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Prepartum maternal cortisol concentrations on postnatal cortisol concentration and immunoglobulin absorption in neonatal dairy calves

Dana Nadine Wooley

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**PREPARTUM MATERNAL CORTISOL CONCENTRATIONS ON POSTNATAL CORTISOL
CONCENTRATION AND IMMUNOGLOBULIN ABSORPTION IN NEONATAL DAIRY
CALVES**

A Thesis

**Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science**

in

**The Interdepartmental Program
of Animal and Dairy Sciences**

by

Dana N. Wooley

**B.S., Louisiana State University – A&M College, 2008
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ABSTRACT

The role of glucocorticoids on intestinal closure in neonates has recently become an area of interest but a definitive mechanism remains to be identified. It is known that glucocorticoids enhance immunoglobulin absorption in dairy calves, but the role of maternal glucocorticoids at parturition is not clear. In the present experiment, we obtained plasma and milk samples from primiparous and multiparous Holstein cows ($n=24$) to measure cortisol at 72, 48 and 24 hours before and 3, 6, 12, 24 and 48 hours after spontaneous parturition. After parturition, calves ($n=24$) were immediately removed from their dams and a blood sample was taken from the calf before colostrum ingestion (3 hours postpartum). Calves were sampled at 6, 12, 24 and 48 hours postpartum to measure plasma cortisol concentration and determine the concentration of plasma immunoglobulin G (IgG). Milk cortisol concentration in cows was significantly lower compared with plasma (5.4 ng/ml vs. 10.2 ng/ml) ($P<0.0001$). Cortisol in plasma and milk at 24 hours prepartum was significantly lower compared with 3 hours postpartum (4.2 ng/ml vs. 11.2 ng/ml) ($P<0.05$). Calf plasma cortisol was significantly elevated at 3 hours postpartum (258.1 ng/ml) and declined thereafter to 60.6 ng/ml by 48 hours postpartum ($P<0.0001$). Calf IgG in plasma was significantly lower at 3 hours postpartum (134 mg/ml) compared with 12 and 24 hours postpartum (2,324 mg/ml and 3,015 mg/ml, respectively), indicating minimal concentrations of antibody in plasma before colostrum ingestion. Calves were divided into “low” ($n=11$) and “high” ($n=9$) cortisol groups based on mean plasma cortisol concentration in their dams. Although the difference in cortisol at 3, 6 and 12 hours postpartum between cows of low cortisol and high cortisol (20.5 ng/ml vs. 39.1 ng/ml, 1.6 ng/ml vs. 19.1 ng/ml and 2.9 ng/ml vs. 13.4 ng/ml, respectively) was significantly different ($P<0.05$), there was no significant difference between cortisol or IgG concentration in low cortisol compared with high cortisol calves. The present results indicate that maternal cortisol concentration at parturition does not influence calf cortisol concentration at birth or IgG concentration.

CHAPTER I

INTRODUCTION

It has been widely accepted that the mechanism initiating parturition in the bovine and other ruminant species is the secretion of adrenocorticotrophic hormone (ACTH) from the fetal anterior pituitary (Liggins, 1967; Kindahl et al., 2004). ACTH stimulates production of cortisol from the fetal adrenal cortex to stimulate production of 17α -hydroxylase (P450c17) from the placenta (Wendorf et al., 1983), which is necessary to convert placental progesterone to estrogen. This in turn stimulates release of prostaglandin $F_{2\alpha}$ from the endometrium to cause luteolysis (Königsson et al., 2001). Fetal cortisol is significantly elevated beginning 7 to 9 days before parturition and continues to increase during the last 3 to 5 days of gestation (Taverne et al., 1988). Maternal cortisol is elevated during the last 4 days prior to parturition and declines by 3 to 7 days after parturition, but does not reach peak concentrations until delivery (Adams and Wagner, 1970). High maternal glucocorticoids have been attributed to the stress and difficulty of parturition (Hydbring et al., 1999; Mehrzad et al., 2002). However, maternal glucocorticoids are responsible for other processes during parturition, such as accelerating mammary growth and initiating lactation (Collier and Tucker, 1978). In addition, there is a positive correlation between milk and serum glucocorticoid concentrations at parturition, but not at any other stage of gestation (Schwalm and Tucker, 1978). High maternal cortisol could be due to fetal cortisol crossing the placental barrier. Through *in vitro* experiments, Hoffman et al. (1976) concluded that very little transfer of cortisol between maternal and fetal compartments seem to occur.

The decline of progesterone noted prior to parturition corresponds with initiation of copious milk secretion (Smith et al., 1973), providing evidence that the high progesterone during gestation suppresses lactation. Progesterone acts by suppressing the ability of prolactin to increase the number of its receptors in the mammary gland (Haslam and Shyamala, 1979), as well as blocking glucocorticoid receptors in mammary tissue (Collier and Tucker, 1978). Mammary uptake and binding of glucocorticoids increase with the onset of lactation (Patterson and Linzell, 1974) and act to regulate secretion of α -lactalbumin and β -casein (Ray et al., 1986). Glucocorticoids in the mammary gland also act to induce differentiation of the lobule-alveolar system and are essential to allow prolactin to subsequently induce synthesis of milk proteins (Tucker et al., 1971).

Colostrogenesis, or the prepartum transfer of immunoglobulins from maternal circulation into mammary secretion, begins prior to parturition. During this period, up to 500 g per week of immunoglobulins are transferred into mammary secretions (Brandon et al., 1971). Maximum

entry rates of immunoglobulins in mammary secretions occur between days 3 and 1 prepartum, and an increased production and greater turnover for plasma immunoglobulins occurs around the time of parturition (Sasaki et al., 1976). Serum IgG concentration in maternal circulation has been found to reach 5.7 mg/ml between days 28 and 24 prepartum and decline to 1.4 mg/ml between day 4 prepartum and parturition in dairy cows (Guy et al., 1994a). Glucocorticoids can influence the amount of immunoglobulins present in colostrum and, therefore, influence the degree of passive immunity in the neonate. Schwalm and Tucker (1978) found that glucocorticoids are elevated in the milk at parturition, although overall concentrations of glucocorticoids in milk are lower than in serum (Patterson and Linzell, 1974). Shutt and Fell (1985) found that milk and serum cortisol concentrations during established lactation are 0.35 ng/ml and 4.5 ng/ml, respectively. In contrast, colostrum and serum cortisol concentrations just before parturition have been reported to be 4.4 ng/ml and 16.6 ng/ml, respectively, indicating that 60% of the cortisol in colostrum may be protein bound.

Acquisition of passive immunity is critical during the first 24 hours of neonate life (Brignole and Stott, 1980; Wesselink et al., 1999). Glucocorticoids can have a significant influence on the amount of immunoglobulins in colostrum and also on the amount of immunoglobulins absorbed by the neonate. Cows induced to calve before term with dexamethasone have lower colostral immunoglobulin concentration than in cows not induced to calve, as well as their calves having significantly lower serum IgG concentrations at 24 hours after birth than calves from cows not induced to calve (Field et al., 1999). In contrast, cows injected with dexamethasone before delivery by Caesarian section at 90% of gestation length, and their calves given intrauterine ACTH treatment, was associated with improved metabolic, endocrine and organ weight parameters as compared with calves delivered in the same manner, but were treated postpartum with ACTH (Schmidt et al., 2004). It appears that immaturity is responsible for the slower uptake of colostral immunoglobulins in calves born from cows induced to calve (Johnston and Stewart, 1986). The presence of glucocorticoids in late gestation may actually enhance absorption in the premature calf and reduced immunoglobulin absorption is more likely due to immaturity than to high plasma glucocorticoid concentrations. Postpartum injections of glucocorticoids to calves, however, enhance immunoglobulin absorption. Injecting calves with ACTH results in greater serum IgG concentrations than calves injected with metyrapone (a synthetic drug that decreases cortisol secretion) (Johnston and Oxender, 1979). Decreased cortisol concentrations may reduce the ability of or time available for the calf to absorb colostral immunoglobulins, whereas, increased serum cortisol concentrations increase IgG concentrations. Lambs born with higher cortisol tend to have higher

immunoglobulin concentration (Chen et al., 1999). A similar trend exists in other domestic species, such as pigs (Patt and Eberhart, 1976; Body and Hogg, 1981) and lambs (Hough et al., 1990). Our hypothesis was maternal cortisol being taken up by the mammary gland during colostrogenesis may be transferred to the neonate after birth via colostrum and enhance immunoglobulin absorption.

Our goal for Experiment 3.1 was to identify a diurnal variation in cortisol secretion in dairy cows in order to eliminate time of day as a source of variation. Our goal for Experiment 3.2 was to eliminate milk cortisol concentrations obtained from two milking techniques as a source of variation. Our goal for Experiment 4.1 was to determine whether maternal cortisol before and after parturition influences calf cortisol levels or immunoglobulin G concentration.

CHAPTER II

LITERATURE REVIEW

HORMONE PATTERNS IN THE COW AROUND PARTURITION

Parturition is a chain of events that occurs when the fetus has properly matured and has the ability to survive outside the uterus. While in utero, the fetus is on a life support system, which must be maintained until the fetus has the ability to survive outside the uterine environment. Liggins (1969) first demonstrated that parturition is under endocrine control using a sheep model. It was found that one critical component of parturition is the maturation of the fetal hypothalamic-pituitary-adrenal (HPA) axis, which is capable of producing stimulatory or inhibitory hormones. The fetal HPA axis serves to initiate parturition by secreting adrenocorticotrophic hormone (ACTH) from the anterior pituitary, which stimulates production of cortisol and other glucocorticoids from the adrenal cortex (Kindahl et al., 2004). The primary source of progesterone in the early to mid-gestating cow is the corpus luteum (Hoffman et al., 1979) and there is a significant decrease in progesterone production within the first 24 to 48 hours before calving (Smith et al., 1973a). This decrease is due to the utilization of progesterone by the placenta to allow conversion of progesterone to estrogen.

Although the placenta contains all of the necessary components to synthesize estrogen, it lacks the essential enzyme 17 α -hydroxylase (P450c17) (Arosh et al., 2004). The secretion of cortisol from the fetal adrenal cortex is essential to stimulate production of 17 α -hydroxylase to convert progesterone to estrogen. Estrogen produced in the placenta is transported to maternal circulation to stimulate production of oxytocin receptors in the endometrium. The endometrium produces prostaglandin F_{2 α} (PGF_{2 α}) to induce luteal regression. The sharp increase of estrogen production has been suggested to be a major factor in placental expulsion. Cows with lower concentrations of estrogen at the time of parturition have been noted as having a higher incidence of retained placentas (Pimentel et al., 1987). Production of PGF_{2 α} not only contributes to regression of the corpus luteum, but also has been proposed to regulate uterine contractions, dilation of the cervix and separation of fetal membranes (Königsson et al., 2001). There are many processes regulating parturition. In order for parturition to occur, a very intricate, synchronous coordination of physiological processes are required. These systems can influence fetal and maternal well-being.

Role of the Fetal Hypothalamic-Pituitary-Adrenal Axis in Parturition

Glucocorticoids are essential for initiation of parturition in cattle. Extensive research in cattle has demonstrated activation of the fetal hypothalamic-pituitary-adrenal axis and secretion of cortisol from the adrenal gland is vital to the process of parturition (Smith et al., 1973a; Adams and Wagner, 1970; Hoedemaker et al., 1990). Adams and Wagner (1970) conducted one of the first of many studies on the role of corticoids in parturition. Maternal plasma corticoid concentrations did not increase during the last 4 days prior to parturition compared with 5 to 7 days before parturition and 3 to 7 days after parturition (Adams and Wagner, 1970). Prepartum rise in plasma glucocorticoids may act by causing a decline in corpus luteum function and serve to facilitate parturition. Adrenocorticotrophic hormone has been reported to be luteolytic in the intact heifer, but not in the hysterectomized heifer (Brunner et al., 1969). However, in contrast to the pattern noted by Adams and Wagner (1970), Smith et al. (1973a), who reported a sharp peak in maternal glucocorticoid concentration ~2 days before parturition. After which, concentrations of glucocorticoids returned to basal concentrations by 2 days after parturition. This variation was reported to reflect differences in parity because maternal glucocorticoid concentrations reported by Smith et al. (1973) were measured in heifers and not in multiparous cows. Fetal cortisol concentrations remain relatively low over the period of 5 to 8 months of gestation and begin to increase gradually at 9 months of gestation (Takeishi et al., 1989). Additionally, fetal cortisol concentrations are significantly elevated beginning 7 to 9 days before parturition and continue to increase during the last 3 to 5 days of gestation (Taverne et al., 1988).

Induction of parturition can be achieved by injecting the cow with synthetic glucocorticoids as early as 3 to 4 weeks before expected calving (days 254 to 265) with parturition occurring ~7 days later (Beardsley et al., 1974; Beardsley et al., 1975; Muller et al., 1975; Chew et al., 1978; Königsson et al., 2001, Takagi et al., 2002). Parturition may also be induced using two intramuscular injections of prostaglandin $F_{2\alpha}$ two weeks before term and cows will calve in ~5 days (Kask et al., 2000, Kornmatitsuk et al., 2002). However, the use of synthetic glucocorticoids and prostaglandins to induce parturition pose a high risk of impaired reproductive performance (i.e., increased risk of dystocia, retained placenta and delayed uterine involution). Prostaglandin has recently been used as a model to identify the mechanisms involved in these postpartum processes (Kask et al., 2000, Kornmatitsuk et al., 2002). It is interesting to note that the mechanisms involved in inducing parturition becomes more sensitive when dams are injected closer to term (i.e., as the fetus approaches maturity). A study by Adams and Wagner (1970) investigated the effect of synthetic glucocorticoids on inducing

parturition at varying stages of gestation. Animals that were injected before 245 days of gestation were not successfully induced to calve. Parturition was successfully induced in cows injected after 245 days of gestation. However, the interval from injection to fetal expulsion increased as the animals were injected earlier in pregnancy.

Moreover, maternal crypt epithelial cells demonstrate increasing glucocorticoid receptor immunoreactivities during the second and third trimester of pregnancy (Boos et al., 2000). This stage-dependent presence of glucocorticoid receptor immunoreactivities confirms the results of Adams and Wagner (1970). During late pregnancy and parturition, the bovine intercaruncular uterine wall expresses glucocorticoid receptors and exhibits a cell type-specific distribution pattern (Schäubli et al., 2008). Near the end of gestation, various cell types of the intercaruncular uterine wall demonstrate specific sensitivities to glucocorticoids, which may result in cell type-specific reaction patterns. Maternal surface epithelial cells express enzymes for endometrial prostaglandin synthesis during pregnancy (Fuchs et al., 1999). This suggests that glucocorticoids directly stimulate endometrial and placental prostaglandin $F_{2\alpha}$ synthesis and secretion leading to luteolysis and parturition, or premature birth if given during late pregnancy.

