversion of lactate to glucose by the placenta. Amino acids are the third leading nutrient used for fetal fuel. The fetus takes up about 1.5 gm of nitrogen/kg of fetus per day. This exceeds the amount of nitrogen

accumulated by the fetal lamb (about .65 gm/kg of fetus per day) and NH_3 is excreted in the form of urea. This indicates that amino acid are catabolized by a gluconeogenic pathway by the fetus or placenta. Fructose concentrations are great in fetal fluids and appears to be the preferred form of fetal carbohydrate storage. Long chain fatty acids are transported to the fetus to provide essential fatty acids but furnish negligible quantities of energy to the fetus.

Endocrine Control During Pregnancy

Studies relating the concentrations of hormone in serum or plasma to metabolic events do not account for possible changes in blood flow or changes in the number of receptors on or in the target tissue (Convey, 1974; Trenkle, 1978). However, endocrine involvement in the homeorhetic control of partitioning of nutrients can not be overlooked.

Placental lactogen, secreted by the conceptus, increases during the last one-third of pregnancy in the goat(Kelley et al., 1976; Currie et al., 1977; Hayden et al., 1979). Placental lactogen is similar in structure to growth hormone and prolactin and may bind to tissues with receptors for growth hormone and prolactin (Currie et al., 1977; Chan et al., 1978). Receptors for placental lactogen appear in the mammary gland, liver, and adipose tissue (Chan et al., 1976; 1977; Currie et al., 1977). Therefore, placental lactogen may cause lipolysis of maternal adipocytes to provide nutrients for the fetus or cause the maternal liver to release enzymes to cause lipolysis. This

is a possible homeorhetic control by the fetal-placental unit.

Progesterone and estrogen may have a homeorhetic control in partitioning nutrients during pregnancy. The progesterone to estrogen ratio can alter blood flow to the uterus (Huckabee et al., 1970; Caton et al., 1974) and blood flow to the uterus is influenced by the conceptus (Greiss and Anderson, 1970; Ford, 1985). As blood flow to the uterus increases, nutrient flow to the fetus increases.

Norepinephrine and insulin are maternal hormones that have a homeostatic control in partitioning nutrients and may aid in generating a constant flow of nutrients to the fetus. In late pregnancy, norepinephrine stimulates lipolysis (Metz and van den Bergh, 1977) that, through expensive pathways, fat can be converted into glucose or lactate for fetal use. Insulin is lipogenic under most conditions, but in rats in late gestation, adipose tissue has a diminished responsiveness to insulin (Scow et al., 1964; Kelley et al., 1974; 1976). A homeorhetic signal from the conceptus (placental lactogen) may alter the insulin receptor population in adipose tissue making adipose tissue available for lipolysis.

Relationship Between Glucose And Insulin With Body Energy Reserves And Reproduction

Most research relating glucose and insulin with body energy reserves and reproduction has been conducted in human subjects. Glucose intolerance in man is characteristic of

several states, including age, obesity, and diabetes. The changes in metabolism associated with glucose intolerance are unclear. Changes in the ability of the pancreas to secrete insulin in response to a glucose challenge or a change in the ability of the target tissue to respond to increased insulin after glucose are two possible causes.

Obese humans require greater amounts of insulin to maintain "normal" blood glucose concentrations (Karam et al., 1963; Bagdade et al., 1967). Prior to and after a glucose tolerance test, obese men had greater concentrations of insulin in serum than did nonobese men (Bagdade et al., 1967).

The amount of insulin in extravascular spaces in humans increases with age (McGuire et al., 1979). Extravascular insulin includes insulin bound to receptors, suggesting that the insulin receptor population changes with age. However, reduced concentrations of insulin in plasma of aged humans may be a function of the ability of the pancreas to secrete insulin. The rate of entry of new insulin into peripheral circulation was reduced in older subjects compared to diabetic and moderately obese subjects (McGuire et al., 1979).

In ruminants, secretion rates of insulin are greater in obese heifers than in lean heifers (McCann and Reimers, 1985a). However, obese heifers were less sensitivity to the glucoregulatory effects of exogenous insulin compared to lean heifers.

Rats have greater glucose tolerance and increased concentrations of insulin in serum at proestrus and estrus, when concentrations of estrogen, progesterone, and gonadotropins in plasma are greatest (Bailey and Matter, 1971). During metestrus in the rat, increased concentrations of progesterone may be associated with glucose tolerance values and concentrations of insulin that are intermediate to those at estrus and proestrus. Similarly, estradiol and progesterone treatment enhanced secretion of insulin and plasma insulin response to glucose treatment during pregnancy in rats (Costerini and Kalkhoff, 1971). In women, day of the menstrual cycle does not influence the response to glucose tolerance tests and plasma insulin (Spellacy et al., 1976). In humans with diabetes mellitus, estrogen treatment reduces daily insulin requirements (Spiegelman, 1940).

Dunn et al., (1972) demonstrated that distribution of glucose was not effected by stage of the estrous cycle of ewes. Reimers et al., (1982) observed that basal concentrations of insulin in heifers were greater at estrus than at diestrus. In addition, insulin secretion is greater at estrus than at diestrus for obese heifers (McCann and Reimers, 1985b), suggesting that insulin secretion may be associated with concentrations of estradiol and progesterone during estrus and diestrus. Progesterone treatment reduces glucose removal from peripheral circulation, whereas estrogen treatment enhances or has no effect on removal of

glucose from blood in rats (Costrini and Kalkhoff, 1971; Ashby et al., 1981). However, it is not known if steroids can directly or indirectly cause an increase in tissue uptake of glucose.

Effect Of Prepartum Energy On Birth Weight

In cattle, dystocia is a major cause of perinatal calf losses (Anderson and Bellows, 1967). Calf birth weights are associated with dystocia. Cows giving birth to calves with heavy birth weights tend to have a greater incidence of dystocia (Bellows et al., 1971b; Rice and Wiltbank 1972; Laster and Gregory, 1973). Therefore, early studies tried to regulate calf birth weight by restricting precalving energy intake.

Reduced prepartum energy intake that results in a thin body condition of the cow influences calf birth weight (Turman et al., 1964), but has little effect on calving difficulty (Young, 1970; Tudor, 1972; Laster, 1974; Bellows and Short, 1978). However, prepartum energy also influences postpartum reproduction. Cows restricted in energy intake (Wiltbank et al., 1964; Bellows and Short, 1978; Dunn and Kaltenbach, 1980) or the amount of supplemental protein offered prepartum (Mobley, 1982; Rasby, 1983; Garmendia, 1984; Selk, 1986) have extended intervals from parturition to first estrus.

Cows and heifers that lost moderate amounts of body energy reserves prepartum had calves with birth weights that

were similar to calves from cows and heifers maintaining body condition (Mobley, 1982; Rasby, 1983; Wettemann et al., 1986; Spitzer et al., 1986). Even though calf birth weight was not influenced by moderate losses of body condition prepartum, cows that lose body energy reserves have longer postpartum anestrous periods (Mobley, 1982; Rasby, 1983; Selk, 1986).

Sows that lost weight and backfat during lactation have extended periods of anestrus (Reese 1982a; 1982b). In addition, subsequent litter size is similar but piglet weight is reduced for sows losing weight and backfat compared to sows maintaining weight and backfat during lactation (Reese et al., 1982b).

Collectively, these results indicate that birth weight is influenced by prepartum nutrient intake. Reducing nutrient intake prepartum reduces birth weights; however, to optimize postpartum reproduction, prepartum nutrition must be adequate.

Body Energy Reserves And Reproductive Function

The importance of body energy reserve at calving on subsequent reproductive performance has been documented. Reproductive performance of beef cows vary during the production cycle depending on the amount of body energy reserves available (Wiltbank et al., 1962; Donaldson et al., 1967; Baker, 1969; Croxton and Stollard et al., 1976; Lowman et al., 1976; Stollard et al., 1976; Dunn and Kaltenbach,

1980; Dziuk and Bellows, 1983; Richards et al., 1986; Selk, 1986). Similarly, a specific body composition (percent lean, percent water, and percent fat) must be attained before estrous cycles in rats are initiated (Kennedy and Mitra, 1963; Frisch et al., 1975; Frisch et al., 1977) and before reinitiation of menstrual cycles in women (Frisch, 1973; Frisch and McArthar, 1974). Amenorrhea began in women when body fat decreased to less than 10 to 15 percent (Frisch et al., 1974). Similarly, anestrus in beef cows began when body fat was reduced below percent (Richards et al., 1986).

Eighty percent of the cows with a body condition score of 7 (1=emaciated; 9=obese) exhibited estrus cycles by 60 days postpartum regardless of weight changes before or after calving (Whitman, 1975). Wettemann et al., (1982) demonstrated in spring calving cows, that percentage change in condition score from November to calving (March) was correlated with days to first estrus (r=.61) and days to conception (r=.62). A 20 percent decrease in body condition score between November and calving was associated with an additional 15 days to first estrus compared to cows that maintained body condition between November and calving.

Anestrous cows with poor body condition (body condition score 2; where 1=thin; 5=fat) during the breeding season had small inactive ovaries and uteri that lacked tonicity (Lowman, 1982). Indicating that the ovary and uterus are sensitive to body energy reserves available. In addition, Mobley, (1982) demonstrated that cows losing body energy

reserves in late gestation have reduced concentrations of progesterone in plasma.

The relationships between body energy reserves and pituitary and hypothalamic functions have not been clearly defined. Most experiments that relate nutrient intake with hypothalamic and pituitary function fail to monitor body energy reserves of the experimental animals. Cows that maintain body energy reserves have greater basal LH and greater concentrations of LH after GnRH (Rutter and Randel, 1984). Cows slaughtered with body condition scores (1=extremely thin; 5=extremely fat) of 4.4±.2, 3.1±.1, and 1.6±.3 had similar pituitary weights (Moss et al., 1982), but GnRH in the stalk-median eminence, preoptic area, and hypothalamus proper did not differ among cows.

These data suggest that when evaluating nutritional effects on reproductive function, body energy reserves must be monitored. Body energy reserves can be estimated using a body condition scoring system (Wagner,1985; Richards et al., 1986). Wagner (1985) demonstrated a strong correlation (r=.92) between live body condition score and total energy content (mcal) of the carcass.

Influence Of Nutrition On Gonadotropins, Ovarian Progesterone And Estrogen, And Thyroxine

Nutritional influence on pituitary function is unclear and inconsistent. Cattle restricted in energy intake had greater (Gombe and Hansel, 1973; Whisnant et al., 1985), similar (Hill et al., 1970; Dunn et al., 1974; Spitzer et

al., 1978), or decreased (Apgar et al., 1975) concentrations of luteinizing hormone (LH) in serum compared to cattle with ample energy intake. However, cows on a restricted energy ration elicited a greater LH response after GnRH compared to cows not restricted in energy intake (Beal et al., 1978; Whisnant et al., 1985). In addition, serum LH tended to be reduced in dairy cows fed a ration that contained 12.7% crude protein compared to cows fed a ration containing 16.3% or 19.3% crude protein (Jordan and Swanson, 1979). The LH response to GnRH was reduced in cows fed a 12.7% crude protein ration compared to cows fed a 16.3% or 19.3% crude protein ration. Limiting protein intake to postpartum beef cows may effect the ability of the pituitary to store and/or release LH (Nolan et al., 1984).

Pituitary weight does not appear to be influenced by energy intake of the animal. Pituitary weights for cows fed restricted amounts of energy were similar to those for cows fed ample energy (Beal et al., 1978; Moss et al., 1982). The effect of diet on pituitary content of LH is unclear. Pituitaries from intact cows fed a restricted energy diet contained less LH compared to pituitaries from cows not restricted in energy intake (Beal et al., 1978). In contrast, pituitaries from cows fed ample energy had less LH in the pituitary gland compared to cows fed a moderate or restricted energy ration (Moss et al., 1982). However, there appears to be no difference in the concentration of GnRH in the stalk-median eminence, preoptic area, or hypothalamus

proper of cows due to energy intake or body condition score at slaughter (Moss et al., 1982).

Blood metabolites that are energy precursors at the cellular level appear to influence pituitary function. Heifers fed monensin, a feed additive that influences the ruminal propionate to acetate ratio (Richardson et al., 1976), released greater quantities of LH after GnRH, elicited greater maximum concentrations of LH after GnRH, and had greater areas under the LH response curves (Randel and Rhodes, 1980). In addition, infusion of propionate into the rumen of heifers resulted in greater amounts of LH after treatment with GnRH (Rutter et al., 1983). Rats given metabolic inhibitors that inhibit glycolysis and oxidative phosphorylation exhibited a reduced GnRH stimulated release of LH (Sen et al., 1979).

Most of the experiments that regulate the energy intake of the animal and then correlate energy intake with pituitary and ovarian function fail to monitor body condition (fat reserve) of the animal. Inconsistencies in the data previously mentioned may indicate that energy reserve of the animal plays an important role in pituitary and ovarian function. Cows maintaining body condition had greater basal LH and greater GnRH induced LH release compared to cows that did not maintain body condition (Rutter and Randel, 1984).

Information on the effect of nutrition on follicle stimulating hormone (FSH) is sparse. Ewes fed a high energy

diet tended to have greater concentrations of FSH in serum (Rhind et al., 1985). However, pituitaries from cows on a high energy diet had reduced concentrations of FSH compared to cows fed a ration that had a low energy content (Moss et al., 1982). In addition, rats restricted in energy intake failed to exhibit the increase in FSH that normally occurs after unilateral ovariectomy (Meredith and Butcher, 1985).

The influence of energy consumed on ovarian steroids is inconsistent. Cows with restricted energy consumption had greater (Donaldson et al., 1970; Dunn et al., 1974), similar (Gombe and Hansel, 1973; Apgar et al., 1975; Spitzer et al., 1978;), or reduced (Donaldson et al., 1970; Gombe and Hansel, 1973) concentrations of progesterone in plasma. Cows losing weight and body condition in late pregnancy had reduced concentrations of progesterone and estrone in plasma compared to cows maintaining weight and body condition (Mobley et al., 1983).

The ovary containing the corpus luteum (Spitzer et al., 1978) and the corpus luteum (Gombe and Hansel, 1973; Beal et al., 1978) weighed less in cows fed a restricted amount of energy. Corpora lutea from cows restricted in energy intake produced less progesterone in vitro compared to cows on unrestricted energy diets (Apgar et al., 1975).