This would seem logical as a protective mechanism to prevent interruption of pregnancy during natural stress situations. Even so, prenatal stress does have a significant influence on the calf after birth. Prenatal stress can cause increased birth and pituitary weights and greater cortisol concentrations compared with calves not stressed in utero (Henry et al., 1994; Lay et al., 1996; Lay et al., 1997). These studies imposed physiological stress via transport or handling of the dam. Results suggests prenatal stress on the neonate serve to precondition or imprint on endocrine glands associated with growth, reproduction, and tolerance to biological stressors.

Most early research examining the role of the fetal hypothalamic-pituitary-adrenal axis was conducted in the sheep. In the fetal sheep, before 120 days of gestation (average gestation length 148 days), the majority of cells in the fetal zona fasciculata (inner zone) of the adrenal gland can be classed as immature, having little smooth endoplasmic reticulum and mitochondria. Between 120 days of gestation and term, growth of the adrenal cortex is associated with an increasing proportion of mature cells in the zona fasciculata. Within 24 to 48 hours of parturition this zone consists almost entirely of mature cells (Robinson et al., 1979). In contrast to the intact fetal lamb near term, the hypophysectomized fetus has low plasma concentration of cortisol and, without the prepartum cortisol surge, parturition does not occur and gestation is prolonged (Liggins et al., 1969).

A study conducted by Robinson et al. (1983) investigated the effects of fetal hypophysectomy on cell populations of the adrenal cortex and the sensitivity of the adrenal cortical cells to exogenous ACTH. Fetal sheep were hypophysectomized on day 100 of gestation and infused with saline until day 135 of gestation. This resulted in smaller, less mature zona fasciculata cells than intact fetuses of the same gestational age. However, infusion of ACTH in hypophysectomized fetuses induced a complete change in the cell population of the zona fasciculata and after 48 hours of infusion all the cells in this zone in both hypophysectomized and intact fetuses were mature. It was concluded that the enhanced response to ACTH that develops in the last 14 to 20 days of gestation seems to be related to the increase in the proportion of mature cells that occurs during this period (Robinson et al., 1983).

Poore et al. (1998) conducted a similar study in which intact and hypophysectomized fetal lambs (hypophysectomy at 115 days of gestation) received a continuous infusion of ACTH until the end of the study (day 140 of gestation). Parturition can occur in both intact and hypophysectomized fetuses provided with a constant infusion of ACTH. Adrenal cortical growth and adrenal maturation were similar in all groups. Therefore, it seems unlikely that ACTH infusion had a collective effect upon the fetal adrenal gland. Poore et al. (1998) concluded that the presence of ACTH is required to prevent adrenal atrophy and allow adrenocortical maturation. However, an increase in ACTH concentration in late gestation does not appear to be an absolute prerequisite for parturition and constant plasma ACTH concentrations appear to be sufficient for adrenal maturation. Fetal lambs that were pituitary stalk-sectioned on day 115 of gestation and continuously infused with cortisol took longer to deliver when compared with intact fetuses, suggesting that a connection between the fetal hypothalamus and pituitary is not essential for parturition to occur (Nathanielsz et al., 1978). In contrast, hypothalamic-pituitary disconnection at days 125 or 135 of gestation in fetal lambs results in failure to deliver at term (Deayton et al., 1994). This demonstrates that hypothalamic connection to the pituitary needs to be maintained until at least day 135 of gestation for the initiation of parturition at term. All of these findings together bring into question the precise role of ACTH in adrenal development during late gestation and leave unanswered the relationship between enhanced adrenal responsiveness and basal cortisol secretion on the timing of parturition.

Although the fetal hypothalamic-pituitary-adrenal axis is an intricate process to facilitate parturition, many investigators have speculated the role of maternal glucocorticoid secretion in parturition. Patel et al. (1996) investigated the effect of fetal number and stage of gestation on maternal cortisol concentrations in the cows. Fetal number and stage of gestation did not

influence concentrations of cortisol throughout gestation except on the day of parturition. Twin-bearing cows had greater concentrations of cortisol compared with singleton cows. No relationship between the stage of gestation, fetal number, and peripheral circulating cortisol supports the possibility of a limited role of maternal cortisol in parturition.

Parturition is a natural event that involves stress and pain to the dam. Therefore, many studies have attributed high maternal glucocorticoids to stress and severity of distinct phases during parturition. In dairy heifers and goats, cortisol concentrations increase in association with onset of labor, continue to rise during expulsion, and peak when the calf or first kid is born (Hydbring et al., 1999). Additionally, cortisol concentrations did not differ between heifers needing assistance during calving and those that did not. Concentrations of cortisol change with the phases of labor, regardless of duration, in both cows and goats (Hydbring et al., 1999). Although it is easy to attribute high cortisol concentrations to the stress of labor, glucocorticoids are also responsible for other processes during parturition, such as accelerating mammary growth and initiating lactation. High progesterone concentrations during pregnancy competitively inhibit binding of glucocorticoids to the glucocorticoid-binding site in mammary tissue and thereby inhibit lactogenesis (Collier and Tucker, 1978). In addition, there is a positive correlation between milk and serum glucocorticoid concentration at parturition, but not at any other stage of gestation in the cow (Schwalm and Tucker, 1978).

It has also been proposed that fetal cortisol crosses the placental barrier and influences maternal cortisol. In the epitheliochorial placenta, found in cows and other ruminants, maternal and fetal blood are separated by maternal endothelium, maternal connective tissue, the endometrium, the trophoblast, fetal connective tissue and fetal endothelium. The rise in plasma corticoids seen just prior to parturition in the cow may be the result of an increase in steroid secretion by the fetal adrenal system with transport across the placenta to the maternal circulation. Hoffman et al. (1976) conducted a study where indwelling catheters were placed in the maternal jugular and uterine veins, umbilical artery and vein and the fetal vena cava and blood samples were collected to measure cortisol concentrations. Plasma cortisol in maternal circulation was elevated on the day of surgery and continued to decrease until day 3 post-surgery. In contrast, the cortisol concentration in fetal circulation did not rise on the day of surgery, but rather it continually increased until day 3 post-surgery. It was concluded that very little transfer of cortisol between maternal and fetal compartments seems to occur considering plasma cortisol in the maternal and fetal circulations were moving in opposite directions during the sampling period (Hoffman et al., 1976).

This was further warranted upon the discovery of a placental enzyme, 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1), which is expressed in the ovine uterus during pregnancy (Yang et al., 1996). The bioavailability of glucocorticoids is regulated by 11 β -HSD1 enzyme. In cattle, it acts to interconvert physiologically active glucocorticoids to their inactive metabolites. Therefore, the fetus is protected from the high maternal cortisol by the presence of 11 β -HSD1, which oxidizes cortisol to the biologically inactive cortisone. In baboons, placental 11 β -HSD1 receptors are regulated by estrogen and the late-gestational rise in estrogen is associated with increased 11 β -HSD1-enzyme expression (Pepe et al., 2001). These results suggest that maternal cortisol does not play a significant role in initiation of parturition. Moreover, dexamethasone is not degraded by 11 β -HSD1 (Shun and Myatt, 2003), which indicates that maternal injection of synthetic glucocorticoids has the ability to initiate parturition.

Role of Progesterone and Estrogens in Parturition

The domestic cow is a species that depends on a source of progesterone throughout most of gestation. The primary source of progesterone during pregnancy is the corpus luteum. It is essential to embryo survival that the corpus luteum begins secreting ~100 μ g of progesterone by day 15 post-ovulation (Staples and Hansel, 1961). Moreover, the corpus luteum must remain functional by secreting progesterone and maintaining pregnancy for 180 to 200 days and throughout pregnancy for normal length of gestation, normal delivery of the calf, and expulsion of the fetal membranes (Estergreen Jr. et al., 1968). The importance of the corpus luteum during early pregnancy has been well documented and was confirmed in many early studies in which removal of the corpus luteum or ovariectomy before 200 days of gestation resulted in abortion of the fetus (McDonald et al., 1953; Johnson and Erb, 1962; Estergreen et al., 1968; Pope et al., 1969; Chew et al., 1978). Eissa and El-Belely (1990) reported plasma concentrations of progesterone in dairy cattle fluctuate between 8.9 and 9.7 ng/ml until the third month of pregnancy and decrease significantly to 6.9 ng/ml by the fourth month of pregnancy. Progesterone further decreases at ~258 days of gestation (Erb et al., 1968) and this decline has been attributed to the utilization of progesterone in the fetal-placental unit due to steroid conversion (Hoedemaker et al., 1990) by the enzyme 17 α -hydroxylase.

It has been hypothesized that an extra-ovarian source of progesterone is present after 200 days of gestation because concentrations of progesterone in jugular venous plasma remain high after the third month of gestation and the concentrations of progesterone in ovarian venous plasma are significantly lower from 199 to 237 days (Stormshak and Erb, 1960; Erb et al., 1968; Hoffman et al., 1979). Extra-ovarian sources of progesterone in the ovariectomized cow appear

to be inadequate because gestations are generally shorter along with a higher incidence of retained placenta (Pope et al., 1969). These two conditions can be avoided by injection of progesterone (Chew et al., 1978) or implanted progestin compounds (Johnson and Erb, 1962) until day 274 of gestation.

In contrast, administration of RU 486, a progesterone antagonist, on days 277 and 278 of gestation does not result in retained placentas (Li et al., 1991); however, this could be due to the fact that injections were given closer to term, which would allow normal maturation of the placenta. It has been suggested the extra-ovarian source of progesterone, which has the ability to maintain pregnancy, is derived from the adrenal gland (Wendorf et al., 1983) or the placenta (Hoffman and Schuler, 2002). Indeed, the adrenal gland is capable and does secrete progesterone in the cow (Balfour et al., 1957), however, the amount of progesterone is generally less than 10 µg/ml (Gorski et al., 1958, Stormshak and Erb, 1960).

A study by Wendorf et al. (1983) was conducted to test the hypothesis that the adrenal gland is capable of secreting adequate amounts of progesterone following ovariectomy and/or adrenalectomy to maintain pregnancy. Treatments consisted of sham operation, ovariectomy, adrenalectomy and adrenalectomy plus ovariectomy on day 215 of gestation. It was concluded that the adrenal gland as an extra-ovarian source of progesterone is likely because (1) pregnancy was terminated in the absence of the ovaries and the adrenals but maintained with either one or both were present, (2) 2 to 4 ng/ml of progesterone were present in the blood of ovariectomized cows but there was none after ovariectomy plus adrenalectomy and (3) there were slightly lower plasma progesterone concentrations in adrenalectomized cows than in control cows. In addition, a study conducted by Pimentel et al. (1986) compared the secretion of progesterone by the fetal-placental tissue at 250 days of gestation with that at 270 days of gestation after removal of the corpus luteum. Results indicated that tissues within the uterus were capable of progesterone secretion at 250, but not 270, days of gestation. The amount of progesterone secreted by the placenta at 250 days of gestation is small, compared with that produced by the corpus luteum. Production of progesterone by the placenta, in close proximity to the site of action of this steroid, may make the placenta the more critical source of progesterone compared with corpus luteum for maintenance of pregnancy beyond 250 days of gestation.

At ~40 days before parturition, maternal plasma estradiol-17β is low (25 pg/ml) and begins to rise ~20 days before parturition in dairy cattle (Hunter et al., 1970; Smith et al., 1973; Robertson, 1974) and peak at 450 pg/ml at the time of parturition (Robertson, 1974; Robertson

and King, 1979). Total plasma estrogen increases steeply from the 4th to 6th month of pregnancy (178 pg/ml), fluctuates within a narrow range until late pregnancy, increases sharply to 203 pg/ml 5 days before parturition, and peaks on the day of calving (460 pg/ml) (Eissa and El-Belely, 1990). This is followed by an abrupt decline to 198 ± 16 pg/ml ~12 hours after parturition.

In contrast, there is a sharp peak in estrone sulphate in the allantoic fluid relatively early in pregnancy followed by a second rise around mid-gestation, and a subsequent decline towards late pregnancy in the dairy cow (Robertson and King, 1979). The initial rise in estrone sulphate concentrations can be attributed to the increase in the mass of the placental tissue, fetal membranes and fetus. It was proposed that the major source of estrogens in the pre-partum cow is ovarian-derived progesterone in the peripheral circulation. This speculation was later eradicated given that cows induced to calve with dexamethasone in combination with progesterone exhibit low estradiol-17 β despite elevated plasma progesterone (Hoffman et al., 1979; Fairclough et al., 1984). The rise in maternal jugular plasma estrogens and utero-ovarian estrogens parallel one another, however, the differences in concentrations between the two sources are significantly different (Peterson et al., 1975), indicating that the increase in peripheral estrogen before parturition in the cow is primarily of fetal-placental origin. However, it has been suggested that passage of steroids across the placenta to the maternal circulation is unlikely and that each compartment (fetal and maternal) is distinctly different. This hypothesis is supported by the fact that conjugated estrone is only twice as high in the umbilical artery compared to the uterine vein (13.63 ng/ml vs. 7.95 ng/ml) whereas conjugated estradiol-17 α is ~15 times higher in the umbilical artery compared with the uterine vein (36.85 ng/ml vs. 2.32 ng/ml) (Hoffman et al., 1976; Ferrell et al., 1983). In addition, concentrations of estrogen in the uterine vein are significantly higher compared with peripheral plasma estrogen during the last week of gestation (Comline et al., 1974).

Estrogens appear to play a role in maturation and expulsion of the placenta. Cows with proper expulsion of their placenta have significantly higher concentrations of estrogen from 6 to 1 days prepartum compared with cows with delayed or only partial expulsion of the fetal membranes or cows with fully retained placenta (Grunert et al., 1989). Serum estrogens are lower (290 vs. 601 pg/ml) in cows induced to calve with dexamethasone plus estradiol benzoate than in cows not induced to calve 2 days prior to parturition (Beardsley et al., 1974a,b). Cows induced to calve also experience a 50% higher incidence of retained placenta vs. 4% in cows not induced to calve (Chew et al., 1978). Thus, dexamethasone plus estradiol benzoate may decrease the incidence of retained placentas compared with dexamethasone alone. In contrast, the use of PGF_{2 α} or PGF_{2 α} plus estradiol-17 β to induce parturition demonstrated no difference in

retained placenta between the two groups (Henricks et al., 1977a,b). Additionally, during induced parturition, the duration of the increased estrogen concentration is reduced to 2 or 3 days compared with 6 to 10 days in dairy cows undergoing spontaneous parturition at normal gestation length (Wischral et al., 2001). It can be concluded that not only does estrogen concentration appear to be important, but also the duration of estrogen secretion for complete maturation and expulsion of the placenta.

A current hypothesis of bovine parturition suggests that cortisol secreted by the fetal adrenal gland 4 to 5 days before parturition acts to induce estradiol synthesis in the placenta. The rise in fetal cortisol parallels the maternal rise of estrogen in the uterine vein and peripheral concentrations of estrogen are much lower compared with estrogen concentrations in the uterine vein (Comline et al., 1974), suggesting cortisol from the fetal adrenal gland is acting to stimulate the enzymes necessary for placental estrogen synthesis. In vitro studies on ovine placental tissue demonstrated that dexamethasone treatment in near term fetuses show a decrease in utero-ovarian vein and placental progesterone production and an increase in 17α -hydroxylase activity (Anderson et al., 1975).

Further investigations confirmed that the placenta contains the enzymes necessary for the conversion of 17α -hydroxyprogesterone to estrone and the enzyme C-17,20 lyase and 17α -hydroxylase are activated or stimulated in late gestation (Steele et al., 1976; Flint et al., 1978; Ricketts et al., 1980; Schuler et al., 1994). Furthermore, concentrations of estrone and estradiol- 17β are greater before luteectomy (surgical removal of the corpus luteum) at 270 days than before luteectomy at 250 days of gestation (Pimentel et al., 1986). This implies that progesterone may be less available for secretion at 270 days because progesterone and its precursors are being utilized for estrogen synthesis. Studies in cattle have demonstrated that bovine fetal villi tissue is capable of converting pregnenolone and androsteindione precursors to estrogen compounds (Gross and Williams, 1988a). Results also indicated a decrease in fetal villi progesterone synthesis and an increase in estrogen synthesis immediately before parturition, which would indicate that progesterone is no longer the primary end product of fetal villi steroid synthesis and that conversion to other steroid products (e.g., androsteindione and estrogens) occurs during this peripartum period. Incubation of fetal villi minces with dexamethasone or cortisol increases fetal villi steroid synthesis (Gross and Williams, 1988; Hoedemaker et al., 1990) and the action of corticoids on estrogen synthesis by the placenta during late gestation is through increased C-17,20 lyase enzyme activity.

Role of Prostaglandins and Oxytocin in Parturition

Synthesis of estrogen by the bovine placenta is followed by a sharp increase in prostaglandin production (Fairclough et al., 1984; Gross et al., 1988a,b) and cows infused with estrone from 246 through 250 days of gestation show an increase in prostaglandin production (Pimentel et al., 1986). Synthesis of prostaglandins by the bovine uterus is largely dependent on the presence of oxytocin receptors (Fuchs et al., 1996). Increased numbers of oxytocin receptors are found in the endometrium from approximately day 250 of gestation to term, and coincide temporally with the slow decrease in plasma progesterone concentrations (Fuchs et al., 1992). The subsequent decrease in progesterone production, together with increased estrogen and up-regulation of cyclooxygenase-II (COX-II), stimulates prostaglandin synthesis and ultimately luteolysis (Schuler et al., 2006b). The day before parturition, $\text{PGF}_{2\alpha}$ increases rapidly and highest concentrations are reached at the time of fetal expulsion (Königsson et al., 2001). Exogenous administration of oxytocin at day 250 of gestation results in a release of prostaglandins from the uterus (Taverne et al., 2001). This suggests that a functional coupling mechanism exists between uterine oxytocin receptors and prostaglandin production and release.

Initial studies investigating the role of prostaglandins in parturition demonstrated that fetal sheep administered glucocorticoids to induce parturition results in increased concentrations of $\text{PGF}_{2\alpha}$ in maternal cotyledons and the myometrium during labor (Liggins and Grieves, 1971; Edqvist et al., 1978). Gross and Williams (1988b) demonstrated the ability of the bovine fetal placental cells to synthesize and metabolize prostaglandins and reported that fetal placental principal cells appear to be the primary source of prostaglandin synthesis in the form of $\text{PGF}_{2\alpha}$. Arosh et al. (2004) detected tissue-specific expression of $\text{PGF}_{2\alpha}$ receptors and COX-II at the fetal-maternal-placental barrier, suggesting that prostaglandin synthesis and signalling components contribute to fetal-maternal communication and regulate uterine activities during the initiation of parturition.

Prostaglandins not only play a role in luteal regression (Fairclough et al., 1984), but also in placental separation. It was proposed that cows retaining their fetal membranes have earlier activation of $\text{PGF}_{2\alpha}$ synthesis (Peter and Bosu, 1987), leading to abnormal or incomplete placental maturation. Induction of parturition can be achieved using $\text{PGF}_{2\alpha}$ at day 260 of gestation; however, this results in a much greater incidence of retained placenta when used this early in gestation (Henricks et al., 1977b; Kask et al., 2000). Incidence of retained placenta in cows spontaneously calving has been linked to prepartum diet and a positive correlation exists

between glucose and prostaglandin (Chassagne and Barnouin, 1992). Oxytocin concentrations in maternal-placental tissues in dairy cows with retained fetal membranes are significantly lower than cows not retaining fetal membranes (Takagi et al., 2002). This suggests that weak uterine contractions after parturition may be due to lower oxytocin concentrations and subsequently decrease prostaglandin release in cows with retained fetal membranes.

Oxytocin is a potent endogenous uterotonic agent stimulating the myometrium directly, or indirectly by increasing production of uterine prostaglandins (Fuchs et al., 1996). Around parturition, oxytocin is released from the posterior pituitary after appropriate stimulus, such as pressure on the cervix. Oxytocin receptor numbers in the endometrium are greater compared with the myometrium throughout gestation and show a steep rise at parturition (Fuchs et al., 1992). Physiological doses of oxytocin have been shown to elicit an increase in myometrial activity in pregnant cows as early as day 260 of gestation (Taverne et al., 2001). The magnitude of the oxytocin-induced PGF_{2α} release is dependent on the stage of gestation and is correlated to endometrial oxytocin receptor numbers, which reaches maximum concentration at the time of parturition (Fuchs et al., 1996).

PERIPARTURIENT IMMUNOSUPPRESSION

In order to understand the mechanisms of periparturient immunosuppression, it is important to have a basic understanding of the immune response to stress. The stress response is a conserved, physiological coping reaction to adverse environmental conditions. Such conditions are as diverse as physical and/or psychological constraint, injury, trauma, poor climate or nutrition, and others. The immune response, stress, and inflammation are a set of responses aimed at the neutralization of stimuli perturbing body homeostasis.

The complex interaction between the immune system and the stress/inflammation complex has mainly developed on the basis of a diversified system of cytokines and chemokines. In particular, cytokines are protein hormones released by white blood cells (WBC) that are chemical messengers and are the foundation of the complex crosstalk between the brain and the immune system. A pro-inflammatory cytokine like interleukin-1 (IL-1) induces activation of the hypothalamic-pituitary-adrenal axis. Other pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α and IL-6 play a pivotal role in mounting and directing the inflammatory response. TNF-α is produced mainly by macrophages but also by activated T cells and natural killer cells, and is a major mediator of inflammation. A foreign substance known as an antigen stimulates an adaptive immune response. The types of recognition molecules are antibodies and T cell receptors. Antibodies are proteins called immunoglobulins. They are borne

on the surface of B lymphocytes or secreted by cells derived from B lymphocytes. T cell receptors are borne on the surface of T lymphocytes. Lymphocytes are activated when they are stimulated to move from their recognition phase, in which they simply bind with particular antigens, to a phase in which they proliferate and differentiate into cells that function to eliminate the antigens.

Activation of effector cells, such as macrophages occurs when they are stimulated to carry out their protective function. Most T cells also bear other transmembrane proteins, which serve as accessory molecules. These are one of two types: CD4 and CD8. Certain CD4+ cells activate cell-mediated immunity and suppress the humoral response, but others activate humoral and suppress cell-mediated immunity. There is a delay between the time of antigen introduction and the response to it in an immunized subject. This delay occurs because the T helper cells with receptors on their surface for that particular antigen require time to arrive at the antigen site and become activated and secrete IL-2 and TNF. TNF causes endothelial cells of the blood vessels to express on their surface certain molecules to which leukocytes adhere: first neutrophils and then lymphocytes and monocytes. TNF also causes the endothelium to secrete inflammatory cytokines such as IL-8, which increase the mobility of leukocytes and facilitate their passage through the endothelium. Finally, TNF stimulates the endothelial cells to change shape, favoring both leakage of macromolecules from blood into the tissues and passage of cells through the vascular system lining. As monocytes pass out of blood vessels, they become activated macrophages. They phagocytize particulate antigen, secrete mediators that promote local inflammation, and secrete cytokines and growth factors that promote healing.

The interaction between the immune and neuroendocrine system is bidirectional, as exemplified by the action/response pattern of glucocorticoids. Environmental and metabolic stress enhances the secretion of glucocorticoids. Their anti-inflammatory activity is related to the down-regulation of IFN- γ and pro-inflammatory cytokines such as IL-1, IL-2, IL-3, tumor necrosis factor- α (TNF- α), IL-6, and IL-8. Infection, injury, or inflammation activates production of regulatory cytokines, which stimulate the release of circulating glucocorticoids from the pituitary-adrenal axis (Sordillo et al., 1995). Cytokines with known neuroendocrine effects are IFN's that enhance steroidogenesis, IL-1, IL-2, IL-6 that increase blood concentration of ACTH and glucocorticoids (Shun and Myatt, 2003). The immune response to an antigen leads to the differentiation of the native T helper (Th) cells to Th0 and, then, Th1 or Th2. Cytokines produced by Th1 cells stimulate the immune response and Th1 cell proliferation, inhibiting the production of cytokines secreted by Th2. Th1 cytokines (IFN- γ , TNF- α , and IL-2) generate a considerable pro-inflammatory response, often associated with tissue damage, while those arising from Th2

(IL-4, IL-5, IL-10) show helper functions for B lymphocytes, enhancing the production of IgM, IgE, and subclasses of IgG antibody. Cortisol concentrations that inhibit IL-2 production lead to an increase in IL-4, which drives the differentiation of Th0 lymphocytes to the Th2 subpopulation, with a concomitant increase of immunoglobulins. Furthermore, the effect of corticosteroids can vary with respect to blood concentration and as a result, for instance, when cortisol output is high the immune system secretes pro-inflammatory cytokines, specifically, in cattle, IL-6 (Koets et al., 1998).

Immune Response During the Peripartum Period

The peripartum period is known to be a time when host immune responses are diminished. Essential lymphocyte effector functions such as proliferation, antibody development, and cytokine production are reduced during this time. Lymphocyte blastogenesis increases 2 and 3 weeks before parturition and, by the first week after parturition, lymphocyte blastogenesis is markedly impaired (Kehrli et al., 1989a). In addition, Nagahata et al. (1992) reported significantly decreased numbers of plaque-forming cells in lymphocytes from cows at parturition and 3 days after parturition compared to those lymphocytes obtained 14 days after parturition. Lymphocytes that are positive for the CD8+ leukocyte antigen can express either cytotoxic or suppressor effect functions. It was found that CD8+ lymphocytes are of the suppressor compared with the cytotoxic nature prior to and immediately following parturition (Shafer-Weaver and Sordillo, 1997).

Alterations in proportions of lymphocyte subsets and their ability to migrate to the appropriate locations may affect the local and systemic immune responses. Variations in T lymphocyte subsets occur more as a result of pregnancy and lactation than health status in the dairy cow (Van Kampen and Mallard, 1997). Moreover, mononuclear cells from periparturient cows produce significantly higher TNF- α than mid to late lactating dairy cows (Sordillo et al., 1995). However, it was also reported that elevated pro-inflammatory cytokines TNF- α , IL-1, and IL-6 during late gestation are not necessarily detrimental to the course and outcome of pregnancy but may be a part of a regulatory network (Koets et al., 1998). Significant increases in random migration of neutrophils are evident 2 weeks before parturition and then decrease dramatically by the first week after parturition (Kehrli et al., 1989b). Furthermore, there is a gradual increase in the number of neutrophils in blood as calving approaches, followed by a sharp decrease after calving. The number of lymphocytes in blood decrease before calving, being the lowest on the day before calving (Saad et al., 1989).

T cells also play an important role in the immune system by virtue of their ability to recognize antigens with a high degree of specificity, to act as effector cells, and to regulate the nature and intensity of the immune response (Saad et al., 1989). Populations of T helper cells decline during the periparturient period in a pattern very similar to that reported for loss of immune cell function (Kimura et al., 1999). Interleukin-2 is one of many T cell-derived cytokines of major importance in the regulation of immune responses. Colostrum samples obtained during the last week of gestation have considerably lower IL-2 activity when compared with those obtained 14 days prior to calving and decreased IL-2 correlates with diminished immune cell function and increased susceptibility to mastitis (Sordillo et al., 1991).

Factors affecting the ability of a prepartum cow's immune system to cope with the stress of labor have been evaluated. Blood leukocytes are greater in primiparous dairy cows around parturition and during early lactation and there is more leukocyte and milk leukocyte viability suppression during the periparturient period in multiparous cows (Mehrzhad et al., 2002). This suggests that oxidation-reduction reactions in primiparous neutrophils could be more functional than those of multiparous cows. Cows selected for high milk yield have significantly greater circulating neutrophils and mononuclear cells compared with cows with average milk production (Detilleux et al., 1995). And finally, cows in the fourth or greater lactation suffer greater periparturient impairment of neutrophils function (Gilbert et al., 1993) and could be a factor mediating their increased susceptibility to certain postpartum complications.