Thyroxine, a metabolic hormone produced by the thyroid gland, adjusts the rate of metabolism in animals to changing climatic conditions. Animals exposed to elevated ambient temperatures have reduced concentrations of

thyroxine in serum, reduced rectal temperatures, and reduced feed intakes compared to their counterparts exposed to cooler environments (El-Nouty et al., 1976).

Feeding thyroprotein depresses thyroid stimulating hormone (TSH, Wagner et al., 1979) and there appears to be an inverse relationship between TSH and LH (Louw et al., 1964). The inverse relationship between TSH and LH may account for anestrus that occurs in thyroprotein fed cows (Wagner and Hansel, 1969) and triiodothyronine treated sheep (Howland et al., 1966). Concentration of thyroxine in serum has been related to milk production. The mean concentration of thyroxine in serum appears negatively related to total milk yield ($r = -.76$; Hart et al., 1978).

The mechanism or mechanisms that control postpartum anestrus in beef cows remains unclear. Prepartum nutrition and body energy reserves influence the length of the postpartum anestrous period. It is possible that changes in prepartum hormones in response to changes in nutrient intake and body energy reserves carry over into the postpartum period and dictate the length of the time from calving to first estrus. Experiments to evaluate the effects of nutrient intake and body energy reserves on prepartum endocrine responses and fetal development would aid in linking these changes to postpartum reproduction. In addition, experiments relating energy intake and body condition with pituitary and ovarian function would aid in evaluating nutritional influences on reproductive

performance of cattle.

CHAPTER III

INFLUENCE OF NUTRITION AND BODY CONDITION ON FETAL AND PLACENTAL DEVELOPMENT, AND ESTROGENS AND PROGESTERONE IN PLASMA AND PLACENTAL FLUIDS OF BEEF COWS

ABSTRACT

Mature pregnant Hereford cows (n=17) were used to determine the effect of nutrition and body energy reserves on fetal development, concentrations of nutrients and estrogens in placental fluids, and progesterone and estrogens in maternal plasma. On day 145 of gestation, cows were blocked by breeding date into two groups and fed to achieve a thin (TH; n=8) or moderate (M; n=9) body condition (1=emaciated; 9=obese) by day 195 of gestation. Cows were then fed to maintain weight and body condition. Body weights, body condition scores, estrogens, and progesterone in plasma were determined weekly between days 200 and 256 of gestation. On day 256±1 of gestation, cows were infused intravenously with a 50% glucose solution to determine glucose and insulin responses. Cows were slaughtered on day 259±1 of gestation, and amnionic and allantoic fluids were sampled and analyzed for concentrations of protein, fructose, and estrogens. Body weights and BCS were less ($P<.01$) for TH (418±26 kg; 3.7±.2) compared to M (509±25 kg;

5.7±.2) cows at slaughter. Nutrient intake and body condition did not influence ovarian, pituitary, follicular fluid, caruncular, or fetal weights, pituitary LH content, number of caruncles, fetal crown rump length, or volume of placental fluids. Uterine weights were less (P<.07), but chorioallantoic weights were heavier (P<.07) in TH (1.29±.09 kg) compared to M (1.06±.09 kg) cows and cotyledonary weights were greater (P<.05) for TH (1.87±.16 kg) than for M (1.44±.15 kg) cows. Total fructose in amnionic fluid was reduced (P<.01) in TH (1.7±.8 g) compared to M (4.8±.8 g) cows. Glucose disappearance, and concentrations of progesterone, estrone sulfate, and insulin in maternal plasma were similar for TH and M cows. Concentrations of estradiol and estrone in maternal plasma were greater for TH compared to M cows between days 240 and 260 of gestation. We conclude that nutrient intake and body energy reserves of beef cows during late gestation influence placental weights, fructose in amnionic fluid, and concentrations of estrone and estradiol in maternal plasma and body energy reserves must be determined when evaluating nutritional effects on reproductive function of beef cows. Increased concentrations of estrogens in plasma of TH cows prepartum may influence postpartum reproduction.

(Key Words: Beef Cow, Body Condition, Nutrition, Estrogens, Fetal Development)

Introduction

Prepartum nutrition influences postpartum reproductive performance of beef cows (Wiltbank et al., 1964; Dunn et al., 1969). Cows that lose weight and body condition prior to calving have extended intervals from parturition to first estrus (Dunn and Kaltenbach, 1980; Wettemann et al., 1982; Selk, 1986). Restricting the amount of energy consumed prepartum may reduce calf birth weights (Turman et al., 1964; Bellows and Short, 1978).

During late gestation, gonadotropin secretion is regulated by ovarian and fetal-placental hormones (Nalbandov and Casida, 1940). The effect of prepartum energy intake on postpartum reproductive performance may be dictated by the fetal-placental unit. Nutrient demands by the fetal-placental unit on the dam are great because substrates for fetal growth are derived from the dam (Everitt, 1964; Battaglia and Meschia, 1978). Pregnant cows supplemented to lose weight and body condition had reduced concentrations of estrone and progesterone in plasma during the last 30 days of gestation compared to cows supplemented to gain or maintain weight and body condition (Mobley et al., 1983). The influence of prepartum changes in maternal hormones on postpartum reproduction is unclear. The objectives of this experiment were to determine the influence of body condition and nutrient intake between days 200 and 260 of gestation in beef cows on fetal development and concentrations of hormones in maternal plasma and fetal fluids.

Materials and Methods

Mature Hereford cows (n=17) were exposed to bulls with chinball markers during May and June and observed daily to determine the day of breeding. The day of conception was verified via rectal palpation 45 and 60 days after the observed day of breeding. Cows were maintained on native range until about day 145 of gestation; then, cows were divided into two groups based on day of conception and managed in a dry lot. Thin (n=8) cows were fed to achieve a body condition score of 4 (BCS; 1=emaciated; 9=obese; Wagner, 1985) and moderate (n=9) cows were fed to achieve a body condition score of 6 by day 195 of gestation. Cows in each treatment were fed to maintain weight and body condition between days 195 and 256 of gestation. Cows were weighed and scored for condition weekly. Diets of native hay and 41% cottonseed meal cubes were calculated to achieve desired weights and body condition scores.

Between days 200 and 256 of gestation, blood (40 ml) was obtained each week via jugular venipuncture. Blood samples were mixed with 25 mg of oxalic acid, immediately cooled in ice to 5 C, and centrifuged (5,000 xg for 15 min) within 2 h. Plasma was decanted and stored at -10 C until analyzed for progesterone, estradiol, estrone, and estrone sulfate.

The concentrations of progesterone in plasma were quantified by radioimmunoassay (Lusby et al., 1981). The

intra-assay and inter-assay coefficients of variation were 7.6% and 14.5%, respectively. When 5 ng of progesterone were added to steer plasma, 4.5±.1 ng (n=9) were recovered.

The concentrations of estrone in plasma were quantified by radioimmunoassay (Mobley, 1982). The intra-assay and inter-assay coefficients of variation were 9.6% and 14.9%, respectively. When 1 ng of estrone was added to steer plasma, 1.03±.11 ng (n=9) were recovered.

Concentrations of estradiol in plasma were quantified by radioimmunoassay (Hallford et al., 1979). The intra-assay and inter-assay coefficients of variation were 5.7% and 8.9%, respectively. When 100 pg of estradiol were added to steer plasma, 98±5 pg (n=9) were recovered.

Concentrations of estrone sulfate were quantified by radioimmunoassay (Mobley, 1982). The intra-assay and inter-assay coefficients of variation were 11.9% and 14.3%, respectively. When 1 ng of estrone sulfate was added to steer plasma, 1.1±.1 ng (n=9) were recovered.

On day 255 of gestation, cows were confined to metabolism stalls and both jugular veins were cannulated. One jugular cannula was used for glucose infusion and the contralateral cannula was used for blood sampling. After one day of adaptation, cows were infused intravenously with 250 ml of a 50% glucose solution. Blood samples (20 ml) were obtained every 30 min for one h prior to glucose treatment, every 15 min for the first 2 h after treatment, and every 30 min for an additional 2 h post glucose treatment. Blood

samples were added to tubes containing 16 mg of sodium fluoride[1] and 12 mg of oxalic acid. Samples were immediately cooled in ice to 5 C and centrifuged (5,000 xg for 15 min). Plasma was decanted and stored at -10 C until analyzed for concentrations of glucose (Sigma Chemical Co., St. Louis) and insulin (Selk, 1986). The intra-assay and inter-assay coefficients of variation for the insulin radioimmunoassay were 5.5% and 15.1%, respectively. When 1 ng of insulin was added to cow serum, 1.1$\pm$.1 ng (n=2) were recovered. The intra-assay and inter-assay coefficients of variation for the glucose assay were 2.2% and 9.5%, respectively. When 50 mg of glucose were added to cow plasma, 50.6$\pm$1.5 (n=2) were recovered.

Insulin was iodinated with ^{125}I using a procedure similar to that described by Greenwood et al., (1963). Thirty-five microliters of phosphate buffer (.5M; pH=7.5) were added to a reaction vial containing 5 micrograms of insulin[2]. One millicurie of ^{125}I[3] in 10 microliters of phosphate buffer (.05M; pH=7.0) was added to the reaction vial and allowed to equilibrate for about 2 min. Ten micrograms of chloramine-T in 10 microliters of phosphate buffer (.05M) were added to the mixture, as an oxidizing agent, and allowed to react for 2 min. Forty micrograms of

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[1]Sigma Chemical Co., St. Louis, Mo.

[2]Bovine Insulin Lot #615-70N-80, Eli Lilly Co., Indianapolis, IN.

[3]Cintichem, Inc., Tuxedo, NY.

sodium metabisulfite in 20 microliters phosphate buffer (.05M) were added to the mixture, as a reducing agent, and allowed to react for 1 min. An anion exchange column was used to separate ^{125}I-insulin and free ^{125}I. The column was composed of a disposable 3 cc syringe and disposable stopcock. The syringe was packed with a thin layer (3 mm) of glass wool and moistened with 2 ml of phosphate buffer (.05M). Anion exchange resin[4] was swelled in phosphate buffer (.05) then layered (1.5 ml of resin) in the syringe. The resin in the column was rinsed with 2 ml of phosphate buffer (.5M), 2 ml of 5% BSA[5] in phosphate buffer (.05M), and 3 ml of phosphate buffer (.05M). When three tenths of a ml of phosphate buffer (.05M) remained on top of the column the stopcock was closed and the column was ready for the iodination mixture. The mixture in the reaction vial was layered on the separation column and the stopcock opened. After the mixture penetrated the column bed the stopcock was closed. The reaction vial was rinsed with 100 microliters of phosphate buffer (.05M) and the rinse was layered on the column. Finally, 2 ml of phosphate buffer (.05M) were added to the column and effluent of the column was collected in .5 ml phosphate buffered saline in .1% gelatin (.01M; pH=7.0).

On day 259$\pm$1 of gestation, all cows were slaughtered.

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[4]Analytical Grade Anion Exchange Resin, AG 1-10 200-400 Mesh Chloride Form, Bio-Rad Laboratories, Richmond, CA.

[5]Bovine Serum Albumin No. a-7888 RIA Grade Fraction, Sigma Chemical Co. St Louis.

At the time of slaughter (within 35 min of death), the allantoic and amnionic fluids were sampled using a sterile syringe. Fluids were cooled in ice to 5 C, centrifuged (5,000 xg for 30 min), and stored at -10 C until analyzed. The remaining amnionic and allantoic fluids were aspirated[6] and total amnionic and allantoic fluid volumes were determined. Estradiol, estrone, estrone sulfate, fructose (Roe, 1954), and protein (Lowry et al., 1951) in allantoic and amnionic fluid were quantified. In addition, concentrations of PGF (Geisert et al., 1986) and calcium (Alexander, 1971) were quantified in allantoic fluid. The intra- and inter-assay coefficients of variation for the fructose assay were 1.0% and 2.7%, respectively. When 500 µg of fructose were added to bovine allantoic fluid, 484±6 µg (n=4) were recovered. The intra- and inter-assay coefficients of variation for the protein assay were 5.0% and 7.5%, respectively. When 50 µg of bovine serum albumin were added to bovine allantoic fluid, 48±1 µg (n=4) were recovered.

Concentrations of PGF in allantoic fluid were determined in a single assay and the intra-assay coefficient of variation was 5.5%. The concentrations of calcium in allantoic fluid were quantified with a calcette calcium analyzer (Precision Systems, Inc., Sudburg, MA). The system uses ethylene glycoltetraacetic acid (EGTA) for

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[6]Precision vacuum Pump Model DD 2, Robbins and Myers Inc., Gallipolis, OH.

fluorometeric titration of calcium in aqueous solutions (Alexander, 1971). Concentrations of calcium in allantoic fluid were quantified in a single assay and the intra-assay coefficient of variation was 3.3%.

Pituitary glands were removed and weighed within 48 min of exanguination. The posterior lobe was dissected from the anterior lobe and weight of the anterior lobe of the pituitary was determined. Anterior pituitaries were immediately frozen on dry ice and stored at -10 C until analyzed for LH content by radioimmunoassay (Hallford et al., 1979). The anterior pituitary was prepared for assay of LH similar to a procedure described by Schoenemann et al., (1985). Each anterior pituitary was minced with a scalpel and diluted in phosphate buffered saline (.01M; pH=7.0) to achieve a concentration of 50 mg of tissue/ml of buffer. The tissue in buffer was maintained at 5 C and homogenized with a Sorvall Omni mixer at high speed for 45 seconds, centrifuged (3,000 xg for 30 min), and the extract was decanted. Pituitary extracts were diluted 1:5,000 in phosphate buffered saline containing 1% lypholyzed egg white (.01M; pH=7.0). Pituitary LH content was quantified in one radioimmunoassay in which the intra-assay coefficient of variation was 2.5%. Aliquots of 50, 100, and 150 µl of pituitary extract were parallel to the standard curve composed of .1, .2, .4, .8, 1.6, 3.2, and 6.4 ng of LH. The

first antibody was prepared from B225[7] and used at a dilution of 1:100,000. Radiolabled ligand was prepared from LER-1374A-ovine LH[8] and ^{125}I. Bovine LH (NIH-LH-B9) was used as the standard.

The fetal-placental unit was obtained at slaughter. Fetal sex, weight, and crown rump length were recorded. In addition, the caruncles and cotyledons were separated and uterine weight, caruncle number, and caruncular weight determined. Cotyledons were dissected from the chorioallantoic membrane and weights were recorded.

The corpus luteum was removed from the ovary and total ovarian and corpora lutea weights were determined. Ovaries were minced, blotted on a paper towel, and weighed to determine follicular fluid weights (Casida et al., 1968) as an indicator of follicle size and number. Dry ovarian weights were determined after desiccation in an oven at 90 C for 54 hours.