The adrenal gland may be an important convergence point between the immune and endocrine systems. The increase in neutrophils 4 days prior to and up to the day of parturition is accompanied by an increase in corticosteroids (Guidry et al., 1976). IL-6 stimulates the release of corticosterone from adrenal cells and this cytokine has the ability to act synergistically with ACTH (Salas et al., 1990). Because IL-1, ACTH, and angiotensin II regulate IL-6, and this cytokine stimulates corticosterone release. IL-6 may play an important paracrine role in integrating the signals derived from these systems (Judd and MacLeod, 1992). As IL-6 induces release of acute-phase proteins along with glucocorticoids from the adrenal gland, and regulates the secretion of various hormones from neuroendocrine and endocrine tissues, it is possible that stress-induced increase in plasma IL-6 contributes to the maintenance of homeostasis (Zhou et al., 1993). The down-regulation of glucocorticoid receptors in neutrophils during the periparturient period may be involved in neutrophil dysregulation. This is evident in cows with reduced glucocorticoid receptor expression in blood neutrophils are associated with increased serum cortisol concentrations, leukocytosis and neutrophilia during the peripartum period (Preisler et al., 2000b).

Glucocorticoid receptors are down regulated in mononuclear leukocytes in association with increased adrenal secretion of cortisol at calving. Parturition has been found to cause a 42% reduction in lymphocyte glucocorticoid receptor expression (Preisler et al., 2000a). The functional capacity of mononuclear leukocytes is inhibited in periparturient cows at a time when blood cortisol concentrations are high and it is possible that glucocorticoid receptor down-regulation is also associated with altered function of lymphocytes (Preisler et al., 2000a). One mechanism of the anti-inflammatory action of glucocorticoids is to induce dramatic down-regulation of L-selectin and CD18 adhesion molecules on blood neutrophils (Burton et al., 1995). Glucocorticoid-induced suppression of L-selectin, which accompanies neutrophilia, is likely mediated by a direct effect of glucocorticoid receptor activation on intracellular reservoirs of L-selectin mRNA, occurring predominantly in blood neutrophils (Weber et al., 2004). In addition, macrophage inhibitory factor is an important protein mediator that regulates the host immune response and exhibits both glucocorticoid-antagonistic and growth regulatory properties. Macrophage inhibitory factor (MIF) immunoneutralization enhances lymphocyte exiting from the blood during stress-induced lymphocyte redistribution, consistent with a functional interaction between MIF and glucocorticoids on immune cell trafficking (Fingerle-Rowson et al., 2003).

Effects of Immunosuppression on Subsequent Calf Health

The vital role of the mammary gland in providing local host defense and passive protection to the newborn has long been recognized. The calf is born with a functional immune system and is capable of responding to certain antigenic stimuli (Ishikawa, 1987), but the system is thought not to be operating at optimum capacity. Therefore, colostrum is largely responsible for providing protective antibodies and possibly lymphocytes to the calf (Riedel-Caspari and Schmidt, 1991a,b). It was postulated that colostral leukocytes and lymphocytes pass through the intestinal wall of the neonate and act to stimulate and regulate the blastogenic response and enhance T helper cell-dependent formation of antibodies (Riedel-Caspari and Schmidt, 1991a,b). Because passive immunity is highly dependent on mechanisms that allow the transport of antibodies and cells from the blood across the endothelium into epithelial tissue and then into the mammary gland, the status of the immune system of the peripartum cow has an important influence on calf health. For example, whether the cow has been vaccinated or exposed to infectious disease, has produced protective antibodies, and has transported these into the mammary gland could influence colostral quality and subsequent calf health.

Initial studies conducted using newborn piglets indicated that lymphoid cells (both T and B lymphocytes) originating from the common mucosal immune system of the sow were transported into the mammary gland and then transferred via colostrum into the digestive tract of the neonate where they were absorbed into their circulation (Tuboly et al., 1988). Subsequent studies using porcine maternal colostrum leukocytes that had been labeled with fluorescein isothiocyanate confirmed that these cells were able to migrate intracellularly between duodenal and jejunal cells (Williams, 1993). By 24 hours postpartum, maternal colostrum lymphocytes from sows were detected in the liver, lungs, lymph nodes, spleen, and gastrointestinal tissues of the piglets (Williams, 1993). Later studies by Reidel-Caspari and Schmidt (1991a,b) indicated that calves fed milk containing colostrum cells had higher blastogenic responses to mitogen, higher lysozyme activity, and increased uptake of *Streptococcus agalactiae* than did calves fed milk that had been depleted of leukocytes. In studies in which calves were orally infected with *E. coli* and then fed colostrum, the calves receiving colostrum leukocytes shed significantly fewer bacteria (Riedel-Caspari, 1993).

Further studies showed that ingestion of maternal colostrum leukocytes immediately after birth stimulates development of the neonatal immune system and these maternal leukocytes enhance development of antigen-presenting capacity (Reber et al., 2005). Transfer of maternal colostrum leukocytes affects maturation of neonatal monocyte cells and neonatal lymphocytes (Reber et al., 2008a,b). Calves that received maternal colostrum leukocytes had lower expression of leukocyte markers associated with general physiological stress compared with calves that received acellular colostrum (Reber et al., 2008a). Collectively, these studies would indicate that colostrum leukocytes enter the neonatal circulation and contribute to passive immunity and resistance to infectious disease. Whether the type and quantity of transferred cells or the status of the immune response in the peripartum cow govern the quality of this response remains to be elucidated. In contrast, animals with greater serum antibody responses also supply greater concentrations of specific antibody to the mammary gland (Mallard et al., 1997). Therefore, prepartum vaccinations and the ability to maternal transfer of antibodies and cells to the mammary gland are both important to colostrum quality.

MAMMOGENESIS, LACTOGENESIS AND COLOSTROGENESIS

As science and research have manipulated milk production in dairy cattle, mainly to meet consumer supply and demand, it is not uncommon for herds of Holstein cows to average 305-day lactation yields of 13,000 kg of milk. Such prodigious production of milk requires very efficient mammary glands and careful attention to the feeding and management of these high-

producing animals. The mammary gland contains a variety of tissue and cell types. The mammary gland is also one of the only tissues in mammals that can repeatedly undergo growth, functional differentiation, and regression (Akers et al., 1978, Nickerson and Akers, 1984). Mammary growth and development are markedly stimulated by the onset of ovarian activity at puberty (Ball et al., 2000). Generally little or no true lobule-alveolar development occurs before a heifer's first conception and this period is associated with creation of a framework to allow proliferation of the secretory cells needed for lactation, such as extension of the duct system and increased growth of the adipose and connective tissue.

In the early stages of pregnancy, the duct system continues to develop with appearance of a rudimentary lobule-alveolar system by ~5 months of gestation (Akers and Nickerson, 1983, Woodward et al., 1993). After initial formation of lobule-alveolar tissue during the second half of gestation, individual alveoli continue to increase in size, and new alveoli are added until most of the mammary area is filled with alveoli. Once lactation is established, the rate of mammary cell proliferation is markedly lower than during other stages of mammary development. Hormonal regulators of the mammary gland are classified as mammogenic, lactogenic, and galactopoietic (Barnes et al., 1985). Mammogenic hormones (or growth factors) have an effect on growth and development of the mammary gland. Lactogenic molecules act to promote the structural and/or biochemical differentiation of the alveolar epithelial cells to synthesize and secrete milk. Galactopoietic agents function to maintain or enhance milk production once lactation is established. The former two hormonal regulators will be focused on and discussed in further detail, as well as formation, content, and function of colostrum in milk.

Milk Synthesis

Milk synthesis depends on the metabolic pathways necessary to supply the precursors needed to produce the protein, fat, and carbohydrate in milk. The primary substrates extracted from the lactating mammary gland are glucose, amino acids, fatty acids, and minerals (Akers et al., 1981). For ruminants because of bacterial fermentation of dietary carbohydrates, acetate and β -hydroxybutyrate are also critical substrates (Bequette et al., 1998). Glucose is the direct precursor for lactose, ribose, and much of the glycerol needed for triglyceride synthesis. All essential and many nonessential amino acids are derived from the bloodstream. The nucleotides needed for synthesis of RNA and DNA and lactose synthesis are produced by the epithelium. Fatty acids for production of triglycerides are derived from the bloodstream, mobilization from body stores, as well as synthesis by the alveolar cells. Volatile fatty acids derived from fermentation of dietary carbohydrates provide acetate for generation of ATP and

propionate to generate a sufficient quantity of blood glucose for energy production. Following ingestion of complex carbohydrates or more simple sugars, glucose enters the mammary cell and is phosphorylated to form glucose-6-phosphate (Tucker, 1981). At this point, glucose can be utilized in one of three pathways: 1) glycolysis to produce pyruvate and a limited amount of ATP, 2) formation of galactose (necessary for lactose production), or 3) formation of pentose phosphates. The other primary volatile fatty acid, β -hydroxybutyric acid is derived from microbial fermentation and is mostly used by mammary cells to synthesize fatty acids (Schanbacher et al., 1997).

The disaccharide lactose is the most common carbohydrate found in milk. Lactose synthase, the functional enzyme required for lactose synthesis, is a combination of uridine triphosphate (UTP), uridine diphosphate (UDP), glucose, and galactose to form lactose + UDP. Uridine diphosphate galactose-4-epimerase (final enzyme required for lactose synthase production) in mammary tissue appears well before parturition, but its activity is increased near the onset of lactation (Rosen et al., 1999). Milk proteins are synthesized from amino acids and these mammary specific proteins include caseins and whey proteins (Goodman et al., 1983; Filep and Akers, 2000). The caseins in cow's milk accounts for ~80 percent of the specific milk proteins. Protein synthesis in mammary cells follows the pattern described for many other tissues (Heald and Saacke, 1972).

Hormonal Regulation of Mammogenesis and Lactogenesis

It was established in early research that hormonal control of lactation is, in part, under the influence of estrogen. An injection of estradiol-17 β alone or in combination with progesterone for 7 days initiates lactation (Willet et al., 1972; Smith and Schanbacher, 1973; Croom et al., 1976; Collier et al., 1976). Ovariectomized heifers supplemented with progesterone and estradiol-17 β produce alveolar cell histologies similar to those of intact, 5-month-gestating heifers (Sud et al., 1968). Cellular tissue studies indicate that cows induced to lactate with estradiol-17 β and progesterone undergo critical periods of cellular proliferation and differentiation, undergo lactogenesis, and exhibit histological and ultrastructural characteristics associated with actively secreting mammary tissue (Croom et al., 1976; Collier et al., 1976). With impending parturition, estrogen in blood is the first hormone to increase (Hunter et al., 1970; Smith et al., 1973; Robertson et al., 1974). Estrogen is involved in initiation of lactation during the periparturient period in two ways: 1) estrogen stimulates the release of prolactin from the anterior pituitary gland into circulation, and 2) estrogen increases the number of prolactin receptors in mammary cells (Janowski et al., 1988; Belvedere et al., 1996; Berry et al., 2001).

Mammogenesis during pregnancy coincides with increased secretion of both estrogen and progesterone to synergistically induce lobule-alveolar growth (Randel and Erb, 1971). The decline of progesterone prior to parturition was reported to correspond with initiation of copious milk secretion (Smith et al., 1973), and provided correlative evidence that progesterone suppresses lactation. Progesterone inhibits lactation by suppressing the ability of prolactin to increase the number of prolactin receptors in the mammary glands (Haslam and Shyamala, 1979), and blocking glucocorticoid receptors in mammary tissue, which suppresses the lactogenic activity of glucocorticoids (Collier and Tucker, 1978). Studies in pre-partum goats indicated that progesterone is metabolized within the mammary gland and that this plays a role in mammary progesterone homeostasis (Stewart, 1983), suggesting that progesterone uptake and metabolism could possibly contribute to the regulation of mammary activity in late pregnancy.

Prolactin was discovered to be critically important for initiation of lactation in dairy cows during the periparturient period. Serum prolactin is 65% higher at 260 days than at 90 days of gestation (Oxender et al., 1972; Kann and Denamur, 1974). This increase in prolactin in late gestation can be blocked with bromocriptine, which results in markedly reduced subsequent milk yield, and exogenous prolactin can reverse this effect of bromocriptine (Akers et al., 1981). Unlike laboratory species, increasing prolactin concentration in the plasma of lactating cows with exogenous prolactin during early or late lactation does not alter milk yield or composition. Prolactin treatment also decreases the milking-induced release of endogenous prolactin (Plaut et al., 1987), indicating a long-term negative feedback loop. The formation of alveoli in organ cultures of mammary tissue primed with estradiol and progesterone was dependent upon the presence of prolactin (Dijane et al., 1975; Collier et al., 1977), and is required for formation of the lobule-alveolar structure of the mammary gland. Basal concentrations of prolactin in the plasma of lactating cows averages 10 ng/ml in the winter vs. 44 ng/ml in the spring in addition to milk yields being greater in the spring (Kensinger et al., 1979). Therefore, milk yield is significantly affected by season, temperature, and photoperiod.

Growth hormone was originally considered to be involved in lactogenesis. Early studies where growth hormone was injected into late-pregnant cows did not initiate lactation nor show observable signs of early onset of lactation (Simmons et al., 1994). Further *in vitro* studies showed that growth hormone was not lactogenic when added to bovine mammary tissue slices (Goodman et al., 1983). A surge in growth hormone is evident at parturition (Ingalls et al., 1973), but the surge is maximal around the time of delivery of the calf, which is too late to explain its role in onset of lactation. It was later confirmed that growth hormone has only indirect effects on

mammary gland function (Sinowatz et al., 2000) and that growth hormone has a more galactopoietic than lactogenic nature. The major function of cortisol in the mammary gland is to induce differentiation of the lobule-alveolar system and is essential to allow prolactin to later induce synthesis of milk proteins. Injections of glucocorticoids into non-lactating cows induced onset of lactation (Tucker, 1981). Glucocorticoids bind to specific receptors in mammary tissue (Gorewit and Tucker, 1976) and act to regulate secretion of α -lactalbumin and β -casein (Ray et al., 1986). Mammary uptake and binding of glucocorticoids increase with onset of lactation and are positively correlated with uptake of glucose into mammary tissue (Patterson and Linzell, 1974).

Synthesis and Secretion of Colostrum

Colostrumogenesis, or the prepartum transfer of immunoglobulins from maternal circulation into mammary secretions, begins prior to parturition. During this period, up to 500 g per week of immunoglobulins are transferred into mammary secretions (Brandon et al., 1971). The transfer of passive immunity involves the mammary accumulation of immunoglobulins in colostrum, the large bulk of which comes from the maternal bloodstream. Maximum entry rates of IgG₁ and IgG₂ in mammary secretions are between days 3 and 1 prepartum. Greater production and turnover of plasma IgG₁ occurs around the time of parturition, which can account for the large accumulation of IgG₁ in colostrum (Sasaki et al., 1977). Serum IgG concentrations in maternal circulation reach ~5.7 mg/ml between days 28 and 24 prepartum and decline to 1.4 mg/ml between day 4 prepartum and calving (Guy et al., 1994a).

It was speculated that the initial prepartum decline in progesterone, which coincides with the established time of onset of colostrumogenesis, might be the initiating signal. In addition, because transfer of IgG₁ into colostrum is initiated during the last weeks of gestation and rapidly declines prior to onset of lactation, one could speculate that the hormones that initiate lactation may also suppress colostrumogenesis. Studies in which lactation was initiated in nonlactating, nonpregnant cows found that prior to onset of lactation, IgG₁ concentrations of mammary secretions steadily rose to resemble that of colostrum (Smith and Schanbacher, 1973; Winger et al., 1995). These findings supported the notion that estrogen both alone or in combination with progesterone initiates lactation and likely stimulates IgG₁ receptor activity.

Another study investigated the effects of estrogen and progesterone, either alone or in combination with each other, on colostrum formation (Smith et al., 1971). Estrogen plus progesterone caused colostrum formation and mean quantities of IgG₁ removed from the mammary glands was 79.1 g for treatment animals and 14.0 g for animals receiving no

treatment. Treatment with estrogen alone resulted in colostrum formation whereas progesterone alone resulted in no colostrum formation. These results suggest that the changing estrogen and progesterone profiles the last 4 to 6 weeks of pregnancy exert a controlling influence on selective transport of IgG₁ to bovine lacteal fluid and, thus, colostrum formation.

In addition to estrogen and progesterone, circulating prolactin concentrations also play a role in colostrogenesis and onset of lactation. Administration of prolactin is associated with increased lactogenic activity, decreased secretion of IgG₁, and decreased IgG₁ receptor expression (Barrington et al., 1999). In addition, explants of mammary tissue from cows in late gestation incubated with prolactin showed decreased IgG₁ receptor expression (Barrington et al., 1997b), which implies that in addition to its positive lactogenic effect, prolactin decreases expression of the bovine mammary IgG₁ receptor. Further studies on IgG₁ receptor expression demonstrated epithelial expression of IgG₁ receptors in mammary tissue from cows producing colostrum but not cows in lactation (Barrington et al., 1997a). It was speculated that mammary cells possess two types of IgG₁ receptors: low affinity receptors, which are present throughout lactation, and higher affinity receptors, which appear one week before parturition.

Evidence for local regulation of colostrum formation in dairy cattle has also been implicated. Unilateral milking twice-daily 10 days before parturition results in cumulative secretion of IgG₁ and milk production and prolactin secretion are higher for milked compared with unmilked sides (Brandon and Lascelles, 1975; Guy et al., 1994b) and cumulative secretion of IgG₁ is higher for nonresponders. Differences between left and right udder halves indicate that local control is important to the regulation of colostrogenesis. Differences in IgG₁ between response groups indicate that maintenance of transport and dilution are important determinants of IgG₁ concentration in colostrum. Prepartum milking also hastens differentiation and activity of mammary epithelial cells in responding cows and it was hypothesized that in the presence of IgG₁ receptors, increased cellular activity is associated with increased transport of IgG₁ (Akers et al., 1977). Subsequently, transport of IgG₁ declined as milk yield continued to increase (Akers et al., 1977), implying that receptors may have been down-regulated in association with premature lactogenesis. This suggests a delicate balance exists in the process that regulates receptor function, cellular transport activity, and the onset of milk secretion.

This was further emphasized in studies in which prepartum milking was employed and then ceased. Concentrations of immunoglobulins in mammary secretions increased over a 7-day period of involution and there was a transitory increase in selective index for IgG₁ (Watson et al., 1972). The magnitude of selective IgG₁ transfer can be influenced by local factors in the

gland associated with diminished milk secretion or resorption. Many factors influence the amount of immunoglobulins present in colostrum. Injection of glucocorticoids in nonlactating cows in late gestation results in decreased concentration of IgG₁ after injection and no characteristic decrease in serum concentration of IgG₁ just before calving was found (Brandon et al., 1975). This supports the notion that changes in the magnitude of selective transfer of IgG₁ are regulated by de novo synthetic activity of the gland rather than a direct endocrine influence. Injection of dexamethasone and measurement of immunoglobulins in serum of calves fed colostrum from the mother results in failure of passive transfer of immunity to these calves (Field et al., 1989).

Additionally, age and lactation number are also factors correlated with the amount of immunoglobulins in colostrum. Cows in first lactation contain less IgG₁ in colostrum and also produce less total colostrum than older cows or those in advanced lactation (Devery-Pocius and Larson, 1983). Total immunoglobulins will reach a maximum in the third and fourth lactations, almost doubling in amount compared with the first lactation (Devery-Pocius and Larson, 1983). This suggests that the mammary transport system for IgG₁ becomes fully developed when the cow reaches maximum capacity for milk production.

Mammary Uptake of Glucocorticoids and Excretion in Milk

Glucocorticoids play an important role in initiation and maintenance of lactation. At the time of parturition, maternal glucocorticoids are significantly elevated in the blood and milk. During this time, cortisol secretion rate and plasma cortisol concentrations are significantly greater compared with non-lactating cows or cows in established lactation (Paterson and Linzell, 1974). In goats, the rate of cortisol secretion between days 33 and 138 of gestation is much lower than the rate 2 days prepartum or in early lactation (Paterson and Linzell, 1971). It is possible that mammary tissue in late pregnancy is extracting a greater amount of circulating cortisol that enters the mammary gland. Schwalm and Tucker (1978) found that glucocorticoids are elevated in the milk at parturition, although the overall concentrations of glucocorticoids in milk are lower than in serum. In addition, they also found no correlation between glucocorticoids in milk and serum, except near parturition. In contrast, concentrations of corticosterone in serum are similar to those in milk (Schwalm and Tucker, 1978). This suggests there is preferential uptake of corticosterone by the mammary gland, the mammary gland catabolizes cortisol, or conversion of cortisol to corticosterone occurs in the mammary gland. The second possibility seems the least likely given that only 3 to 4% of secreted cortisol is taken up by the mammary gland (Paterson and Linzell, 1974). Concentrations of cortisol in mammary tissue decrease 2-

fold with induction of lactation (Shirley et al., 1973), indicating that the lactating mammary gland is metabolizing cortisol at an increased rate.

Bovine mammary cells cultured *in vitro* possess a specific mechanism for the binding of cortisol (Tucker et al., 1971c,d). Additionally, mammary tissues from lactating cows possess specific binding components for corticoids (Gorewit and Tucker, 1975). Glucocorticoid binding proteins of bovine mammary tissue have different electrochemical characteristics than bovine serum binding components, confirming that the glucocorticoid receptor in mammary tissue is not cortisol binding globulin (Gorewit and Tucker, 1976c). Glucocorticoids are taken up by the mammary gland shortly after onset of milking (Gorewit and Tucker, 1976a). This seems to parallel the fact that cortisol concentration in blood is also increased with initiation of milking (Bremel and Gangwer, 1978). *In vitro* studies showed that mammary tissue slices from lactating cows bound more glucocorticoid molecules than tissue from cows that were pregnant and nonlactating or from cows that were nonpregnant and nonlactating (Gorewit and Tucker, 1976b). Results indicate binding of glucocorticoids may be related to lactation. Shutt and Fell (1985) found milk and serum cortisol concentrations during established lactation were 0.35 ng/ml and 4.5 ng/ml, respectively.

In contrast, milk and serum cortisol concentrations in colostrum were 4.4 ng/ml and 16.6 ng/ml, respectively. This indicated that 60% of the cortisol in colostrum might be protein bound. A further study showed that uptake of cortisol in mammary tissue is reduced in both lactating and nonlactating tissue when progesterone is added to culture medium (Collier and Tucker, 1978). Furthermore, the ability of progesterone to inhibit cortisol uptake in mammary tissue is reduced as lactation progresses. The inhibition of cortisol binding by progesterone constitutes the mechanism(s) of progesterone for inhibition of lactogenesis. Upon these findings, Collier and Tucker (1978) proposed the mechanisms for this inhibition.

In nonlactating mammary tissue, progesterone and cortisol are distributed evenly within epithelial cell cytoplasm. The availability of cortisol binding sites is influenced by the concentration of progesterone and cortisol in the cytoplasm. Increased progesterone concentrations (as during gestation) would competitively inhibit binding of glucocorticoids to the glucocorticoid-binding site and inhibit lactogenesis. In contrast to nonlactating tissue, progesterone and cortisol are not distributed evenly in lactating mammary tissue. The aqueous phase of the cytoplasm now contains high concentrations of cortisol while the fat droplets of the epithelial cell contain high concentrations of progesterone. Uneven distributions of cortisol and

progesterone, along with the increased secretion of milk fat, act to remove the inhibitory effect of progesterone, allowing the binding of cortisol to increase, and lactation can proceed.

CALF IMMUNOGLOBULIN ABSORPTION

Smith and Erwin (1959) first proposed that the permeability of the intestinal wall of the young calf to colostral immunoglobulins is transitory. Ingestion of colostrum has marked effects on gastrointestinal tract development and function, and affects digestive enzymes (Toofanian et al., 1973; Le Huerou-Luron et al., 1992). Furthermore, colostrum influences gastrointestinal hormones (Blum and Hammon, 2000) and absorptive capacity (Lecce and Morgan, 1962). In pre-term calves, the small intestinal epithelium is immature and brush border enzyme activities differ in part from those in full-term calves (Bittrich et al., 2004). In newborn calves, the microvilli are well developed, tubules or invaginations in the apical cytoplasm are extensive, and the intestine is permeable to heterologous proteins (Staley et al., 2004). Feeding high amounts of first colostrum enhances survival of the mature mucosal epithelial cells, whereas the lack of colostrum decreases epithelial growth (Blättler et al., 2001). Experimental results demonstrate that feeding a formula containing only trace amounts of growth factors and hormones but similar amounts of nutrients as colostrum reduced villus size in the jejunum and decreased crypt cell proliferation in the duodenum and ileum (Blättler et al., 2001). Results indicate that colostral bioactive components have effects beyond those of nutrients and, increase proliferation in parts of the small intestine.

Further studies demonstrated that vacuolated epithelium is present in the ileum of day 1 but not day 8 calves (Bittrich et al., 2004). During the first 8 days of life, villus sizes in the jejunum increase, crypt depth increase in the small intestine and the colon, and crypt cell proliferation increase in the duodenum and jejunum (Bittrich et al., 2004). Immunoreactive immunoglobulins, presumably originating from colostrum, have been localized within the enterocytes in both proximal (non-vacuolated) and distal (vacuolated) regions of the small intestine (Trahair and Robinson, 1989) suggesting the mechanism of intestinal closure is orderly epithelial cell renewal. Transfer of intracellular material into the core of the villus requires solvent factors and this is supported by findings that protein enters the intestinal epithelial cells in calves fed immunoglobulins in chloride solution (Hardy, 1969). The active components of colostrum may affect the small intestine via the circulation before the colostrum reaches the terminal ileum. Other substances such as lactate, pyruvate, and butyrate readily pass across the epithelium of the upper small intestine into the portal vessels and act on the terminal ileum via the circulation (Hardy, 1969).

Factors Affecting Acquisition of Passive Immunity

It has been shown that termination or closure of intestinal permeability to colostral immunoglobulins in the calf occurs spontaneously with age at a progressively increased rate after 12 hours postpartum (McCoy et al., 1970; Stott et al., 1979a; Hadorn et al., 1997). Furthermore, the gut is impermeable to colostral proteins by 24 hours after birth (McCoy et al., 1970). As colostrum feeding is delayed, cessation of absorption is also delayed up to the time of spontaneous closure (Stott et al., 1979a). Colostrum intake by calves within the first 24 hours of life is needed not only for an adequate immune status, but also to produce the additional important and favorable effects on metabolic and endocrine traits and on vitality.

Commercial dairy operations commonly remove calves from their dams within hours after parturition and bottle-fed the necessary amount of colostrum needed for gut closure. Calves left to suckle with their dams usually do not obtain sufficient colostrum to provide passive immunity (Brignole and Stott, 1980) and approximately half of the calves that are left with their dams may not receive colostrum when they are together for up to 24 hours (Wesselink et al., 1999). It is estimated that cessation of absorption spontaneously occurs around 24 hours after birth and calves should be removed from their dams by 6 hours postpartum and fed colostrum to guarantee that they receive a sufficient amount of colostrum and immunoglobulins necessary for passive immunity. Knowing the time of parturition is important to ensure the calf receives colostrum within 6 hours of birth.

A study conducted by Matte et al. (1982) reported that when colostrum was given at 6 hours after birth, 65.8% of the ingested IgG appeared in the plasma. This percentage declined rapidly to reach 46.9%, 11.5%, 6.7% and 6.0% when colostrum was given at 12, 24, 36 and 48 hours after birth, respectively. Therefore, delay of colostrum intake can have severe consequences on calf health and production. The concentration of immunoglobulins present in colostrum can also have an effect on acquisition of passive immunity. The amount of immunoglobulins present in colostrum declines rapidly after parturition, as early as the second milking around 12 hours after parturition (Stott et al., 1981). After a second milking, it is questionable whether immunoglobulin concentration is sufficient to allow attainment of a serum IgG concentration in the neonate considered acceptable for reliable transfer of passive immunity.

This is supported by the findings that calves that receive high quality colostrum (84 mg/ml IgG) have a greater concentration of IgG and total serum protein than calves that receive low quality colostrum (31.2 mg/ml of IgG) (Jaster, 2005). In addition, calves fed 2 liters of high

quality colostrum at 0 and 12 hours after parturition have a greater concentration of IgG after 24 hours than calves fed a similar IgG concentration one time at 0 hours (Jaster, 2005). This suggests that feeding calves two separate feedings of high quality colostrum maximizes colostral IgG intake. This can certainly pose a management problem because, as previously stated, the concentration of immunoglobulins in colostrum decreased significantly just 12 hours after parturition.