Analyses of variance were used to analyze cow, fetal, uterine, placental, and ovarian characteristics. Analyses of variance of prepartum endocrine responses were conducted using ordinary least squares. The experimental design was a split-plot in which cows were nested within treatment. Repeated measurements over time were taken on cows, and day was the subplot. If a significant group*day interaction

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[7]Supplied by Dr, G. Niswender, Colorado State University, Fort Collins, Colorado.

[8]Supplied by Dr. L. Riechert, Jr., Albany Medical College, Albany, New York.

existed, then response curves for hormone concentrations were characterized by a day trend that was analyzed by polynomial regression. Tests of heterogeneity of regression coefficients were used to determine differences in day trends for prepartum endocrine responses between cows with thin or moderate body condition.

Results and Discussion

Body condition scores (BCS) and weights averaged 5.0±.2 and 5.4±.3 and 428±36 and 460±30 kg for thin and moderate cows, respectively, on day 145 of gestation. Between days 200 and 256 of gestation, body condition scores and body weights (Table 1) of thin cows were less ($P<.01$) than those for moderate cows. In addition, BCS and body weights at slaughter were less ($P<.01$) for thin compared to moderate cows (Table 1).

Concentrations of estradiol in plasma of cows with thin and moderate BCS between days 200 and 256 of gestation were best described by a second order polynomial regression equation (Table 2). Daily trends for concentrations of estradiol in plasma were not parallel ($P<.03$; Table 3) between groups. Estradiol concentrations in thin cows gradually decreased from 24±4 pg/ml on day 200 of gestation to 18±3 pg/ml on day 221 of gestation (Figure 1). From day 221 to day 256 of gestation, concentrations of estradiol in plasma increased to 34±3 pg/ml. Estradiol concentrations in moderate cows increased gradually from 20±4 pg/ml on day 200

of gestation to 24±3 pg/ml on day 256 of gestation. Concentrations of estradiol in plasma were greater in thin than moderate cows during the last two weeks prior to slaughter.

Concentrations of estrone in plasma of cows in thin and moderate body condition between days 200 and 256 of gestation were best described by a fourth order polynomial regression equation (Table 2). Daily trends for concentrations of estrone in plasma were not parallel ($P<.05$; Table 4) between groups. Concentrations of estrone in thin cows increased from 117±37 pg/ml on day 200 of gestation to 174±29 pg/ml on day 214 (Figure 2). From day 221 to 249 of gestation, concentrations of estrone in plasma of thin cows increased from 161±29 pg/ml to 368±31 pg/ml. Concentrations of estrone in plasma of moderate cows averaged 211±21 pg/ml between days 200 of gestation and increased to 288±30 pg/ml on day 249 of gestation.

Concentration of estrone sulfate in plasma of cows in thin and moderate body condition between days 200 and 260 of gestation was best described by a third order polynomial regression equation (Table 2). Daily trends of estrone sulfate were parallel ($P>.10$; Table 5) in thin and moderate cows. From day 200 to 260 of gestation, concentrations of estrone sulfate increased from 2.2±.1 to 3.5±.1 ng/ml (Figure 3).

Prepartum increases in free and conjugated estrogens in plasma and relative concentrations (i.e., estrone sulfate >

estrone > estradiol) agree with previous observations (Smith et al., 1973; Comline et al., 1974; Eley et al., 1981; Collier et al., 1982; Mobley, 1982). The bovine placenta is the major source of estrogen during gestation (Gorski and Erb, 1959; Hoffmann et al., 1976) and the cotyledon is the primary source of placental estrogens (Combine et al., 1976; Hoffmann et al., 1976; 1979). Our results suggest greater production or decreased clearance of estradiol and estrone in thin cows between days 242 and 256 of gestation.

The bovine placentae (Adams and Low, 1965) and ovaries (Sturm and Hannappel, 1978) have sulfatranferase activity. It is believed that conjugation of estrogens of fetal origin takes place in the uterus or conceptus before being transferred to maternal blood (Thatcher et al., 1980). Our results indicate that the estrone sulfate pool in maternal plasma is similar in thin and moderate cows. Greater concentrations of estradiol and estrone in thin cows indicates conversion of estrone sulfate to free estrogens by tissues with the sulfatase enzyme may be greater in thin cows. However, total blood volume in the cow is about 7% of body weight (Mark Richards, personal communication). Thin cows weighed less than moderate cows between days 200 and 256 of gestation; therefore, total maternal blood volume of thin cows was less than that of moderate cows. Cotyledons of thin cows may be producing a similar amount of free estrogens as cotyledons of moderate cows but because the estrogen is secreted into a smaller maternal blood pool the

concentrations of estradiol and estrone in plasma of thin cows may be greater.

Concentrations of progesterone in plasma of cows in thin and moderate body condition between days 200 and 256 of gestation were best described by a fourth order polynomial regression equation (Table 2). Daily trends for concentrations of progesterone in thin and moderate cows were parallel (P>.10; Table 6). Concentrations of progesterone in plasma between days 200 and 256 of gestation were similar for the two groups and averaged 9.2±.4 ng/ml (Figure 4). The range and concentrations of progesterone during this stage of gestation were similar to previous reports (Donaldson et al., 1970; Echternkamp and Hansel, 1973; Mobley et al., 1983).

Glucose disappearance after treatment with 250 ml of a 50% glucose solution was best described by a second order polynomial regression equation (Table 2). Time trends for concentration of glucose in thin and moderate cows were parallel (P>.10; Table 7). Fifteen minutes post glucose treatment, concentrations of glucose in plasma averaged 199±2 mg% and by 240 minutes after glucose treatment concentrations of glucose decreased to 79±3 mg% (Figure 5). Insulin response to glucose treatment was best described by a linear regression equation (Table 2). Time trends for concentrations of insulin in thin and moderate cows were parallel (P>10; Table 8). Fifteen minutes post glucose treatment, concentrations of insulin in plasma averaged

3.4±.3 ng/ml and decreased to .6±.3 ng/ml by 240 minutes after glucose treatment (Figure 6).

Glucose disappearance curves and insulin response curves are similar to those previously reported for cows (Sartin et al., 1985). Our results suggest that glucose disappearance and insulin response after glucose treatment are similar in pregnant cows with thin or moderate body condition. However, McCann and Reimers (1985) suggest that mean secretion rates of insulin were greater in obese than lean heifers. We were studying pregnant cows that were in good body condition and differences in the insulin response after glucose treatment were not observed.

Total pituitary and anterior pituitary weights did not differ for thin and moderate cows (Table 9). Anterior pituitary weights were similar to those observed by Beal et al., (1978) in nonpregnant beef heifers and cows. LH concentrations and contents were similar in thin and moderate cows, and the concentrations of LH in pituitaries of cows in our experiment were similar to those in beef heifers on day 40 of gestation (Schoenemann et al., 1985) and cyclic beef cows (Moss et al., 1982). We found no difference in pituitary LH content; whereas, previous studies indicated inconsistent results. Cows restricted in energy consumed had pituitaries with less (Beal et al., 1978) or greater (Moss et al., 1982) amounts of LH compared to cows fed adequate energy. This suggests that body condition of cows may account for differences in LH content

in the pituitary of adequately and underfed cows.

Schams et al., (1972) demonstrated that during late gestation of beef cows there is a transitory increase in serum LH. This could be associated with increases in pituitary stores of LH or increases in LH release. Increased estrogens in plasma of thin cows during late gestation may inhibit or alter preparation of the pituitary for gonadotropin secretion postpartum.

Fetal weight and crown rump length were not ($P>.10$) on day 260 of gestation for fetuses from cows with thin or moderate body condition (Table 9). Regardless of BCS of the cow, crown rump lengths of male fetuses (33.9$\pm$.7 cm) were greater ($P<.02$) than those for female fetuses (31.6$\pm$.6 cm).

Restricting energy consumed prepartum reduces calf birth weights in heifers (Turman et al., 1964) and cows (Bellows and Short, 1978). Fetal weights, although not significant, were less in thin cows and may indicate that fetal weight gain is more sensitive to nutrient intake and body condition of the dam between day 260 of gestation and parturition.

Wet and dry ovarian, wet corpora lutea, and follicular fluid weights were similar for cows with thin or moderate body condition (Table 10). Corpora lutea weights were similar to those observed in beef cows between days 8 and 12 of the estrous cycle (Beal et al., 1978). Previous reports indicated that cows restricted in energy consumed have lighter ovaries (Spitzer et al., 1978) and corpora lutea

(Beal et al., 1978). However, these experiments were conducted with nonpregnant cows or heifers, and we studied cows in late gestation.

Number of caruncles, total caruncular wet weight, and total placental fluid volumes did not differ for thin and moderate cows (Table 10). Uterine weight was greater ($P<.07$) in moderate compared to thin cows. The chorioallantois, without the cotyledons, was heavier ($P<.07$) in thin compared to moderate cows and cotyledons from placentae of thin ($1.29\pm.09$ kg) cows were heavier ($P<.05$) than cotyledons in moderate ($1.06\pm.09$ kg) cows. Compensatory growth of the placental unit of thin cows may have occurred resulting in no significant difference in fetal growth between thin and moderate cows on day 260 of gestation.

The cotyledon is the major source of placental estrogen (Hoffmann et al., 1976; 1979) and calf birth weights have been positively correlated with maternal concentrations of free and conjugated estrogens (Echternkamp et al., 1984; Guilbault et al., 1985). In addition, calf birth weight (Collier et al., 1980) and bovine placental weight (Head et al., 1981) were reduced during hot summer months compared to cooler months, and reduced calf birth weights were associated with reduced prepartum concentrations of estrogens in plasma (Collier et al., 1982). Collectively, these results suggest that variations in maternal estrogen concentrations among cows in late gestation may be associated with variations in placental size. Thin cows in

our experiment had greater concentrations of estradiol and estrone in plasma between days 240 and 256 of gestation and this may be a result of a greater mass of placental steroidogenic tissue in thin cows.

Free estrogens produced by the cotyledons of thin cows may overwhelm the sulfatransferase system and allow secretion of greater amounts of free estrogen in the maternal blood supply. Estrogens cause an increase in blood flow to the uterus (Huckabee et al., 1970; Ford, 1985) that may increase the amount of nutrients reaching the fetal-placental unit in thin cows. Increased nutrients to the fetus of thin cows would support a growth rate similar to fetal growth in moderate cows; therefore, fetal weights could be similar in thin and moderate cows on day 260 of gestation.

Allantoic fluid volumes were similar in thin and moderate cows; however, amnionic fluid volume in moderate cows tended ($P<.10$) to be greater than in thin cows (Table 11). Concentrations and total fructose, protein, and calcium in allantoic fluid were similar for thin and moderate cows. In addition, concentrations and total protein in amnionic fluid were similar in thin and moderate cows. However, concentrations and total fructose in amnionic fluid were reduced ($P<.01$) in thin cows. Total fructose in amnionic fluid averaged $1.7\pm.8$ and $4.8\pm.8$ g in thin and moderate cows, respectively.

Glucose that is not metabolized in the fetal system is

converted to fructose (Alexander et al., 1955) by the placenta and sequestered in allantoic and amnionic fluids. Fructose in placental fluids is a readily available energy source for the fetus (Battaglia and Meschia, 1978). Metabolism of carbohydrates may differ in fetuses of thin cows compared to fetuses of moderate cows. Less fructose in amnionic fluid of thin cows may suggest that carbohydrates are metabolized at a faster rate by the fetus in thin cows, reducing the stores of fructose. We (Rasby et al., 1982) demonstrated that reducing the amount of protein supplement fed to beef cows in late gestation reduces blood glucose. Therefore, less fructose in amnionic fluid of thin cows may also indicate less glucose in maternal blood. In this experiment, concentrations of glucose in peripheral plasma tended to be less ($P<.15$) for thin cows (69.4 ± 2.2 mg%) on day 256 of gestation compared to moderate cows (74.2 ± 2.4 mg%).

Concentrations and total estradiol, estrone, and estrone sulfate in allantoic amnionic fluids were similar for thin and moderate cows (Table 12) and concentrations of prostaglandin F in allantoic fluid were not influenced by treatment. Relative concentrations and total amounts of hormones in placental fluids are similar to previous observations in fetal fluids of cows in late gestation (Robertson and King, 1979; Eley et al., 1979). Concentrations of estrone and estradiol were similar in allantoic and amnionic fluids; however, concentrations of

estrone sulfate were greater ($P<.01$) in allantoic than amnionic fluids. Relationships of estrogens in allantoic and amnionic fluid are similar to those observed in the ewe in late gestation (Challis and Patrick, 1981).

Results from this experiment suggests that nutrition and body condition influence placental development, concentrations of estradiol and estrone in maternal plasma, and fructose in amnionic fluid. Cows with thin body condition had greater concentrations of estradiol and estrone in plasma between days 240 and 256 of gestation. Total fructose in amnionic fluid was less in thin cows on day 260 of gestation compared to cows in moderate body condition. Finally, fetal weights on day 260 of gestation were not significantly affected by body condition and nutrient intake, but cotyledonary and chorioallantoic weights were greater in thin cows. Enhanced placental development of nutritionally restricted cows may reduce the influence of nutrient intake and body condition of the dam on fetal development between days 145 and 260 of gestation. We conclude that fetal-placental changes occur in the beef cow to compensate for reductions in energy intake and body energy reserves; however, the mechanism(s) that prepartum losses of body energy reserves influence the length of the postpartum anestrous period remains unclear. To decrease the period from calving to first estrus, cows must be in good body condition at calving.

TABLE 1. WEIGHTS (KG) AND BCS OF THIN AND MODERATE COWS BETWEEN DAYS 200 AND 260 OF GESTATION

	Body Condition			
	Thin		Moderate	
Day of Gestation	Weight[a]	BCS[a]	Weight	BCS
200[c]	395±40[b]	4.2±.2[b]	467±44	5.6±.2
207[c]	399±44	4.1±.1	469±39	5.8±.1
214[c]	399±31	4.0±.2	466±52	6.0±.2
221[c]	404±31	4.2±.2	477±32	5.7±.2
228[c]	407±29	3.9±.1	473±46	5.7±.1
235[d]	416±34	4.2±.1	502±47	5.7±.1
242[d]	404±34	4.1±.1	500±41	5.8±.1
249[d]	411±36	4.1±.1	502±37	5.8±.1
256[d]	419±37	3.7±.1	511±40	5.7±.1
Slaughter[c]	418±26	3.7±.2	509±25	5.7±.2

[a]Weights and BCS differ (P<.01) for thin and moderate cows at all days.
[b]Least squares mean±SE.
[c]Weights after 12 h without feed and water.
[d]Weights with free access to feed and water.