Studies have revealed that the amount of colostrum fed can have an effect on transfer of passive immunity (Stott and Fellah, 1983; Jaster, 2005). Stott et al., 1979b proposed that the contact and pinocytotic activation factors in colostrum are limiting parameters in colostral immunoglobulin absorption as well as immunoglobulin concentration. However, conflicting evidence published 4 years later demonstrated that when compared on equal mass, the amount of colostrum fed had less influence on immunoglobulin absorption than did concentration (Stott and Fellah, 1983). Inadequate colostrum consumption can have significant effects on growth and development later in that animal's life. Concentration of serum immunoglobulins in heifer dairy calves at 24 to 48 hours significantly affected average daily gain through the first 180 days of life (Robison et al., 1988). In addition, mortality was 6.78% for heifers with less than 12 mg/ml serum IgG at 24 to 48 hours as compared with 3.33% mortality for calves with greater than 12 mg/ml serum IgG (Robison et al., 1988).

Other factors can influence the amount of immunoglobulin present in colostrum and the efficiency of calf absorption. Total protein in serum is greater in February and March compared with warmer summer months (Donovan et al., 1986). In addition, presence of maternal antibodies in serum and colostrum were highest in calves from second parity cows and dystocia appears to decrease the amount of immunoglobulin absorbed by the calf (Donovan et al., 1986). Therefore, careful management practices must be exercised in commercial dairy operations and close attention must be paid to not only the calf, but the cow as well during the pre-partum and postpartum periods.

Effects of Glucocorticoids on Immunoglobulin Absorption

The effects of stress on calf immunoglobulin absorption are of considerable importance to producers. Stress can increase cortisol and have a significant effect on calf viability. Cows induced to calve with dexamethasone have lower colostral immunoglobulin concentration than in cows not induced to calve. Additionally, calves born from induced cows had significantly lower serum IgG concentrations at 24 hours after birth than calves from cows not induced to calve (Field et al., 1989). In addition, efficiency of absorption of immunoglobulins in calves from cows

induced to calve was half that for calves from untreated cows (Husband et al., 1973). In contrast, cows injected with dexamethasone before delivery by Caesarian section at 90% of gestation length and their calves given intrauterine ACTH treatment is associated with improved organ weight parameters compared with calves delivered in the same manner, but were treated post-partum with ACTH (Schmidt et al., 2004). Given this data, it appears that immaturity is responsible for the slower uptake of colostral immunoglobulins in calves born from cows induced to calve (Johnston and Stewart, 1986). The presence of glucocorticoids in late gestation may actually enhance absorption in the premature calf. Inhibition of immunoglobulin absorption was more likely due to prematurity than to high plasma glucocorticoid concentrations.

Further studies investigated the effects of post-partum injections of glucocorticoids to the calf on immunoglobulin absorption. A study conducted by Johnston and Oxender (1979) examined the effects of injecting calves with either metyrapone, a drug that decreases endogenous cortisol production, or ACTH. Serum IgG concentrations in the ACTH-treated calves was greater than those of the metyrapone-treated calves. This suggests that decreased cortisol concentrations may reduce the ability of, or time available for the calf to absorb colostral immunoglobulins, whereas, the increase in serum cortisol concentrations increased the serum IgG concentrations. Lambs given a high dose of cortisol (5 mg/kg BW every 4 hours) and those given a single-peak cortisol (10 IU ACTH/kg BW at 0 hours) had elevated serum IgG concentrations by 20 hours post-partum compared with controls (Hough et al., 1990). In addition, lambs given metyrapone (5 mg/kg BW every 4 hours) had the lowest IgG concentration and precocious closure to immunoglobulin absorption had occurred by 20 hours postpartum (Hough et al., 1990). This suggests that cortisol enhanced immunoglobulin absorption and prevented premature gut closure to absorption.

Additional studies demonstrated that injection of cortisol to calves results in higher immunoglobulin concentrations than calves not receiving cortisol injections (Whitaker et al., 1996). Even in neonates not receiving injections of cortisol, those born with higher cortisol also tended to have higher immunoglobulin absorption (Chen et al., 1999). Goat kids designated as having “high” cortisol at birth gained ~33% more weight during the first 5 days of life than those designated as having “low” cortisol. Additionally, peak serum immunoglobulin concentration by 18 hours post-partum was greater in kids with “high” cortisol compared to kids with “low” cortisol at birth (Chen et al., 1999). Therefore, cortisol on neonatal immunoglobulin absorption is mostly affected by prematurity and that high cortisol can actually enhance immunoglobulin absorption.

CHAPTER III

CORTICOID FLUCTUATIONS IN COWS

INTRODUCTION

The adrenal gland secretes corticoids in response to many environmental stimuli that cause stress, such as animal handling (Willett and Erb, 1972) or milking (Wagner and Oxenreider, 1972). Circulating cortisol increases upon milking stimulation (Negrao et al., 2004; Smith et al., 1972), and high cortisol concentration after milking has been associated with decreased milk production and the inhibition of milk ejection (Van Reenen et al., 2002). Individual responses to external stimuli vary significantly between animals (Van Reenen et al., 2002; Gorewit et al., 1992). Increases in cortisol concentration upon milking stimulation could, therefore, have an influence on milk production.

A diurnal variation in cortisol secretion has been well documented in other species including humans (Rose et al., 1972), pigs and horses (Bottoms et al., 1972), cats (Krieger et al., 1968) and rats (Guilleman et al., 1959). According to Hudson et al. (1974), there is no clear evidence of a diurnal cortisol secretion pattern in dairy cows. However, other studies have shown that cortisol concentrations are decreased between the hours of 1800 to 0200 compared with concentrations between 0200 and 1800 hours (Wagner and Oxenreider, 1972; MacAdam and Eberhart, 1972). This dissimilarity between cows and other species has been attributed to the sleep-wake cycle of cattle. Ruminants do not enter the deep state of sleep characteristic of other domestic animals (Bell, 1960). This information could be useful when employing a sampling regime that could alter cortisol concentrations due to differences in cortisol release depending on the time of day.

Another factor influencing cortisol release is milk production. High producing cows tend to have lower cortisol concentrations, regardless of their reproductive state (Sartin et al., 1988), which would significantly influence interpretation of results when sampling groups of cows in low or high milk production status.

In an effort to evaluate production characteristics, diurnal variation, and time of routine milking as a source of variation, the objectives of Experiment 3.1 were: (1) to determine if a diurnal variation in cortisol exists between two routine milking times (0200 and 1400), (2) to determine differences in plasma and milk cortisol levels between high and low producing cows and (3) to determine differences in cortisol before and after milking. In an effort to evaluate milk

collection techniques as a source of variation, the objectives for Experiment 3.2 were to determine differences in milk cortisol levels between two milk collection techniques.

MATERIALS AND METHODS

Experimental Design

Experiment 3.1

A group of 24 Holstein cows from the LSU Dairy Research Unit, were fed a total mixed ration consisting primarily of corn silage and alfalfa hay. Body condition scores ranged from 2.5 to 5 on a scale of 1 to 5. Cows were divided into two groups (Low, cows producing less than 40 kg of milk/day (mean \pm SEM days in milk (DIM) was 74.8 \pm 5.5); and High, cows producing greater than 45 kg of milk/day (mean \pm SEM days in milk was 71.6 \pm 5.8)) based on previous and most current lactation records. Cows were taken into the milking parlor during routine milking at 0200 and 1400 hours.

Blood and milk samples were obtained from each cow immediately before and immediately after milking. Teats were prepared for milk collection with iodine spray and antiseptic wipes. Teat cisterns were stripped of milk and ~2 ml were collected from each teat in antiseptic collection tubes (The Coburn Co., Whitewater, WI) before milking and the procedure was repeated after machine milking. Blood samples were obtained by caudal venipuncture using sterile, heparinized collection tubes (Monoject Vacutainers[®], Sherwood Medical, St. Louis, MO).

Experiment 3.2

A group of 10 Holstein cows from the LSU Dairy Research Unit, in varying stages of lactation, were fed a total mixed ration consisting primarily of corn silage and alfalfa hay. Cows in good body condition (body condition scores ranged from 2.5 to 5 on a scale of 1 to 5) were randomly selected from the LSU Dairy Research Unit. Cows were brought into the milking parlor during routine milking at 0200 hours. A milk sample was obtained immediately before the milking machine was placed on the cow. Teats were prepared for milk collection with iodine spray and antiseptic wipes. Teat cisterns were stripped of milk and ~2 ml were collected from each teat in antiseptic collection tubes (The Coburn Co., Whitewater, WI) to obtain a combined four-quarter sample. Another composite milk sample was obtained from total volume of milk removed from each cow during the milking session.

Hormone Determination

Blood samples were centrifuged at 300 x *g* for 30 minutes. The plasma supernatant was then separated and stored in individually labeled 7 ml plastic tubes and frozen at -25°C until further analysis. Milk samples were transferred from antiseptic collection tubes to individually labeled 7 ml plastic tubes and frozen at -25°C until further analysis. Commercial radioimmunoassay kits (Beckman-Coulter Labs, Inc., Brea, CA) were purchased from Beckman-Coulter Labs, Inc. (Brea, CA) to analyze plasma and milk samples for assay of cortisol.

Samples frozen for storage were thawed in a cool water bath (37°C) for each assay. The dairy cow is a ruminant species thus samples were extracted with acetone before proceeding to addition of radiolabelled hormone and antibodies. The radioimmunoassay of cortisol is a competitive binding assay. The assay utilized single antibody system of rabbit anti-cortisol. Radio-labeled hormone used in this assay was I¹²⁵-cortisol. Standard doses provided in the kit were 0.5, 2, 4, 10, 20, and 60 of cortisol in buffer with bovine serum albumin and sodium azide. Briefly, cortisol hormone assay was a competitive binding assay in which the labeled hormone and antibody (rabbit anti-cortisol) was added in a single step. After a 24 hours incubation period at 4°C, samples were washed and centrifuged. After decanting, sample tubes were loaded into the gamma counter for value readings and counted for 1-2 minutes each. Inter- and intra-assay variation was 5% and 9%, respectively.

Statistical Analyses

Experiment 3.1

A three-way analysis of variance (ANOVA) was used to compare the variances within and between cortisol concentrations in AM before, AM after, PM before, and PM after plasma and milk samples. A log transformation was added to the model to improve the distribution of the residual data. To determine if differences in cortisol concentration in low milk producing and high milk producing cows existed, the variable “low” or “high” was added to the model. A P value of <0.05 was used to determine significant differences for this study. SAS version 9.1 was used to analyze each data set.

Experiment 3.2

A paired t-test was used to compare the differences between composite milk samples and four-quarter milk samples. The difference in means and the standard error are reported. A

P value of <0.05 was used to determine significant differences for this study. SAS version 9.1 was used to analyze this data set.

RESULTS

Experiment 3.1

The mean age, days in milk (DIM), and lactation number for low producing cows were 5.3 years, 74.8 days and 2.7, respectively. The mean age, DIM, and lactation number for high producing cows were 4.7 years, 71.6 days and 2.5, respectively (Table 3.1). The cortisol concentrations in plasma and milk for individual experimental cows are presented in Appendix A. The plasma cortisol concentration for all experimental cows for AM before and after milking and PM before and after milking were 64.4, 75.7, 69.0 and 86.4 ng/ml, respectively. Milk cortisol concentrations for all experimental cows for AM before, AM after, PM before and PM after were 12.7, 15.4, 11.3 and 12.4 ng/ml, respectively. Mean plasma cortisol concentration was 73.9 ± 4.8 ng/ml and mean milk cortisol was 12.9 ± 0.9 ng/ml (Table 3.2 and Figure 3.1).

Mean plasma cortisol concentration in all cows was greater compared with mean milk cortisol concentration ($P < 0.0001$). Plasma and milk samples obtained after milking had significantly higher cortisol concentrations compared with samples obtained before milking in both AM and PM milkings ($P < 0.05$) (Table 3.2). Cortisol concentrations in plasma and milk samples obtained in the AM and PM were not significantly different ($P = 0.30$).

Low producing cows had a mean plasma cortisol concentration of 78.8 ± 7.5 ng/ml and a mean milk cortisol concentration of 13.5 ± 0.8 ng/ml. High producing cows had a mean plasma cortisol concentration of 69.2 ± 5.1 ng/ml and a mean milk cortisol concentration of 12.4 ± 0.9 ng/ml (Table 3.3 and Figures 3.2 through 3.5). Low milk producing cows had greater mean plasma cortisol concentrations compared with high producing cows in the PM milking ($P < 0.05$); however, there was no significant difference between the two in the AM milking. Mean plasma cortisol concentrations were significantly higher than overall mean milk cortisol concentrations (70.0 ng/ml vs. 12.9 ng/ml, respectively) ($P < 0.0001$).

Experiment 3.2

The parameters for days in milk (DIM) milk cortisol concentrations for experimental cows are summarized in Table 3.4 and Figures 3.6 and 3.7. The mean milk cortisol concentrations for composite and four-quarter milk samples were 1.2 ± 0.5 ng/ml and 1.2 ± 0.3 ng/ml, respectively.

There was no difference in cortisol concentrations between composite milk samples and four-quarter milk samples ($P=0.97$).

DISCUSSION

The purpose of Experiment 3.1 was to determine diurnal patterns of cortisol in dairy cows by obtaining plasma and milk cortisol samples during AM and PM milkings. There was no significant difference in plasma or milk cortisol between AM and PM milkings. This is in agreement with previous reports in which no diurnal variation of cortisol secretion in cows was observed (Hudson et al., 1974; Gorewit et al., 1992). Other studies have shown that cortisol is lower between the hours of 1800 to 0200 and higher between 0200 and 1800 (Wagner and Oxenreider, 1972; MacAdam and Eberhart, 1972), however, such a trend did not exist in this study. Although diurnal variation of cortisol exists in other mammalian species, such as humans (Rose et al., 1972), pigs and horses (Bottoms et al., 1972), cats (Kreiger et al., 1968) and rats (Guilleman et al., 1959), there appears to be no consistent evidence of a diurnal variation of cortisol levels in dairy cows. Ruminants do not enter the deep state of sleep, as do other domestic animals (Bell, 1960), which may help explain the dissimilarity between cows and other domestic species.

Results of this study and others (Wagner and Oxenreider, 1972; Smith et al., 1972; Rushen et al., 2001; Negrão et al., 2004) confirm that concentrations of cortisol in plasma and milk increase after milking. In both the AM and PM milkings, cortisol in plasma significantly increased from 64.4 ng/ml to 75.7 ng/ml and from 69.0 ng/ml to 86.4 ng/ml, respectively (Table 3.2). Consistent with present results from this study, cortisol concentrations in milk are directly related to cortisol concentrations in the blood and the trends in blood cortisol directly parallel those in milk (Bremel and Gangwer, 1978; Termeulen et al., 1981).