TABLE 2. R^2 AND PROBABILITY LEVELS OF POLYNOMIAL REGRESSION EQUATIONS FOR CONCENTRATION OF ESTRONE (E_1), ESTRADIOL (E_2), ESTRONE SULFATE (E_1SO_4), PROGESTERONE (P_4), GLUCOSE (GLU), AND INSULIN (I) IN PLASMA OF COWS BETWEEN DAYS 200 AND 256 OF GESTATION

	Constituent In Plasma					
Order	E_2	E_1	E_1SO_4	P_4	GLU	I
Linear	.53[a]	.69	.86	.25	.85	.56[c]
	.003[b]	.001	.001	.68	.001	.001
Quadratic	.56[c]	.69	.87	.27	.95[c]	.56
	.018	.37	.002	.09	.001	.37
Cubic	.56	.70	.87[c]	.29	.96	.57
	.62	.14	.05	.11	.06	.21
Quartic	.56	.72[c]	.87	.35[c]	.95	.57
	.67	.05	.43	.001	.06	.50
Quintic		.56		.35		
		.58		.96		

[a]R^2 value.
[b]Probability level.
[c]Order of response curve used.

TABLE 3. ORTHOGONAL COMPARISONS USED TO TEST FOR HETEROGENEITY OF REGRESSION COEFFICIENTS FOR POLYNOMIAL RESPONSE CURVES TO DETERMINE WHETHER DAILY TRENDS FOR ESTRADIOL BETWEEN GROUPS WERE NOT PARALLEL

	Thin versus Moderate			
Error	D.F.	S.S.	M.S.	F
Thin	58	4530.50		
Moderate	56	3237.00		
Total	114	7776.50	68.10	
Thin, Moderate	116	8415.81		
Difference	2	639.31	319.66	4.69**

**(P<.03).

TABLE 4. ORTHOGONAL COMPARISONS USED TO TEST FOR HETEROGENEITY OF REGRESSION COEFFICIENTS FOR POLYNOMIAL RESPONSE CURVES TO DETERMINE WHETHER DAILY TRENDS FOR ESTRONE BETWEEN GROUPS WERE NOT PARALLEL

	Thin versus Moderate			
Error	D.F.	S.S.	M.S.	F
Thin	56	605586.7		
Moderate	57	111978.2		
Total	113	717564.9	6350.1	
Thin, Moderate	117	776265.4		
Difference	4	58200.5	58200.5	2.31*

*(P<.05).

Figure 1. Least-Squares Regressions and Least-Squares Means (■=Thin; +=Moderate) for Concentrations of Estradiol in Plasma of Cows With Thin (TH) or Moderate (M) Body Condition Between Days 200 and 260 of Gestation.

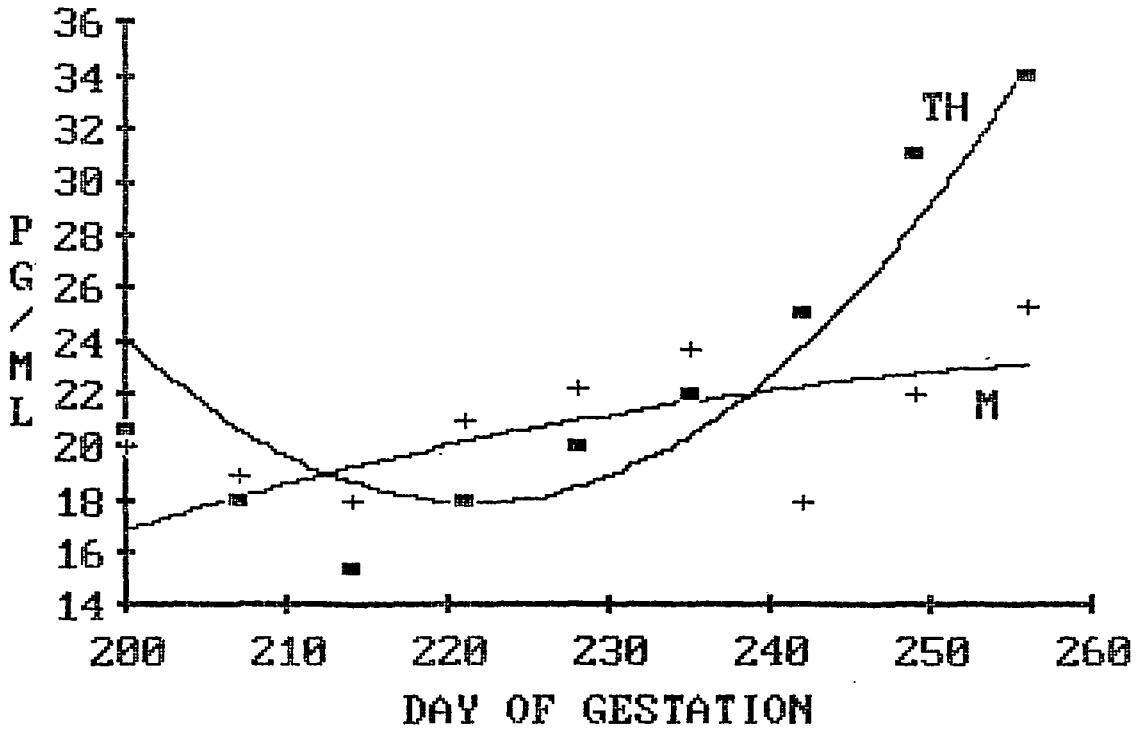
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Figure 2. Least-Squares Regressions and Least-Squares Means (■=Thin; +=Moderate) for Concentrations of Estrone in Plasma of Cows With Thin (TH) or Moderate (M) Body Condition Between Days 200 and 260 of Gestation.

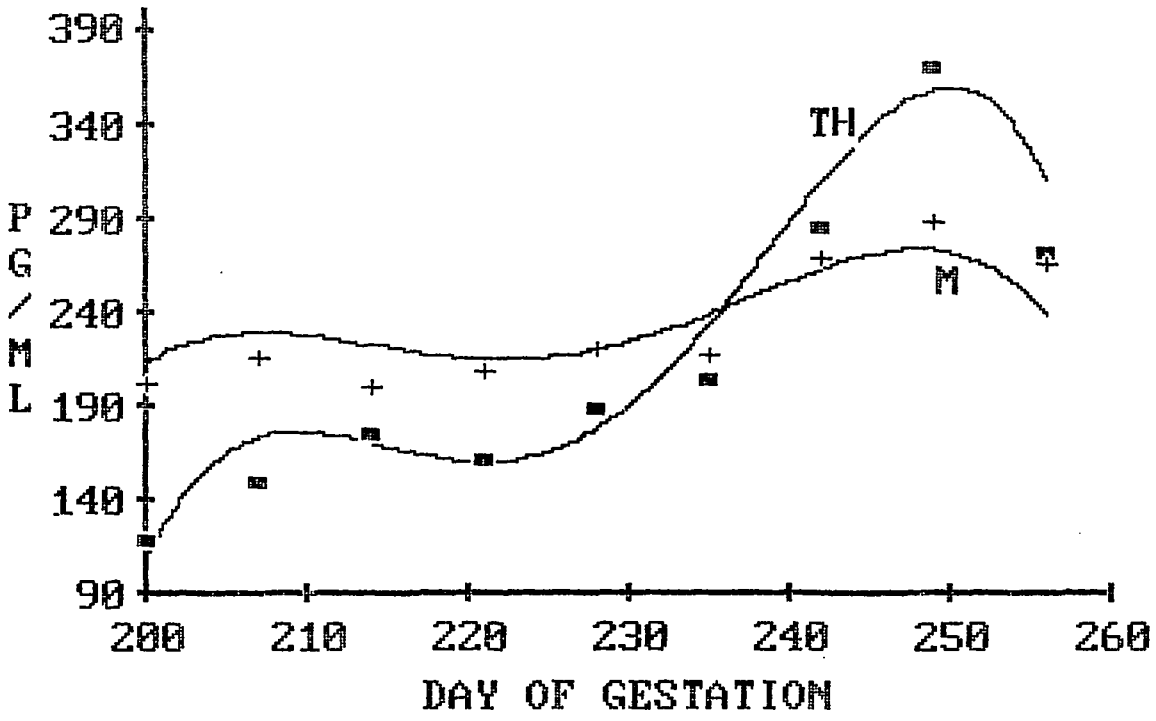
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TABLE 5. ORTHOGONAL COMPARISONS USED TO TEST FOR HETEROGENEITY OF REGRESSION COEFFICIENTS FOR POLYNOMIAL RESPONSE CURVES TO DETERMINE WHETHER DAILY TRENDS FOR ESTRONE SULFATE BETWEEN GROUPS WERE NOT PARALLEL

	Thin versus Moderate			
Error	D.F.	S.S.	M.S.	F
Thin	57	9851591.9		
Moderate	57	10336658.0		
Total	114	20188250	177089.9	
Thin, Moderate	117	20243149		
Difference	3	54899.1	18299.7	.10

TABLE 6. ORTHOGONAL COMPARISONS USED TO TEST FOR HETEROGENEITY OF REGRESSION COEFFICIENTS FOR POLYNOMIAL RESPONSE CURVES TO DETERMINE WHETHER DAILY TRENDS FOR PROGESTERONE BETWEEN GROUPS WERE NOT PARALLEL

	Thin versus Moderate			
Error	D.F.	S.S.	M.S.	F
Thin	53	219.39		
Moderate	53	194.37		
Total	106	413.76	3.9	
Thin, Moderate	110	418.58		
Difference	4	4.82	1.21	.31

Figure 3. Least-Squares Regressions and Least-Squares Means (■=Thin; +=Moderate) for Concentrations of Estrone Sulfate in Plasma of Cows With Thin (TH) or Moderate (M) Body Condition Between Days 200 and 260 of Gestation.

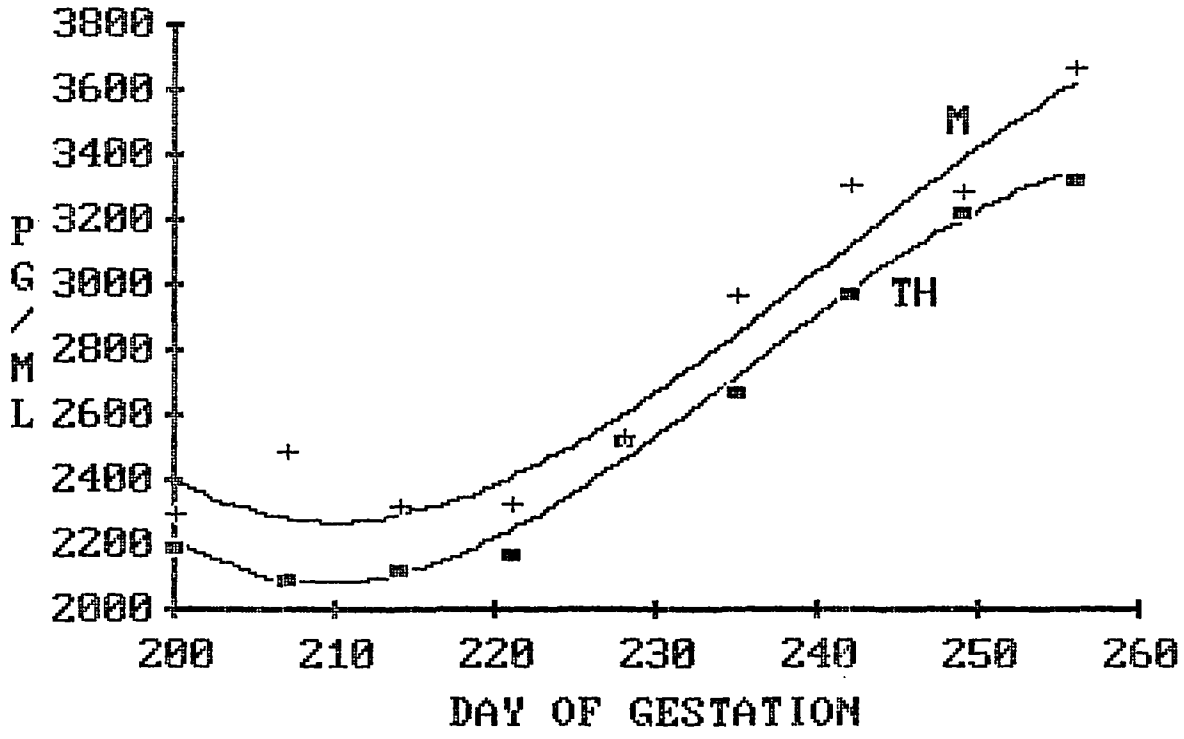
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Figure 4. Least-Squares Regressions and Least-Squares Means (■=Thin; +=Moderate) for Concentrations of Progesterone in Plasma of Cows With Thin (TH) or Moderate (M) Body Condition Between Days 200 and 260 of Gestation.