High cortisol after milking has been associated with decreased milk production and inhibition of milk ejection (Van Reenen et al., 2002). Cows milked in unfamiliar surroundings with or without familiar human contact have been reported to have significantly lower milk yield and higher plasma cortisol concentrations compared with cows milked in their routine milking parlor (Rushen et al., 2001). Individual responses to external stimuli vary significantly between cows (Van Reenen et al., 2002; Gorewit et al., 1992); therefore, individual responses to milking stimuli could be highly variable between cows and could be considered a factor in differences between cows in average milk production.

Table 3.1. Age, days in milk (DIM) and lactation status for low milk producing Holstein cows (n=12) and for high milk producing Holstein cows (n=12).

Low producing (<40 kg)				High producing (>45 kg)			
Cow ID	Age (yr)	DIM	Lactation number	Cow ID	Age (yr)	DIM	Lactation number
520	5	76	3	401	6	85	4
524	5	43	2	402	6	90	3
528	5	99	2	403	6	103	3
604	4	82	2	513	5	66	3
611	4	63	2	514	5	40	3
622	4	66	2	607	4	47	2
624	4	61	2	610	4	94	2
627	4	87	2	613	4	76	3
633	4	45	2	618	4	68	2
718	9	88	5	629	4	84	2
723	9	97	5	635	4	52	2
767	7	91	4	639	4	54	2
Mean±SEM	5.3±0.5	74.8±5.5	2.7±0.4		4.7±0.2	71.6±5.8	2.5±0.2

Table 3.2. Cortisol concentrations in Holstein cows (n=24) before and after AM and PM milkings¹.

Milking time	Cortisol (ng/ml)	
	Plasma	Milk
AM before	64.4±5.4 ^a	12.7±1.4 ^c
AM after	75.7±5.5 ^b	15.4±1.5 ^d
PM before	69.0±12.1 ^a	11.3±1.5 ^c
PM after	86.4±10.3 ^b	12.4±1.5 ^d
Mean±SEM	73.9±4.8	12.9±0.9

¹AM and PM milking conducted at 0200 and 1400 hours.
^{a,b,c,d} Different superscripts within the same column (or row) are different ($P<0.05$).

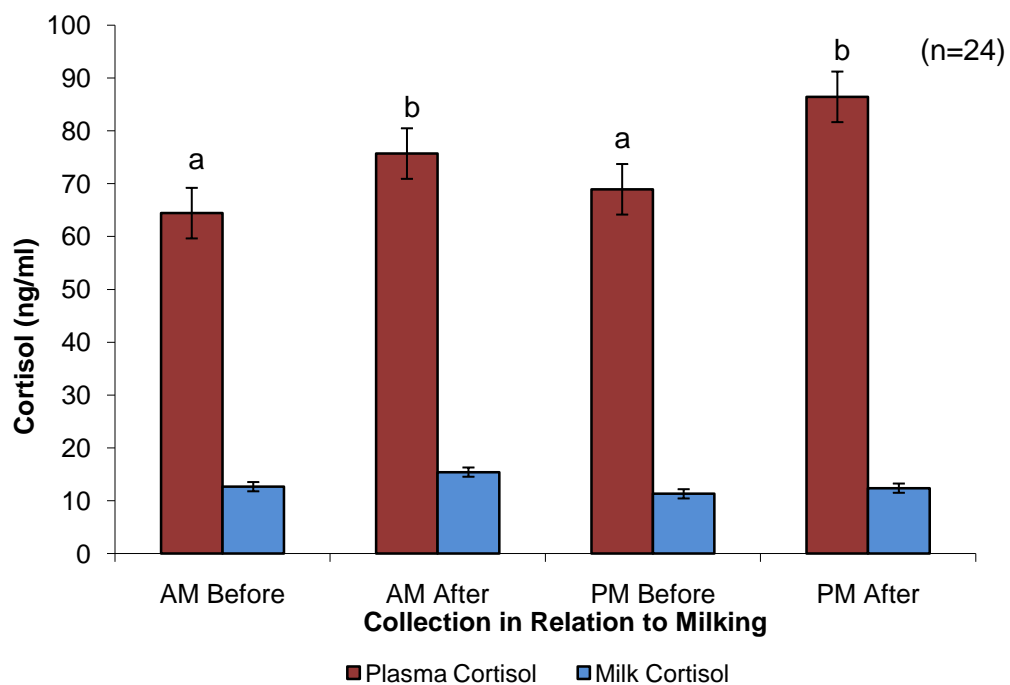


Figure 3.1. Plasma and milk cortisol concentration in Holstein cows before and after two milkings. The AM and PM milkings were conducted at 0200 and 1400 hours.
^{a,b} Different letters indicate significant differences ($P < 0.05$).

Cows were separated into low milk producing and high milk producing groups and comparison of plasma and milk cortisol between the two groups revealed no significant difference in the AM milking. However, low milk producing cows had greater plasma cortisol concentrations compared with high milk producing cows in the PM milking (Table 3.3 and Figures 3.2 and 3.3). Reasons for differences in the PM milking and not the AM milking between the two groups is not known. It could be due to differences in individual milk production in a single milking. Further studies should compare cortisol secretion and milk production from daily milkings instead of separating cows based on average milk production.

High producing cows tend to have elevated growth hormone concentrations (Sartin et al., 1988), which may assist in allowing greater milk production and minimize gluconeogenesis from protein sources during lactation. Transport-induced stress or ACTH injection results in increased potassium and decreased lactose in milk, which decreases tight junction permeability in mammary glands (Stelwagen et al., 1997) and overrides the beneficial effects of cortisol on the mammary gland, decreasing milk production. Induced stress also increases the ratio of plasma cortisol to kilograms of milk produced from 2:3 to 4:3 (Bremel and Gangwer, 1978). Increased cortisol also has an inhibitory effect on prolactin secretion by reducing the lactogenic effect of prolactin (Fox et al., 1981).

Since the quantity of milk produced for individual cows during the experimental period were not reported, we cannot conclude that low producing cows had a higher concentration of cortisol in their milk due to reduced dilution of cortisol in milk. However, it has been reported that the amount of cortisol in milk is in equilibrium with cortisol in the blood and higher concentrations of cortisol in milk parallel cortisol concentrations in plasma (Fox et al., 1981).

The objective of Experiment 3.2 was to evaluate concentrations of cortisol between two sampling techniques. A four-quarter milk sample rather than a composite sample may be necessary to avoid possible variation due to the reproductive status (pregnant, nonlactating) of the cow. The results of Experiment 3.2 indicate there is no significant difference between composite milk samples and combined four-quarter milk samples (Table 3.4). A similar study in dairy cows found no difference in cortisol concentration between foremilk and stripping milk samples (Fox et al., 1981), as opposed to the association of other milk components such as lactose and milk fat (Butler and Des Bordes, 1980).

In the present study, four-quarter milk samples were obtained first and composite samples were obtained after the cow was completely milked and there was no significant

Table 3.3. Cortisol concentration (ng/ml) in low milk producing (n=12) and high milk producing Holstein cows (n=12) before and after AM and PM milkings¹.

Group	AM Before	AM After	PM Before	PM After	Mean±SEM
Low					
Plasma	65.0±6.9	71.2±7.7	79.8±23.1 ^a	99.4±19.7 ^a	78.8±7.5
Milk	12.8±1.7	15.9±2.0	11.9±2.8	13.4±2.6	13.5±0.8
High					
Plasma	63.8±8.5	80.3±8.0	58.0±7.3 ^b	74.5±7.6 ^b	69.2±5.1
Milk	12.5±2.3	14.9±2.4	10.7±1.5	11.4±1.5	12.4±0.9

¹AM and PM milkings conducted at 0200 and 1400 hours, respectively.

^{a,b}Different superscripts within the same column are different ($P<0.05$).

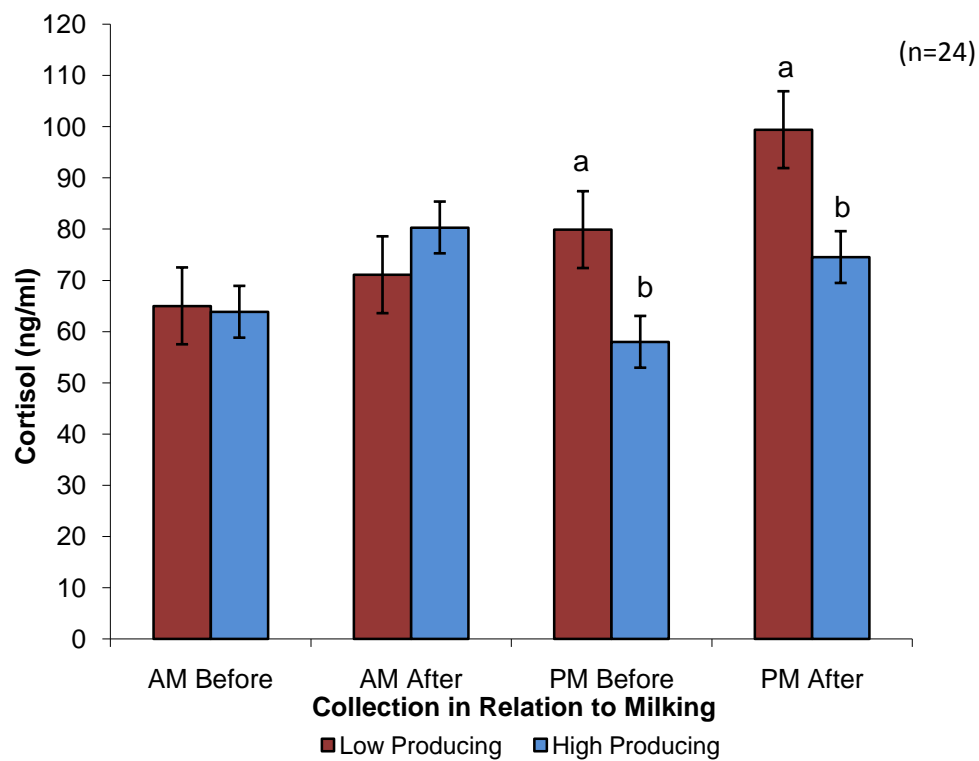


Figure 3.2. Plasma cortisol concentration in low milk producing and high milk producing Holstein cows. The AM and PM milkings were conducted at 020 and 1400 hours.
^{a,b} Different letters indicate significant differences ($P < 0.05$).

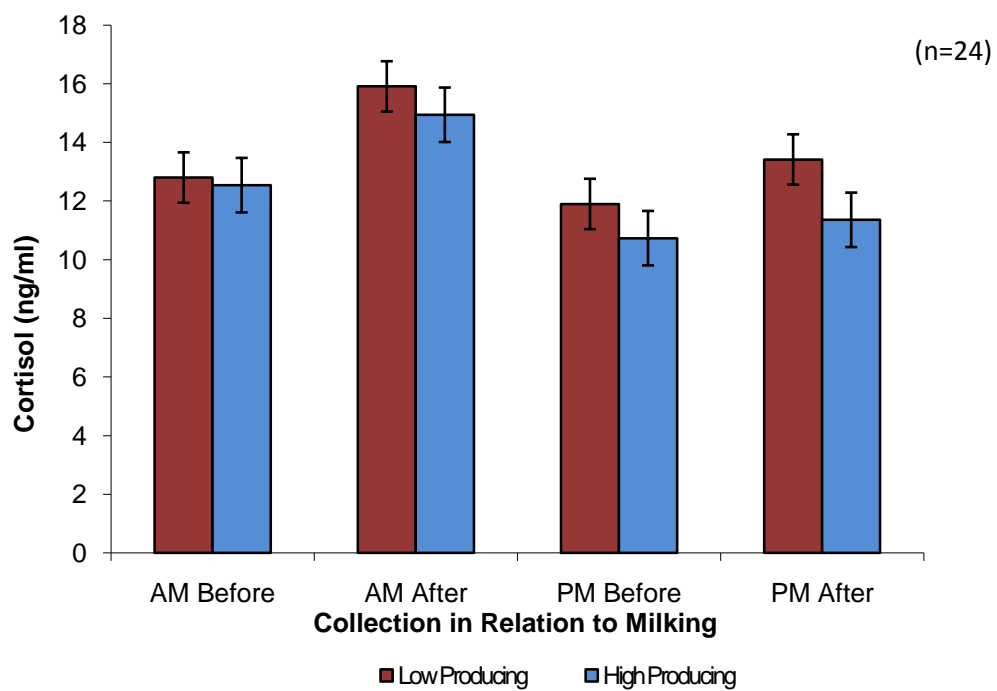


Figure 3.3. Milk cortisol concentration in low milk producing and high milk producing Holstein cows. The AM and PM milkings were conducted at 0200 and 1400 hours.

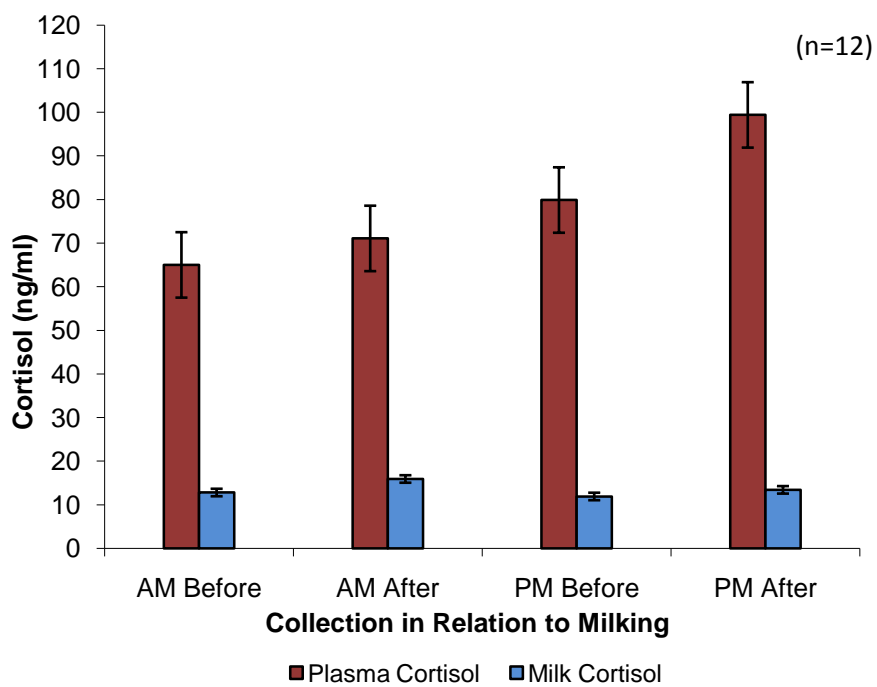


Figure 3.4. Plasma and milk cortisol concentration in low milk producing Holstein cows. The AM and PM milkings were conducted at 0200 and 1400 hours.