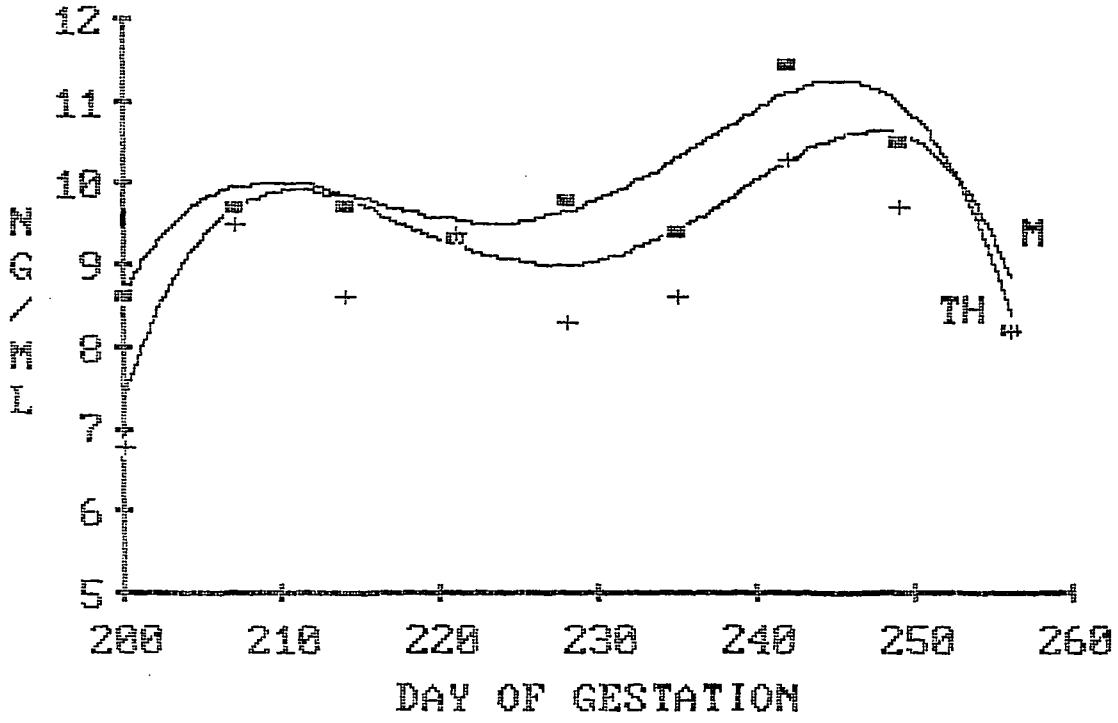
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TABLE 7. ORTHOGONAL COMPARISONS USED TO TEST FOR HETEROGENEITY OF REGRESSION COEFFICIENTS FOR POLYNOMIAL RESPONSE CURVES TO DETERMINE WHETHER TIME TRENDS FOR GLUCOSE BETWEEN GROUPS WERE NOT PARALLEL

	Thin versus Moderate			
Error	D.F.	S.S.	M.S.	F
Thin	67	5324.41		
Moderate	78	8561.69		
Total	145	13886.10	95.77	
Thin, Moderate	147	13999.97		
Difference	2	113.87	56.94	.59

TABLE 8. ORTHOGONAL COMPARISONS USED TO TEST FOR HETEROGENEITY OF REGRESSION COEFFICIENTS FOR POLYNOMIAL RESPONSE CURVES TO DETERMINE WHETHER DAILY TRENDS FOR INSULIN BETWEEN GROUPS WERE NOT PARALLEL

	Thin versus Moderate			
Error	D.F.	S.S.	M.S.	F
Thin	69	160.30		
Moderate	79	105.14		
Total	148	265.44	1.19	
Thin, Moderate	149	267.86		
Difference	1	2.42	2.42	1.35

Figure 5. Least-Squares Regressions and Least-Squares Means (■=Thin; +=Moderate) for Concentrations of Glucose in Plasma After Treatment With 250 ml of a 50% Glucose Solution For Cows With Thin (TH) or Moderate (M) Body Condition.

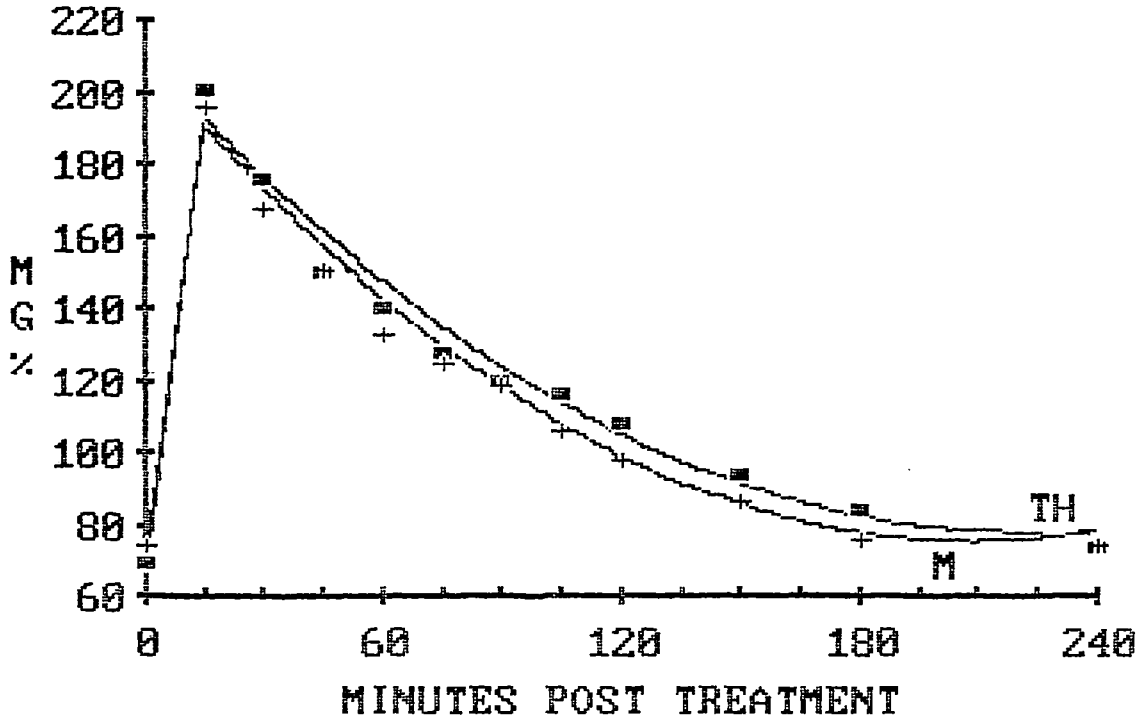
220
200
180
160
M
G 140
%
120
100
80
60
TH
M
0
60
120
180
240
MINUTES POST TREATMENT

Figure 6. Least-Squares Regressions and Least-Squares Means (■=Thin; +=Moderate) for Concentrations of Insulin in Plasma After Treatment With 250 ml of a 50% Glucose Solution For Cow With Thin (TH) or Moderate (M) Body Condition.

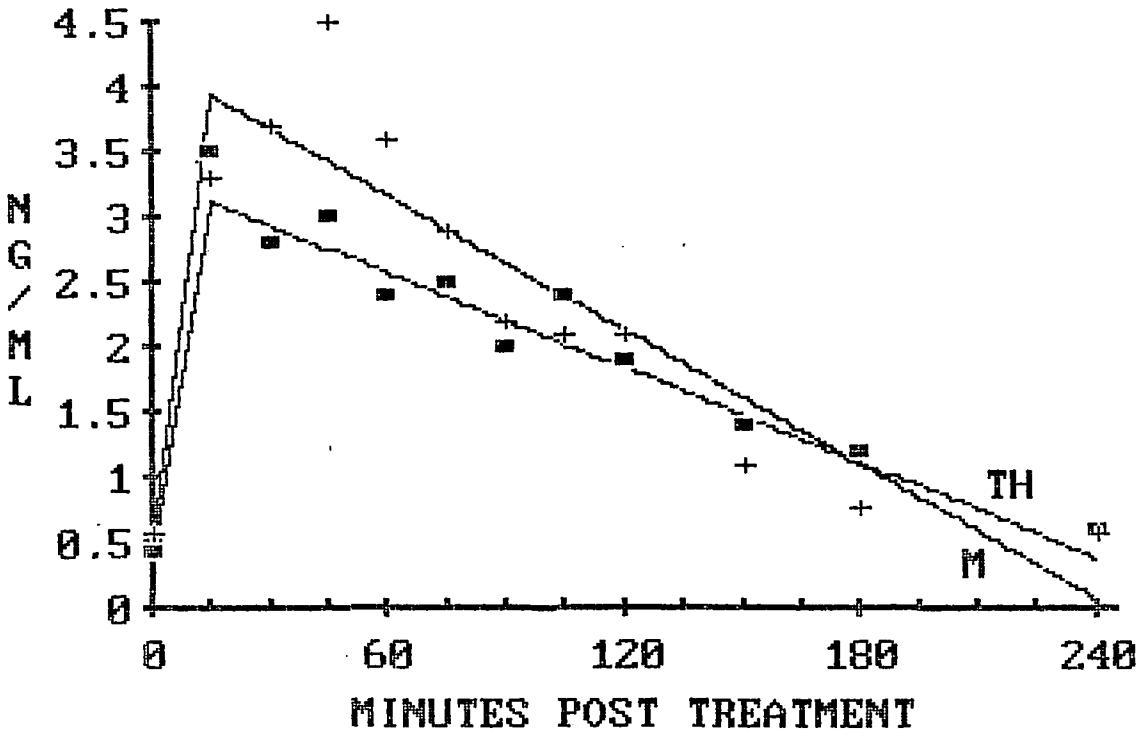
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
NG/ML
TH
M
0
60
120
180
240
MINUTES POST TREATMENT

TABLE 9. CHARACTERISTICS OF COWS AND FETUSES FROM COWS WITH THIN OR MODERATE BODY CONDITION ON DAY 260 OF GESTATION

Criteria	Body Condition	
	Thin	Moderate
Total pituitary weight, g	2.50±.15[a]	2.59±.14
Anterior pituitary weight, g	1.97±.14	2.03±.13
Pituitary LH		
Concentration, µg/mg	.95±.27	1.42±.24
Total, mg	1.80±.59	2.82±.51
Male fetuses, no.	3	4
Female fetuses, no.	5	5
Fetal weight, kg	25.4±1.5	27.5±1.5
Crown rump length, cm	32.1±.7	33.5±.6

[a]Least squares mean±SE.

TABLE 10. OVARIAN AND UTERINE CHARACTERISTICS FOR COWS WITH THIN OR MODERATE BODY CONDITION ON DAY 260 OF GESTATION

	Body Condition	
Criteria	Thin	Moderate
Wet ovarian weight, g	15.28±1.32[a]	15.35±1.25
Dry ovarian weight, g	1.90±.25	1.62±.23
Corpus luteum weight, g	4.18±.45	4.63±.42
Follicular fluid weight, g	1.58±.19	1.87±.17
Number of caruncles	90±8	94±7
Total caruncular weight, kg	2.86±.20	2.81±.18
Uterine weight, kg	3.94±.18[b]	4.39±.17[c]
Cotyledonary weight, kg	1.87±.16[d]	1.44±.15[e]
Placental[f] weight, kg	1.29±.09[b]	1.06±.09[c]
Total placental fluid, l	7.55±7.4	6.91±6.8

[a]Least squares mean±SE.
[b,c]Means not having a common superscript differ P<.07.
[d,e]Means not having a common superscript differ P<.05.
[f]Chorioallantois minus the cotyledons.

TABLE 11. CONSTITUENTS IN AMNIONIC AND ALLANTOIC FLUID OF COWS WITH THIN OR MODERATE BODY CONDITION ON DAY 260 OF GESTATION

Criteria	Body Condition	
	Thin	Moderate
Allantoic fluid		
Volume, l	5.69±.91[a]	4.92±.94
Fructose, mg/ml	1.89±.29	2.37±.27
Total fructose, g	11.2±2.0	9.9±2.1
Protein, mg/ml	6.59±.43	6.78±.40
Total protein, g	36.2±5.5	29.4±5.7
Calcium, mg/dl	8.77±2.44	5.03±2.24
Total calcium, g	46.7±17.1	28.2±17.7
Amnionic fluid		
Volume, l	1.25±.31[b]	2.09±.32[c]
Fructose, mg/ml	1.30±18[d]	2.22±.16[e]
Total fructose, g	1.7±.8[d]	4.8±.8[e]
Protein, mg/ml	1.22±.09	1.21±.09
Total protein, g	15.6±4.3	25.0±4.3

[a]Least squares mean±SE.
[b,c]Means not having a common superscript differ P<.10.
[d,e]Means not having a common superscript differ P<.01.

TABLE 12. HORMONES IN AMNIONIC AND ALLANTOIC FLUID OF COWS WITH THIN OR MODERATE BODY CONDITION ON DAY 260 OF GESTATION

Criteria	Body Condition	
	Thin	Moderate
Allantoic fluid		
Volume, l	5.69±.91[a]	4.92±.94
Estrone, ng/ml	1.20±.24	.89±.21
Total estrone, µg	5.61±1.07	3.13±1.10
Estrone sulfate, ng/ml	229.6±50.2[d]	230.1±46.1[d]
Total estrone sulfate, mg	1.1±.4	.87±.4
Estradiol, pg/ml	64±19	36±17
Total estradiol, ng	168.4±52.2	148.5±54.0
Prostaglandin F, ng/ml	2.56±.42	2.38±.38
Total prostaglandin F, mg	13.6±3.05	10.5±3.2
Amnionic fluid		
Volume, l	1.25±.31[b]	2.09±.32[c]
Estrone, ng/ml	1.65±.42	1.85±.39
Total estrone, µg	1.5±.9	3.8±.9
Estrone sulfate, ng/ml	13.3±4.3[e]	8.4±3.9[e]
Total estrone sulfate, µg	7.6±5.1	14.8±5.2
Estradiol, pg/ml	115±41	84±38
Total estradiol, ng	55.3±42.1	157.9±43.6

[a]Least squares mean±SE.

[b,c]Means in rows not having a common superscript differ P<.10.

[d,e]Means in columns not having common superscripts differ P<.01.

CHAPTER IV

INFLUENCE OF NUTRITION AND BODY CONDITION ON PITUITARY, OVARIAN, AND THYROID FUNCTION OF NONLACTATING BEEF COWS

ABSTRACT

Nonpregnant Hereford cows (n=70) were used to determine the effect of nutrient intake and body condition on reproductive and thyroid function. Body condition scores (BCS; 1=emaciated; 9=obese) of cows averaged 5.0±.2 on July 1, and cows were fed for 4 months to either lose weight and BCS (thin; n=22), maintain weight and BCS (moderate; n=24), or gain weight and BCS (fat; n=24). After November 1, cows received a complete ration so as to maintain weight and BCS. Cows were slaughtered in December (6 thin, 8 moderate, and 8 fat cows) and the subsequent March (16 cows per group). Before slaughter, estrus was synchronized by giving two injections of Prostaglandin $F_{2\alpha}$ (PGF) 11 day apart. Six days after the second PGF injection, cows were simultaneously treated with 100 µg of GnRH (im) and 100 µg of TRH (iv). Serum samples were obtained frequently for 12 h. BCS of cows at slaughter (8 days post PGF) averaged 3.4±.1, 5.3±.1, and 7.1±.1 ($P<.01$) and carcass energy content averaged 243±6, 432±5, and 714±7 mcal ($P<.01$) for thin, moderate, and fat

cows, respectively. Wet ovarian weights were heavier ($P<.001$) and corpora lutea weights were greater ($P<.09$) for fat cows. In December, follicular fluid weights were less ($P<.01$) for thin cows compared to moderate and fat cows, but follicular fluid weights did not differ among groups in March. Pituitary LH content and thyroxine after TRH were not influenced by nutrient intake or BCS. However, thin cows had greater concentrations ($P<.05$) of LH in serum after GnRH than did moderate or fat cows. These data suggest that nutrient intake and body energy reserves of beef cows influence ovarian function serum LH after treatment with GnRH.

(Key Words: Nutrition, Body Condition, Reproduction, LH, Thyroxine.

Introduction

The interval from parturition to first estrus is influenced by age of dam (Wiltbank et al., 1970; Bellows and Short, 1978), suckling frequency (Short et al., 1972; Wettemann et al., 1978; Carruthers and Hafs, 1980a), nutrient intake before and after calving (Wiltbank et al., 1962; Dunn et al., 1969; Corah et al., 1975), and body condition of the dam (Lishman et al., 1979; Dunn and Kaltenbach, 1980; Selk, 1986). The mechanisms that these factors independently or in unison dictate the length of anestrus in beef cows is unclear.

Cattle restricted in energy intake had greater (Gombe

and Hansel, 1973), similar (Hill et al., 1970; Dunn et al., 1974; Spitzer et al., 1978), or decreased (Apgar et al., 1975) concentrations of LH in serum compared to cows fed adequate amounts of energy. Cows fed restricted energy rations had greater concentrations of LH in serum after GnRH than cows not restricted in energy intake (Beal et al., 1978; Whisnant et al., 1985). Inconsistencies among experiments suggest that energy reserves of cows may influence pituitary function. Regardless of energy intake, increased pulsatile release of LH is a prerequisite for initiating estrous cycles in postpartum beef cows (Rawlings et al., 1980; Williams and Ray, 1980; Riley et al., 1981; Lamming et al., 1981; Humphrey et al., 1983).

Content of TSH in the pituitary of ewes is inversely related to LH content (Louw et al., 1964) and may account for the anestrus exhibited in thyroprotein fed cows (Wagner and Hansel, 1969) and triiodothyronine treated sheep (Howland et al., 1966). Alterations in body energy reserves, metabolic rate and thyroid function may effect reproductive function. The objectives of this experiment were to determine the influence of nutrient intake and body condition on pituitary, ovarian, and thyroid function of beef cows.

Materials and Methods

Seventy-two nonpregnant Hereford cows were randomly assigned to one of three feeding regimes to alter weight and

body condition (energy reserves). Two cows were removed from the study because of health reasons. Thirty-five cows were slaughtered in each of two years. Experimental protocol was initiated on July 1 of each year. From July 1 to November 1, cows were fed (Table 13) to either lose weight and body condition (thin; n=11 per year), maintain weight and body condition (moderate; n=12 per year), or gain weight and body condition (fat; n=12 per year). It was anticipated that by November 1, fat cows would have a body condition score (BCS; Wagner, 1985) of 6 or greater, moderate cows would have a BCS score of 5, and thin cows would have a BCS of 4 or less. After November 1, cows were weighed and scored for body condition weekly, and each cow received a complete ration (Table 1) so as to maintain weight and BCS. Cows were slaughtered in December (6 thin, 8 moderate, and 8 fat cows, respectively) and the subsequent March (16 cows per group) each year.

Prior to slaughter, estrus was synchronized by giving two injections of prostaglandin $F_{2\alpha}$ (PGF) 11 days apart. Cows were bled on the days of the first and second PGF injection and 6 days after the second injection of PGF. Blood samples (20 ml) were mixed in tubes containing 12.5 mg of oxalic acid, immediately cooled on ice to 4 C, and centrifuged (5,000 xg for 15 min). Plasma was decanted and stored at -20 C until quantified for concentrations of progesterone (Lusby et al., 1981). Intra- and inter-assay coefficients of variation were 6.0% and 7.2%, respectively.

When 5 ng of progesterone were added to steer plasma, 4.6±.1 (n=9) ng were recovered.

Five days after the second PGF injection, a jugular vein was cannulated[1] and cows were confined to metabolism stalls. On the next day, cows were treated with 100 µg of gonadotropin releasing hormone[2] (GnRH; im) and 100 µg of thyrotropin releasing hormone[3] (TRH; iv). Commencing at 0600 h, serum samples (20 ml) were obtained every 15 min for 30 min prior to treatment. After treatment, samples were obtained every 15 min for the first 2 h, every 30 min for the next 4 h, and then every 2 h until 12 h after treatment.

Pretreatment samples and samples obtained every 15 min for the first 6 h after treatment were quantified for concentrations of LH (Hallford et al., 1979). Antisera (B225[4]) was used at a dilution of 1:100,000. Bovine LH (NIH-LH-B9) was used as the standard. Radiolabled ligand was prepared from LER-1374A-ovine LH[5] and ^{125}I. Luteinizing hormone was iodinated with ^{125}I using a procedure similar to that described by Greenwood et al., (1963). Twenty-five

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[1]Bolab Inc., BB 317-V/10, inside diameter.062 inches, outside diameter .82 inches, Lake Havasu City, Arizona.

[2]LH-FSH-RH (Chloride form), Batch #2, Prepared by NICHD and distributed by NIAMDD NIH, Bethesda, Maryland.

[3]TRH, Sigma Stock # P-6397-L-Pyroglutamyl-L-Histidyl-L-Proline amide Lot # 060F7800, Sigma Chemical Co., St. Louis.

[4]Supplied by Dr. G. Niswender, Colorado State University, Fort Collins, Colorado.

[5]Supplied by Dr. L. Riechert, Jr., Albany Medical College, Albany, New York.

microliters of phosphate buffer (.5M; pH=7.5) were added to a reaction vial containing 2.5 micrograms of LH. One-half millicurie of ^{125}I[6] in 10 microliters of phosphate buffer (.05M; pH=7.0) was added to the reaction vial and allowed to equilibrate for about 2 min. Amounts and reaction times for chloramine-T, sodium metabisulfite and preparation of the anion exchange column are the same as those used for the iodination of insulin and are described in Chapter III. The column effluent of the anion exchange column was collected in 1% lypholyzed egg white in .5 ml phosphate buffered saline (.01M; pH=7.0). Intra- and inter-assay coefficients of variation for the LH assay were 4.7% and 12.2%, respectively. When 5 ng of LH were added to serum from an anestrous cow, 5.1$\pm$.2 ng (n=16) were recovered.

Samples obtained every 30 minutes for the first 6 hours and at 8, 10, and 12 hours after treatment were quantified for concentration of thyroxine (T_4; Pratt et al., 1986). Intra- and inter-assay coefficients of variation for the T_4 assay were 5.4% and 6.8%, respectively. When 50 ng of T_4[7] were added to charcoal stripped cow serum, 53.0$\pm$1.2 ng were (n=10) recovered. Extraction efficiency of ^{125}I added to control samples (n=8) in each assay averaged 78.9$\pm$2.2%; therefore, samples were corrected for procedural losses calculated for each assay.

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[6]Cintichem, Inc., Tuxedo, NY.

[7]T2376 L-Thyroxine Free Acid (Crystalline), Sigma Chemical Co., St. Louis.

Pituitary glands were removed within 45 min of death and weighed. The posterior lobe was dissected from the anterior lobe and weight of the anterior lobe of the pituitary was determined. Anterior pituitaries were immediately frozen on dry ice and stored at -20 C until analyzed for luteinizing hormone (LH) content by radioimmunoassay. The anterior pituitary was prepared for assay of LH similar to the procedure described by Schoenemann et al., (1985). Each anterior pituitary was minced with a scalpel and diluted in phosphate buffered saline (.01M; pH=7.0) to achieve a concentration of 50 mg of tissue/ml of buffer. The tissue in buffer was maintained at 5 C and homogenized with a Sorvall Omni mixer at high speed for 45 seconds, centrifuged (3,000 xg for 30 min), and the extract was decanted. Pituitary extracts were diluted 1:5,000 in phosphate buffered saline containing 1% lypholyzed egg white (.01M; pH=7.0). Pituitary LH content was quantified in a single radioimmunoassay in which the intra-assay coefficient of variation was 2.5%. Aliquots of 50, 100, and 150 µl of pituitary extract were parallel to the standard curve composed of .1, .2, .4, .8, 1.6, 3.2, and 6.4 ng of LH.

Corpora lutea were removed from ovaries and total wet ovarian and corpora lutea weights were determined. In addition, the ovaries were minced, blotted on a paper towel, and weighed to determine follicular fluid weights (Casida et al., 1968). Dry ovarian weights were determined after

desiccation in an oven at 90 C for 54 hours.

One-half of the carcass was obtained at slaughter. Soft carcass tissues were separated from the bone and total carcass energy content and percent fat were determined (Wagner, 1985).

Analyses of variance were used to analyze cow, carcass, pituitary, and ovarian characteristics. Analyses of variance of endocrine responses were conducted using ordinary least squares. The experimental design was a split-plot in which cows were nested within treatment and season slaughtered. Repeated measurements over time were taken on cows, and time sampled was considered the subplot. If a significant treatment*time sampled interaction existed, then response curves for hormone concentrations were characterized by time trends that were analyzed by polynomial regression. Tests of heterogeneity of regression coefficients were used to determine differences in time trends for endocrine responses among cows with thin, moderate, or fat body condition.

Results and Discussion

From July 1 to November 1, thin cows lost about 80 kg and 2 BCS, moderate cows maintained weight and BCS, and fat cows gained about 80 kg and 2 BCS. Between November 1 and slaughter, cows on all treatments maintained BCS (Table 14). BCS at slaughter for thin cows ($3.4 \pm .1$) was less ($P<.001$) than that for moderate ($5.3 \pm .1$) and fat ($7.1 \pm .1$) cows. Cows in all groups maintained weight (Table 14) from November 1

until slaughter and at slaughter weighted 341±9, 394±9, and 483±10 kg (P<.001) for thin, moderate, and fat cows, respectively. Carcass energy content and percent fat in carcasses were greatest (P<.001) for fat cows and less for moderate and thin cows (Table 14).

Analyses of LH response to GnRH included cows that exhibited ovarian luteal activity as indicated by the presence of a corpus luteum at slaughter or plasma progesterone equal to or greater than 1 ng/ml at the time of the first or second PGF injection or at the time of GnRH and TRH treatment. Eighteen (18/22) thin, 24 (24/24) moderate, and 23 (23/24) fat cows had ovarian activity at slaughter and were used in the analyses. Percent fat in the carcasses of the 4 thin cows that did not exhibit ovarian activity was 4.1%. Anestrus occurred when body condition scores (1=emaciated; 9=obese) of nonlactating, cyclic beef cows reached 3.5 (Richards et al., 1986).

Full model analyses of LH and T_4 after GnRH and TRH indicated nonsignificant season slaughtered*treatment, season slaughtered*time sampled, and season slaughtered*treatment*time sampled interactions. Therefore, the reduced model included season slaughtered, treatment, cow(season slaughtered*treatment), and time sampled as a continuous variable.

Total pituitary gland and anterior pituitary weights did not differ among treatments (Table 15). Likewise, concentrations and total pituitary LH content were similar

(P>.10) for thin (3.05±.33 mg), moderate (2.78±.30 mg), and fat (3.66±.36 mg) cows. Pituitary and anterior pituitary weights are similar to those observed in cyclic beef cows and heifers (Beal et al., 1978) and dairy cows (Carruthers et al., 1980b). Pituitary LH content for our cows was similar to that in pituitaries of cows slaughtered when BCS (1=extremely thin; 5=extremely fat) averaged 4.4±.2, 3.1±.1, and 1.6±.3 (Moss et al., 1982).

Our results suggest that nutrition and BCS do not influence pituitary weights in nonlactating cows and agrees with previous results (Beal et al., 1978). It has been demonstrated that energy intake (Beal et al., 1978) and BCS (Moss et al., 1982) of beef cows affects pituitary LH content. Cows with lower BCS and consuming less energy had reduced LH in pituitaries. Cows in these previous experiments were losing weight and body condition until the time of pituitary removal. Cows in our experiment maintained weight for 1 to 4 mo before slaughter, which may influence pituitary LH content.

Season that the cows were slaughtered (March vs. December) influenced pituitary weights and pituitary LH content. Regardless of nutrient intake and BCS, cows slaughtered in March had heavier (P<.02) total pituitaries and anterior pituitaries, and had more (P<.02) LH in the pituitary than did cows slaughtered in December (Table 16). Cows slaughtered in December were maintaining weight and body condition for about one mo before slaughter, whereas,

cows slaughtered in March maintained weight and BCS for about 4 mo before slaughter. The length of time that a cow is maintaining weight and BCS may influence pituitary characteristics. Cows maintaining weight and BCS had greater basal LH and greater GnRH induced LH release compared to cows losing weight and BCS (Rutter and Randel, 1984). Reducing cellular energy substrates does result in decreased pituitary function. Rat pituitaries in medium treated with metabolic inhibitors that alter glycolysis and oxidative phosphorylation released less LH when stimulated with GnRH (Sen et al., 1979).

Fat cows had heavier ($P<.001$) wet ovarian weights than did moderate or thin cows (Table 17). Dry ovarian and wet corpora lutea weights for fat cows tended to be heavier ($P<.09$) than for moderate and thin cows (Table 5). Ovarian and corpora lutea weights were similar to previous reports for beef cows (Gombe and Hansel, 1973; Beal et al., 1978; Spitzer et al., 1978).

In agreement with previous studies, cows that were fed limited amounts of energy had reduced ovarian weights (Gombe and Hansel, 1973; Beal et al., 1978). Thin cows had lighter wet and dry ovarian and corpora lutea weights, suggesting nutrients available to maintain ovarian cell structure and integrity may differ in thin cows. Cows supplemented to lose weight and BCS have reduced concentrations of glucose (Rasby et al., 1982) and increased concentrations of nonesterified fatty acids (NEFA; Garmendia et al., 1984) in plasma. Blood

glucose is the energy substrate used at the cellular level and reduced concentrations may influence ovarian and corpora lutea weights. NEFA indicate fat mobilization.

Secretion of pituitary gonadotropins necessary to maintain ovarian and corpora lutea function may be reduced in thin cows. However, total pituitary LH content of thin, moderate, and fat cows in our experiment were similar. Another reason for altered ovarian weights due to treatment could be that thin cows respond to exogenous PGF differently than fat and moderate cows. For instance, if a thin cow ovulates 24 or 36 h later than a moderate or fat cow after treatment with PGF, the corpus luteum would be lighter at slaughter.

Regardless of nutrient intake and BCS, corpora lutea from cows slaughtered in March (2.03$\pm$.19 g) were heavier ($P<.01$) than corpora lutea from cows slaughtered in December (1.26$\pm$.34 g). Pituitary LH content was greater in cows slaughtered in March compared to December and may influence ovarian luteal cell function in cows slaughtered in March causing heavier corpora lutea.

Analysis of follicular fluid weight indicated a treatment by season slaughtered interaction. Thin cows had the least and fat cows had the greatest amount of follicular fluid ($P<.05$; Table 18) in December. Follicular fluid weights of moderate cows were intermediate to the thin and fat cows. However, in March, follicular fluid weight did not differ among groups.

Thin cows slaughtered in December lost weight and body condition from July 1 to November 1, then maintained weight and BCS for about one mo prior to slaughter. Thin cows slaughtered in March maintained weight and BCS for about 4 mo prior to slaughter. Losses of weight and BCS followed by a short period of maintaining weight and BCS may alter the hypothalamo-pituitary-ovarian axis. Thin cows slaughtered in March may have adjusted to a lighter body weight and BCS resulting in an increase in hypothalamic and pituitary gonadotropin secretion and increased follicular development.

Concentrations of T_4 in serum of cows in thin, moderate, or fat body condition were best described by a third order polynomial regression equation (Table 19). Time trends for concentrations of T_4 were similar ($P>.10$; Table 20) in thin, moderate, and fat cows. At TRH treatment, concentrations of T_4 averaged $69.4\pm.9$ ng/ml for all cows (Figure 7). Concentrations of T_4 in serum increased to 91.7 ± 1.3 ng/ml by 5 h after TRH. T_4 concentrations were similar to those observed in TRH treated steers (Pratt et al., 1986) and cows (Vanjonack et al., 1974).

Our results suggest thyroid function, as indicated by T_4 release after TRH, is similar in thin, moderate, and fat cows. Even though BCS among groups differed, environmental conditions in this experiment were not adverse. Cows were allowed a day adaptation period in environmentally controlled metabolism stalls before TRH treatment and this adjustment may have minimized any differences in thyroid

response to TRH treatment.

Thermal stress is associated with reduced thyroid activity (Gale, 1973). Hypothyroidism results in reduced gut motility (Levin, 1969), and supplementing ruminants with thyroprotein enhances the rate of passage of digesta (Miller et al., 1974; Kennedy et al., 19777). Thyroidectomy tends to prolong the retention time of digesta and increase digestibility, whereas thyroid hormone administration produces the opposite effect in sheep (Kennedy et al., 1977). Hyperthyroidism of ruminants exposed to cold depresses digestibility of the diet by enhancing gut motility and rate of passage (Westra and Christopherson, 1976). Thus, reduced thyroid activity depresses rumen motility and rate of passage but increases digestibility. Concentrations of T_4 in serum prior to TRH treatment were tended to be reduced ($P<.12$) in thin cows (64.04 ± 3.37 ng/ml) compared to moderate (72.69 ± 3.39 ng/ml) and fat (72.69 ± 3.90 ng/ml) cows. This suggests that thin cows may utilize feeds stuffs more efficiently, minimizing the effect of nutrient intake on pituitary and ovarian activity. This supports the reduction in feed required for maintenance of cows in thin versus moderate body condition (Wagner, 1985). Ovarian follicular fluid weights were similar among groups in March, but in December, follicular fluid weights were reduced in thin compared to moderate and fat cows. Cows slaughtered in December were fed a maintenance ration for one mo and cows slaughtered in March were fed a maintenance ration for 4 mo.

Thin cows receiving a maintenance ration for 4 mo may have adapted to their lighter metabolic weight and altered thyroid function. Reduced T_4 release may have reduced gut motility and increased digestibility of the ration and supplied enough nutrients to stimulate pituitary and ovarian activity.

Concentrations of LH in serum of cows in thin, moderate, and fat body condition after GnRH were best described by a fourth order polynomial regression equation (Table 19). Time trends for LH concentrations in serum of thin, moderate, and fat cows were not parallel ($P<.025$; Table 20). Prior to GnRH treatment, concentrations of LH in serum were similar for all treatments and averaged $2.2\pm.59$ ng/ml (Figure 8). At 105 minutes after treatment, concentrations of LH in serum averaged 41.3 ± 3.4, 32.2 ± 3.6, and 33.6 ± 4.1 ng/ml for thin, moderate, and fat cows, respectively. By 360 min after GnRH, concentrations of LH in serum averaged $4.1\pm.8$ ng/ml for all groups. Our results are similar to those of Whisnant et al., (1985) that indicate greater release of LH after GnRH in cows fed restricted amounts of energy compared to cows fed ample energy.

Our results suggest that thin cows release more LH when treated with GnRH than do moderate or fat cows; therefore, pituitary stores of releasable LH in thin cows may be greater than those in moderate and fat cows. The releasable pool of LH in the rat changes during the estrous cycle, but total pituitary LH does not change (Pickering and Fink,

1979). Our results indicate that pituitary LH content is similar among cows in different body conditions; suggesting that greater concentrations of LH after GnRH in thin cows is a result of a greater releasable LH pool. Thin cows may release more LH because endogenous GnRH is absent or reduced. Reduced GnRH may cause an accumulation of LH in releasable pools that inturn cause a hyperresponse or hypersensitivity of the pituitary to an exogenous treatment with a pharmacological dose of GnRH. Another possibility is that thin cows release a similar amount of LH as do moderate and fat cows, but into a smaller blood volume. Regardless of BCS of cows, blood volume is about 7% of body weight (Mark Richards, personnel communication) and thin cows weighed less at the time of GnRH treatment.

We conclude that nutrient intake and BCS do not influence total pituitary weight, anterior pituitary weight, pituitary LH content, nor thyroxine after TRH treatment. However, nutrition and BCS influence ovarian and corpora lutea weights and concentrations of LH in serum after GnRH. Thin cows had lighter ovarian and corpora lutea weights and had greater concentrations of LH in serum after GnRH treatment. Nutrient intake and BCS influence reproductive efficiency of beef cows by altering LH secretion and ovarian function.

TABLE 13. FEEDING SCHEDULE TO ACHIEVE AND TO MAINTAIN COWS WITH THIN, MODERATE, AND FAT BODY CONDITION SCORES

	Body Condition		
	Thin	Moderate	Fat
Cows, no	22	24	24
Interval:			
July 1, 1982 to November 1, 1982	wheat straw ad libitum	.5 kg CSM[a]/ (head*d) + 6.4 kg prairie hay/(head*d)	2.2 kg CSM/ (head*d) + prairie hay ad libitum[b]
November 1, 1982 to slaughter in December or March	each cow received a complete ration[c] to maintain weight until slaughter		

[a]41% cottonseed meal cube.

[b]During the second year, fat cows received 1.5 kg CSM/hd/da and grazed native range between June 1 and September 1.

[c]Composition of the complete ration: 40% rolled corn, 35% dehydrated alfalfa pellets, 21.7% cottonseed hulls, 3.0% cane molasses, and .3% salt and trace minerals.

TABLE 14. BODY CONDITION, WEIGHT, AND CARCASS ENERGY CONTENT OF COWS WITH THIN, MODERATE, OR FAT BODY CONDITION

Criteria	Body Condition		
	Thin	Moderate	Fat
Condition score on November 1	3.2±.1[ab]	5.3±.1[c]	6.9±.1[d]
Condition score at slaughter	3.4±.1[b]	5.3±.1[c]	7.1±.1[d]
Weight on November 1, kg	339±9[b]	394±9[c]	480±10[d]
Weight at slaughter, kg	341±9[b]	394±9[c]	483±10[d]
Carcass energy content, mcal	243±6[b]	432±5[c]	714±7[d]
Fat, %	7.0±.7[b]	12.7±.7[c]	21.6±.8[d]

[a]Least squares mean±SE.
[b,c,d]Means not having a common superscript differ P<.001.

TABLE 15. CHARACTERISTICS OF PITUITARIES FOR COWS WITH THIN, MODERATE, OR FAT BODY CONDITION

Criteria	Body Condition		
	Thin	Moderate	Fat
Pituitary weight, g	2.33±.07[a]	2.40±.07	2.54±.08
Anterior pituitary weight, g	1.96±.07	1.92±.06	2.03±.07
Concentration of pituitary LH, µg/mg	1.59±.16	1.44±.14	1.79±.18
Total pituitary LH, mg	3.05±.33	2.78±.30	3.66±.36

[a]Least squares mean±SE.

TABLE 16. PITUITARY CHARACTERISTICS FOR COWS WITH THIN, MODERATE, OR FAT BODY CONDITION IN DECEMBER AND MARCH

Criteria	Month Slaughtered	
	December	March
Pituitary wt., g	2.32±.07[ab]	2.53±.05[c]
Anterior pituitary wt., g	1.88±.06[b]	2.06±.05[c]
Concentration of pituitary LH, µg/mg	1.49±.15	1.72±.11
Total pituitary LH, mg	2.72±.30[b]	3.61±.23[c]

[a]Least squares mean±SE.
[b,c]Means in columns not having a common superscript differ P<.02.

TABLE 17. OVARIAN CHARACTERISTICS FOR COWS WITH THIN, MODERATE, OR FAT BODY CONDITION

Criteria	Body Condition		
	Thin	Moderate	Fat
Wet ovarian weight, g	12.1±1.4[ab]	14.7±1.4[b]	20.6±1.6[c]
Dry ovarian weight, g	1.80±.18[d]	2.07±.17[de]	2.43±.22[e]
Corpus luteum weight, g	1.01±.37[d]	2.00±.30[e]	1.93±.34[e]

[a]Least squares mean±SE.
[b,c]Means not having a common superscript differ P<.001.
[d,e]Means not having a common superscript differ P<.09.

TABLE 18. FOLLICULAR FLUID WEIGHT (g) FOR COWS WITH THIN, MODERATE, OR FAT BODY CONDITION IN DECEMBER AND MARCH

	Month Slaughtered	
Body Condition	December	March
Thin	1.83±.35[ab]	2.42±.32
Moderate	2.66±.37[bc]	1.84±.30
Fat	3.65±.44[c]	2.51±.44

[a]Least squares mean±SE.
[b,c,d]Means in columns not having a common superscript differ P<.01.

TABLE 19. R^2 AND PROBABILITY LEVELS OF POLYNOMIAL REGRESSION EQUATIONS FOR CONCENTRATION OF LUTEINIZING HORMONE (LH) AND THYROXINE (T_4) AFTER GnRH AND TRH

	Hormone	
Order	T_4	LH
Linear	.75[a] .001[b]	.33 .001
Quadratic	.79 .001	.46 .001
Cubic	.79[c] .03	.71 .001
Quartic	.79 .10	.74[c] .001
Quintic	.79 .41	.74 .16

[a]R^2 value.
[b]Probability level.
[c]Order of response curve used.

TABLE 20. ORTHOGONAL COMPARISONS USED TO TEST FOR HETEROGENEITY OF REGRESSION COEFFICIENTS FOR POLYNOMIAL RESPONSE CURVES TO DETERMINE WHETHER TIME TRENDS FOR T_4 AFTER TRH AMONG GROUPS WERE NOT PARALLEL

	Thin versus Moderate and Fat			
Error	D.F.	S.S.	M.S.	F
Thin	297	32970.92		
Moderate, Fat	584	52984.58		
Total	881	85955.50	97.57	
Thin, Moderate, Fat	884	86234.50		
Difference	3	279.00	93	.95
	Moderate versus Fat			
Moderate	374	35368.14		
Fat	207	17355.90		
Total	581	52724.04	90.75	
Moderate, Fat	749	29402.18		
Difference	3	260.54	86.85	.95

TABLE 21. ORTHOGONAL COMPARISONS USED TO TEST FOR HETEROGENEITY OF REGRESSION COEFFICIENTS FOR POLYNOMIAL RESPONSE CURVES TO DETERMINE WHETHER TIME TRENDS FOR LH AFTER GnRH AMONG GROUPS WERE NOT PARALLEL

	Thin versus Moderate and Fat			
Error	D.F.	S.S.	M.S.	F
Thin	320	17922.67		
Moderate, Fat	694	27817.04		
Total	1014	45739.71	45.11	
Thin, Moderate, Fat	1018	47578.96		
Difference	4	1839.25	458.81	10.19*
	Moderate versus Fat			
Moderate	456	19074.93		
Fat	234	8720.26		
Total	690	27795.19	40.28	
Moderate, Fat	694	27817.04		
Difference	4	21.85	5.46	.13

*(P<.025).

Figure 7. Least-Squares Regressions and Least-Squares Means (■ =Thin; ○=Moderate; + =Fat) for Concentrations of Thyroxine in Serum of Cows With Thin (TH), Moderate (M), or Fat (F) Body Condition.

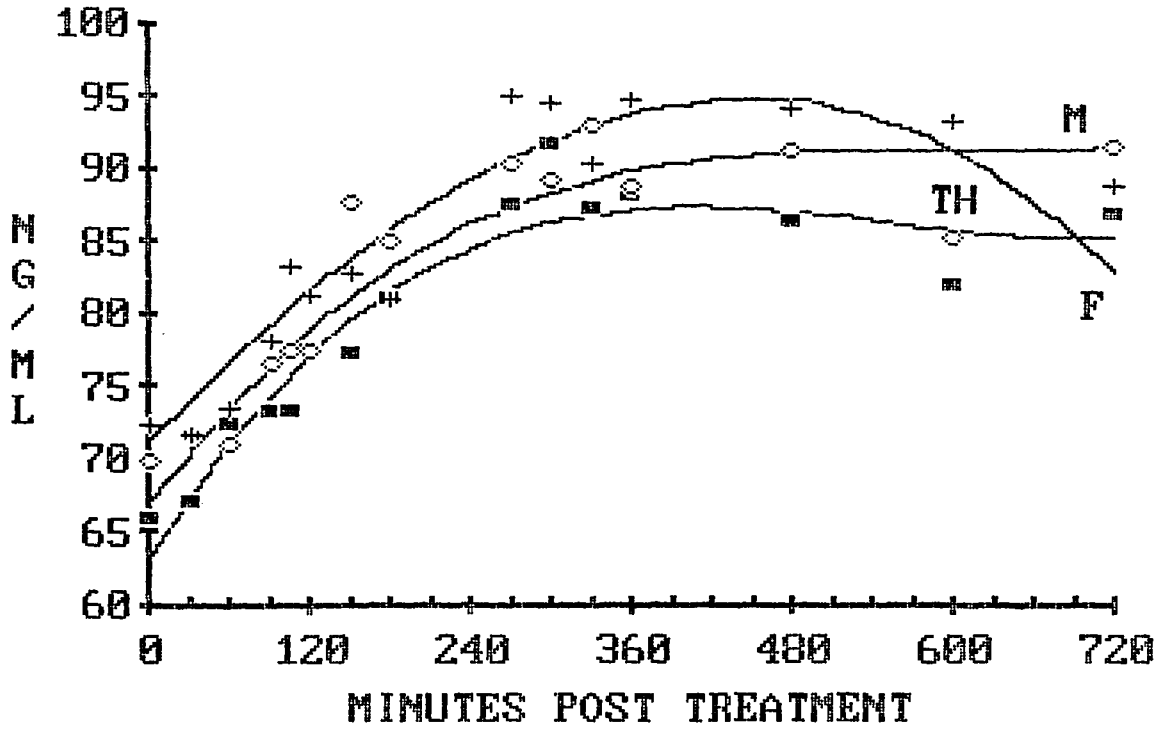
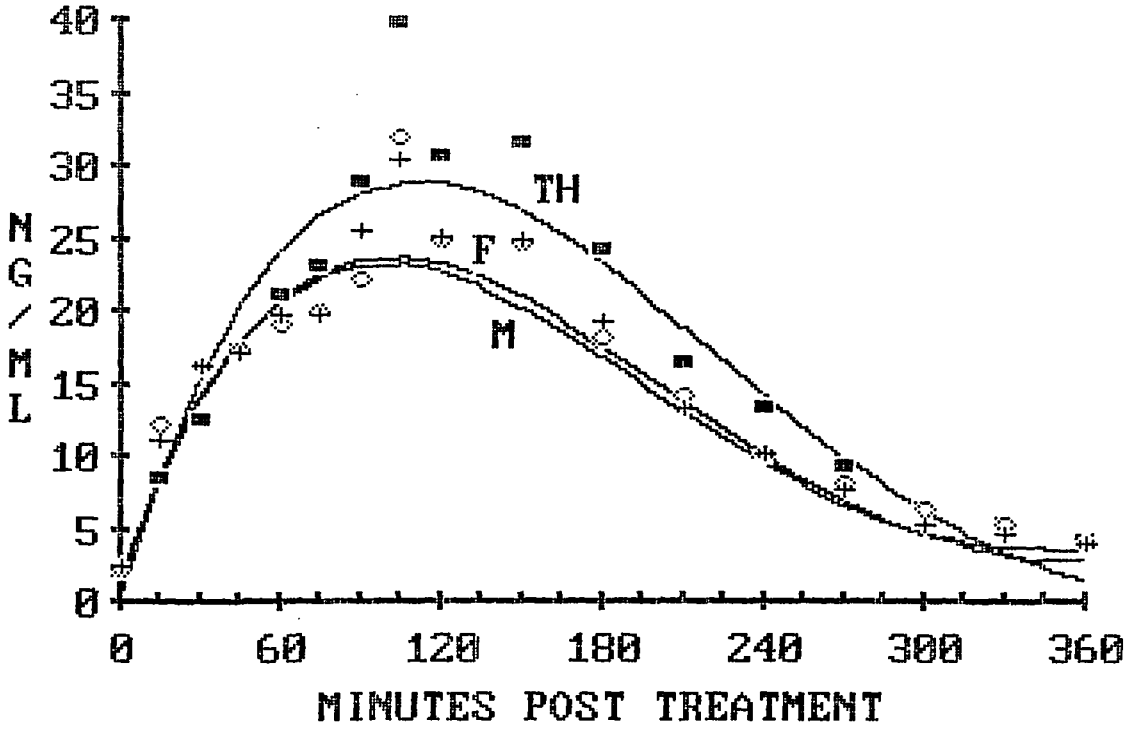

100
95
90
85
80
75
70
65
60
MG/ML
M
TH
F
0
120
240
360
480
600
720
MINUTES POST TREATMENT

Figure 8. Least-Squares Regressions and Least-Squares Means (■=Thin; ○=Moderate; +=Fat) for Concentrations of Luteinizing Hormone in Serum of Cows With Thin (TH), Moderate (M), or Fat (F) Body Condition.


40
35
30
25
20
15
10
5
0
NG/ML
TH
F
M
0
60
120
180
240
300
360
MINUTES POST TREATMENT

CHAPTER V

SUMMARY AND CONCLUSIONS

Two experiments were conducted to evaluate the influence of nutrient intake and body energy reserves on fetal development, pituitary, ovarian, and thyroid function of beef cows. In experiment 1, day of conception was monitored during the breeding season and on about day 145 of gestation, cows were randomly divided into two groups based on breeding date. Body condition score (BCS; 1=emaciated; 9=obese) of cows on day 145 averaged 5, and one group was fed so as to lose BCS (thin; n=8) and the other group fed so as to gain BCS (moderate; n=9) until about day 200 of gestation. After day 200 of gestation, cows were fed so as to maintain weight and BCS. On day 256$\pm$1 of gestation, cows were infused with 250 ml of a 50% glucose solution and blood samples were collected. All cows were slaughtered on day 259$\pm$1 of gestation.

BCS and weight at slaughtered were less in thin cows (3.7$\pm$.2, 418$\pm$26 kg) compared to moderate cows (5.7$\pm$.2, 509$\pm$25 kg). Concentrations of estrone sulfate and progesterone in plasma were similar in thin and moderate cows between days 200 and 260 of gestation. However,

concentrations of estradiol and estrone in plasma of thin cows were greater than in moderate cows between days 240 and 260 of gestation. Glucose disappearance and insulin response after glucose treatment did not differ in thin and moderate cows.

Total pituitary, anterior pituitary, fetal, ovarian, corpora lutea, follicular fluid, and caruncular weights were similar in thin and moderate cows. Pituitary LH content, crown rump length of the fetus, and total estrone, estrone sulfate, estradiol, calcium, prostaglandin F, and protein in placental fluids did not differ in thin compared to moderate cows. However, thin cows had lighter uterine weights and heavier cotyledonary and chorioallantoic weights compared to moderate cows. Total fructose in amnionic fluid was less in thin cows than in moderate cows.

The cotyledon is the major source of estrogens during pregnancy. Greater concentrations of free estrogens (estrone and estradiol) in plasma of thin cows between days 240 and 260 of gestation may be attributed to greater cotyledonary weights of thin cows.

Fetal weights were similar in thin and moderate cows. However, uterine weights were lighter but chorioallantoic weights were heavier in thin cows. Heavier fetal membranes may be able to acquire more nutrients from the maternal system to compensate for the reduced nutrient intake of thin cows and maintain fetal growth. It is possible that the placenta can detect when the dam is in a nutrient deficient

situation and fetal energy constituents are limiting. Reduced or inadequate circulating fetal energy metabolites may stimulate placental growth to offset a deficiency in maternal energy intake to maintain fetal growth.

Estrogens cause an increase in uterine blood flow. Thin cows had greater concentrations of estrone and estradiol in plasma between days 240 and 260 of gestation. Increasing blood flow to the uterus may be another means of offsetting a deficiency in energy intake of the dam. Therefore, if nutrients in the maternal circulation are limiting, increased blood flow to the uterus could allow for sufficient amounts of nutrients to reach the uterus and fetal growth would not be impaired.

Fructose is in great concentrations in fetal fluids and appears to be a storage form of fetal carbohydrates. Fructose in placental fluids is derived from maternal glucose. Reduced concentrations of fructose in amnionic fluid of thin cows may reflect reduced concentrations of glucose in plasma of thin cows. Concentrations of glucose in plasma of thin cows tended to be less ($P<.15$) than in moderate cows on day 256 ± 1 of gestation. In addition, reduced fructose in amnionic fluid may indicate greater glucose metabolism by fetuses of thin cows.

The importance of fructose in amnionic fluid remains unclear. Amnionic membranes are essentially avascular because they are composed of an inner ectoderm and outer mesoderm; therefore, reabsorption of fructose to the fetal

blood supply from amnionic fluid is limited. However, amnionic fluid is continuously being swallowed by the fetus and the fetal gut may be able to acquire nutrients present in amnionic fluid. Nutrients for fetal use are stored in allantoic fluid. Allantoic membranes are vascular because they are composed of an inner endoderm that is vascular and an outer mesoderm; therefore, nutrients in allantoic fluid can be reabsorbed by the fetal blood supply for use by the fetus.

Previous researchers report that limiting energy intake of the dam prepartum reduces calf birth weights. In our experiment, fetal weights were not significantly influenced by maternal nutrient intake between day 145 and 260 of gestation. However, at this stage of gestation, fetuses from thin cows were 2 kg lighter than fetuses from moderate cows. This may suggest that fetal weight gain may be more sensitive to nutrient intake of the dam from day 260 of gestation until parturition.

Nutrient intake prepartum influences reproductive performance of beef cows. Cows losing weight prepartum have extended intervals from parturition to first estrus. Wettemann, (1980) concluded that prepartum changes in hormones may be a mechanism that controls the length of the postpartum anestrous period of beef cows. Cows supplemented to lose weight and body condition in late gestation had reduced concentrations of progesterone and estrone in plasma and had longer interval from calving to first estrus (Mobley

et al., 1983). The present experiment indicates that thin cows have greater concentrations of estrone and estradiol in plasma between days 240 and 260 of gestation. The link between prepartum hormone changes and postpartum reproduction remains unclear. Estradiol and estrone in thin cows in late gestation may set up a negative feedback loop to the anterior pituitary or hypothalamus that carries over into the postpartum period. Increased LH is a prerequisite to estrous cycles post partum. Prepartum concentrations of estrogens may influence pituitary stores of LH influencing postpartum gonadotropin secretion.

Changes in energy constituents in blood of cows prepartum may influence postpartum reproduction. Glucose, an energy source for the cell, is a prime candidate. Selk, (1986) demonstrated that cows that became pregnant during the breeding season had greater concentrations of glucose in plasma compared to cows not becoming pregnant. Cows receiving limited supplemental protein prepartum had lower blood glucose and longer intervals from calving to first estrus (Rasby et al., 1983).

In experiment 2, nonpregnant Hereford cows with an average body condition score of 5 were fed to either lose weight and BCS (thin), maintain weight and BCS (moderate), or gain weight and BCS (fat) from July 1 to November 1. After November 1, cows were individually fed to maintain weight and BCS. Cows were synchronized with two injections of prostaglandin $F_{2\alpha}$ (PGF) given 11 days apart. Six days

after the second injection, cows were treated with 100 µg of GnRH (i.m). and 100 µg TRH (i.v) and concentrations of LH and T_4 in serum were quantified. Eight days after the second PGF treatment, cows were slaughtered and the pituitary gland, ovaries, and carcass were evaluated.

BCS at slaughtered averaged 3.4±.1, 5.3±.1, and 7.1±.1 and carcass energy content averaged 243±6, 432±5, and 714±7 mcal for thin, moderate, and fat cows, respectively. Total pituitary, anterior pituitary, and pituitary LH content were similar in thin, moderate, and fat cows. However, ovarian and corpora lutea weights were lighter in thin compared to fat cows. Thyroxine after TRH did not differ among groups. Concentrations of LH after GnRH were greater in thin compared to moderate and fat cows.

Lighter ovarian and corpora lutea weights may indicate that nutrients available to maintain ovarian cell size and structure are limiting in thin cows. In addition, LH is essential to maintain luteal cell function in beef cows. Lighter corpora lutea may suggest that pituitary LH is limiting in thin cows. Total pituitary LH did not differ among groups, but total pituitary LH does not indicate the amount of releasable LH available. When cows were treated with a pharmacological dose of exogenous GnRH, thin cows had greater concentrations of LH in serum. Collectively this suggests that releasable pools of pituitary LH are greater in thin cows because endogenous GnRH is reduced or absent, and when thin cows are subjected to GnRH treatment there is

a hyperrelease of LH.

It is possible that release of LH after GnRH is similar among thin, moderate, and fat cow. Thin cows weighed less at the time of GnRH treatment and blood volume is about 7% of body weight. Therefore, thin cows may release a similar amount of LH after GnRH as moderate and fat cows but into a small volume of blood. Thus, due to less dilution of LH would be detected as greater concentrations of LH in serum. These results suggest that ovarian and corpora lutea weights are sensitive to nutrient intake and body energy reserves of the animal. Pituitary and hypothalamic function may be adequate in cows fed restricted energy diets, but the ovaries may be less responsive to pituitary secretions.

Our results suggest that nutrient intake and body energy reserves influence placental weights, concentrations of estradiol and estrone, and fructose in amnionic fluid of pregnant cows and ovarian weights, corpora lutea weights, and concentrations of LH after GnRH in nonpregnant cows. However, the link between prepartum hormonal changes and postpartum reproduction is unclear. These experiments do indicate that body energy reserves of cows should be monitored when conducting experiments involving nutrient intake and reproductive function. Finally, energy reserves of cows may account for inconsistencies in studies relating energy intake and pituitary function.

About 70% of the beef cows exposed to bulls during the breeding season wean a calf. Maximum reproductive

performance of beef herds is a key factor in assuring that beef remains a competitive source of protein for human consumption and for producers to realize a profit. Of cows not weaning a calf, about 15 to 20 % fail to become pregnant during the breeding season. In a five year study at Oklahoma State University, the single most important factor influencing whether cows became pregnant during the breeding season was body condition at calving (Selk, 1986). Therefore, the most economically means of increasing reproductive efficiency is to feed cows to attain an adequate body condition prior to calving to insure that the majority of the cows will exhibit estrus during the breeding season. More research is needed to determine the specific range of body energy reserves needed so that reproduction is not impaired. This would not only increase productivity of beef herds, but would also result in feeding regimes that would maximize feed resources.

Understanding the relationship between body energy reserves and hypothalamo-pituitary-ovarian function would aid in linking nutrient requirements and reproduction. These data indicate that pituitary, ovarian, and placental function of cows with thin, moderate, and fat body condition differ. Greater prepartum concentrations of estradiol and estrone in thin cows may establish a negative feedback loop on the pituitary or hypothalamus that results in reduced pituitary function postpartum. Thin cyclic cows appear to have pituitary LH stores similar to those for cows in

moderate or good body condition; however, ovarian characteristics of thin cows differ from cows in good body condition, suggesting that gonadotropin releasing hormone is limiting or the ovary fails to respond to pituitary secretions in thin cows. Cows not pregnant at the end of the breeding season had reduced concentrations of glucose in plasma compared to pregnant cows at the end of the breeding season (Selk, 1986). Blood constituents in cows that are energy precursors for cell function are influenced by nutrient intake (Rasby, 1983; Garmendia, 1984). Manipulating thin anestrous cows to repartition nutrients to the hypothalamo-pituitary-ovarian axis to initiate estrous cycles may be a means of increasing the number of cows becoming pregnant.

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