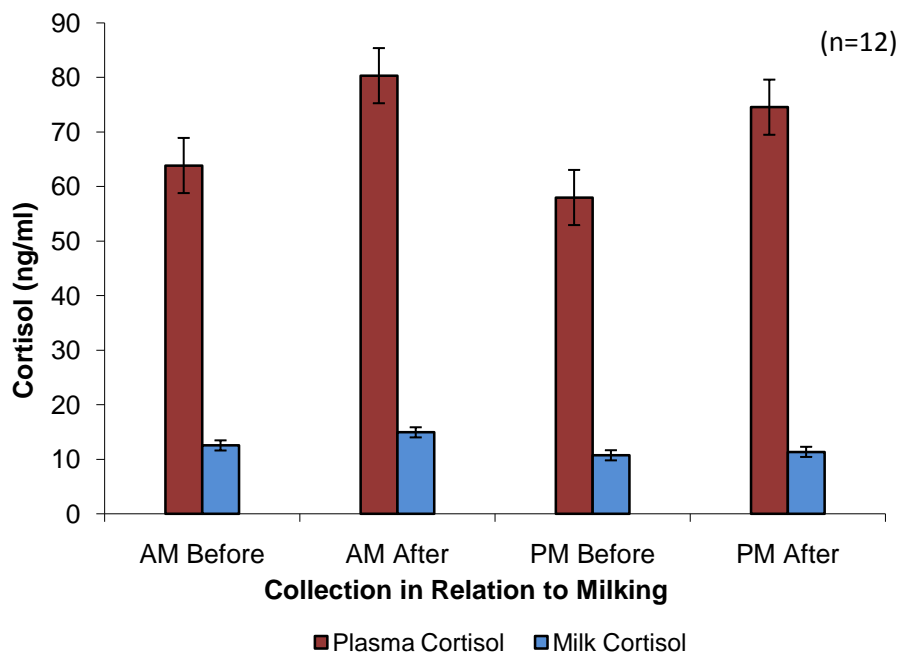


Figure 3.5. Plasma and milk cortisol concentration in high milk producing Holstein cows. The AM and PM milkings were conducted at 0200 and 1400 hours.

Table 3.4. Cortisol concentration in composite and combined four-quarter milk and days in milk (DIM) for experimental Holstein cows (n=10).

Cow ID	Cortisol (ng/ml)		Days in milk
	Composite milk	Four-quarter milk	
1	0.0	1.7	256
2	0.2	0.7	230
3	0.6	1.4	558
4	0.0	0.6	180
5	2.2	0.3	115
6	0.2	2.2	135
7	1.1	0.0	256
8	0.0	0.9	215
9	4.5	0.9	200
10	3.0	3.3	160
Mean±SEM	1.2±0.5	1.2±0.3	231±39

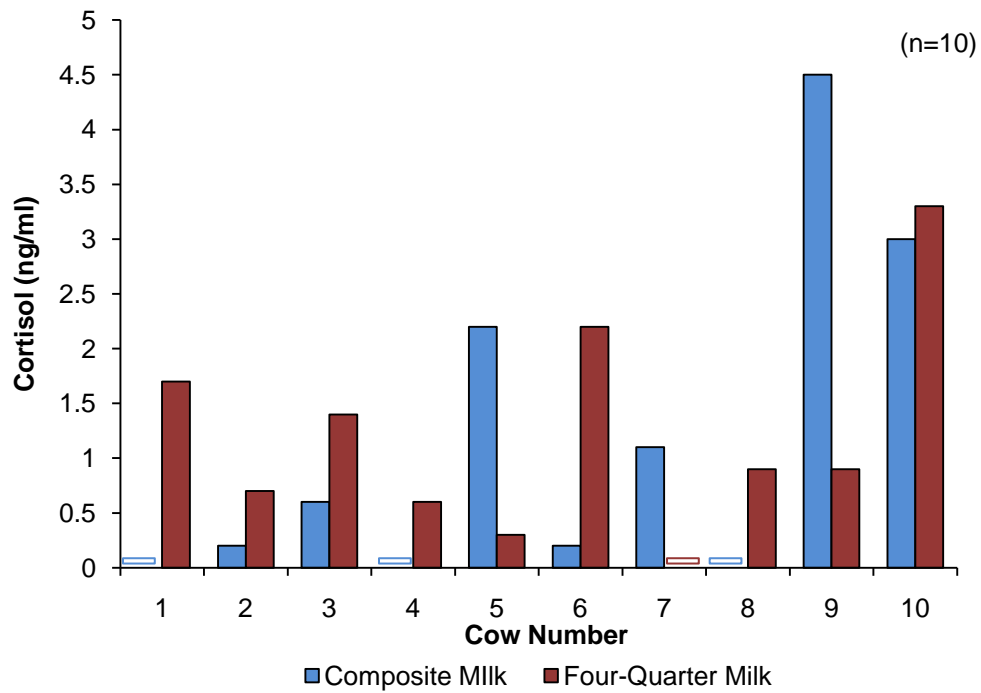


Figure 3.6. Cortisol concentration in composite milk vs. combined four-quarter milk.
Open bars (□) values <0.0001 ng/ml.

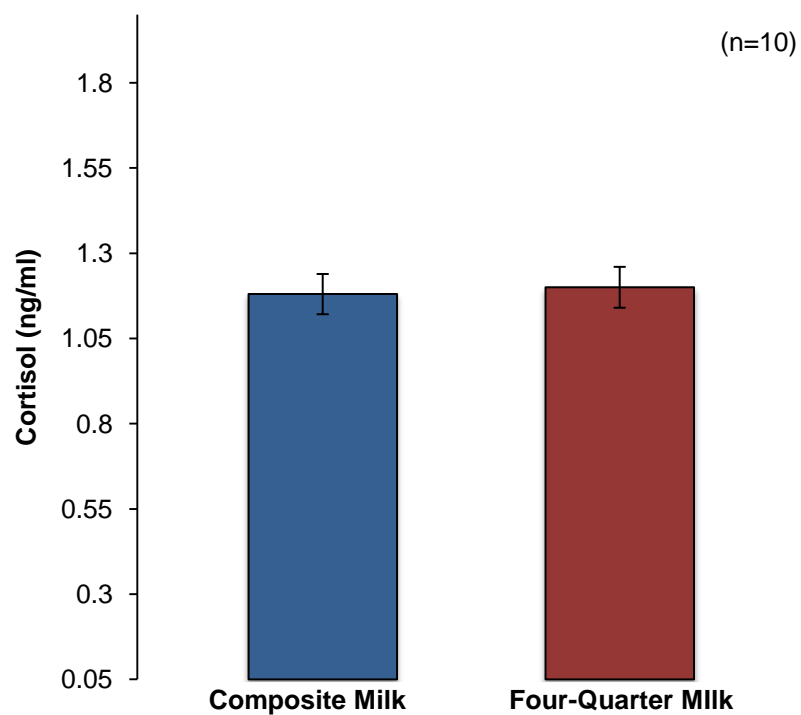


Figure 3.7. Mean cortisol concentration in composite vs. combined four-quarter milk.

difference between the two samples. Therefore, dilution or continued milk synthesis does not affect the amount of cortisol in milk samples obtained by different sampling techniques. We can conclude that there is no difference in milk cortisol concentration between two different sampling techniques.

CHAPTER IV
**PREPARTUM MATERNAL CORTISOL CONCENTRATIONS ON POSTNATAL CORTISOL
CONCENTRATION AND IMMUNOGLOBULIN ABSORPTION IN NEONATAL DAIRY
CALVES**

INTRODUCTION

The rise in fetal cortisol prior to parturition is necessary for parturition to occur in sheep and cows (Liggins 1969; Kindahl et al., 2004). Maternal cortisol concentration in cows is also elevated during parturition (Adams and Wagner, 1970). High maternal cortisol levels during parturition could be attributed to the stress of labor; however, cortisol is also necessary for other physiological processes around the time of parturition. Transport of glucocorticoids into the mammary gland is crucial for formation of colostrum and lactation (Smith et al., 1973; Patterson and Linzell, 1974; Collier and Tucker, 1978). The role of progesterone during the nonlactating state is to suppress the ability of prolactin to increase the number of its receptors in the mammary gland (Haslam and Shyamala, 1979), as well as block glucocorticoid receptors in the mammary tissue (Collier and Tucker, 1978).

Glucocorticoids are necessary for formation of milk components (Ray et al., 1986) and to induce differentiation of the lobule-alveolar system (Tucker, 1981). Mammary binding and uptake of glucocorticoids increase with the onset of lactation (Patterson and Linzell, 1974) and the amount of glucocorticoids present in milk could influence the amount of immunoglobulins present in colostrum (Schwalm and Tucker, 1978) since calves born from cows induced to calve with synthetic glucocorticoids have reduced immunoglobulin absorption (Field et al., 1999). However, it is not known whether the amount of glucocorticoids in colostrum affects passive immunity in neonates.

From a management perspective, it is crucial that newborn calves receive adequate amounts of colostrum during the first 12 to 24 hours of life in order to receive a sufficient amount of immunoglobulins to acquire passive immunity (Brignole and Stott, 1980; Wesselink et al., 1989). Glucocorticoids can have a marked influence on the amount of immunoglobulins present in colostrum. Cows induced to calve with synthetic glucocorticoids have been reported to have lower colostral immunoglobulin concentration than in cows not induced to calve, as well as their calves having significantly lower serum immunoglobulin concentration at 24 hours after birth (Field et al., 1999). It appears that prematurity is responsible for the slower uptake of colostral

immunoglobulins in cows induced to calve or that insufficient mammary growth results in less immunoglobulins being transferred into colostrum (Johnston and Stewart, 1986).

The presence of glucocorticoids in colostrum or blood in the newborn calf may actually enhance immunoglobulin absorption. Injecting calves with adrenocorticotropin hormone (ACTH) results in greater serum immunoglobulin concentration than calves injected with metyrapone (a synthetic drug that decreases cortisol release) (Johnston and Oxender, 1979). Studies in lambs showed that offspring born with higher cortisol levels tend to have higher serum concentrations of immunoglobulin (Chen et al., 1999). This suggests that calves with lower cortisol concentration may have a reduced ability to absorb colostral immunoglobulins.

We hypothesized that the high maternal cortisol levels at parturition may be taken up by the mammary gland during colostrogenesis and transferred to the neonate after birth via colostrum to enhance immunoglobulin absorption. Therefore, our objectives of this study were to determine whether maternal blood and milk cortisol levels before and after parturition influence calf cortisol levels and/or immunoglobulin G (IgG) concentration. A preliminary study conducted at this station produced similar, but incomplete results. Thus, a second experiment was complete with increased sample size and to obtain more extensive sampling (e.g., prepartum milk samples).

MATERIALS AND METHODS

Experimental Design

A group of 10 Holstein cows and 10 Holstein heifers from the LSU Dairy Research Unit were fed a total mixed ration consisting primarily of corn silage and alfalfa. Body condition scores ranged from 2.5 to 5 on a scale of 1 to 5. Each female was monitored for 14 days prior to expected calving (known breeding dates). When calving was suspected to occur within 72 hours, the female was moved to the smaller area and restrained in a holding chute. Teats were prepared for milk collection with iodine spray and antiseptic wipes. Teat cisterns were stripped of milk and ~2 ml were collected from each teat in antiseptic collection tubes (The Coburn Company, Whitewater, WI). Blood samples were obtained by caudal veinapuncture using sterile, heparinized collection tubes (Monoject Vacutainers[®], Sherwood Medical, St. Louis, MO). Blood and milk samples were collected every 24 hours until parturition. Upon calving, calves were immediately removed from their dam and not allowed to nurse. Cows were brought to the milking parlor to obtain first colostrum, and both a blood sample and milk sample were collected within 3 hours of calving in the same manner as previously described. A blood sample was

collected from each calf via jugular venipuncture using sterile, heparinized collection tubes before receiving colostrum. Calves received ~2 liters of colostrum shortly after sampling, and subsequent milk feedings occurred at 5 AM and 5 PM daily until the end of sampling. Quantity and quality, measured using a colostrometer, of colostrum consumed were recorded. Blood and milk sampling of cows and blood sampling of calves continued at 6, 12, 24 and 48 hours postpartum.

Hormone Determination

Blood samples were centrifuged at 300 x g for 30 minutes. The plasma supernatant was then separated and stored in individually labeled 7 ml plastic tubes and frozen at -25°C until further analysis. Milk samples were transferred from antiseptic collection tubes to individually labeled 7 ml plastic tubes and frozen at -25°C until further analysis. Commercial radioimmunoassay kits (Beckman-Coulter Labs, Inc., Brea, CA) were purchased from Beckman-Coulter Labs, Inc. (Brea, CA) to analyze plasma and milk samples for assay of cortisol.

Samples frozen for storage were thawed in a cool water bath (37°C) for each assay. The dairy cow is a ruminant species thus samples were extracted with acetone before proceeding to addition of radiolabelled hormone and antibodies. The radioimmunoassay of cortisol is a competitive binding assay. The assay utilized single antibody system of rabbit anti-cortisol. Radio-labeled hormone used in this assay was I^{125} -cortisol. Standard doses provided in the kit were 0.5, 2, 4, 10, 20, and 60 of cortisol in buffer with bovine serum albumin and sodium azide. Briefly, cortisol hormone assay was a competitive binding assay in which the labeled hormone and antibody (rabbit anti-cortisol) was added in a single step. After a 24 hours incubation period at 4°C, samples were washed and centrifuged. After decanting, sample tubes were loaded into the gamma counter for value readings and counted for 1-2 minutes each. Inter- and intra-assay variation was 5% and 9%, respectively.

Immunoglobulin G Determination

Single radial immunodiffusion (SRID) (VMRD, Inc., Pullman, WA) was used to determine immunoglobulin G concentration in calves throughout the sampling period. The procedure for SRID was conducted as described by Norman and Hohenboken (1981). Briefly, IgG antiserum is incorporated into the agarose gel on the assay plates. Sample antigen added to the wells diffused into the gel containing the antibody and incubated for 24 hours at 37°C. A ring of precipitation forms that is proportional in size to the concentration of the antigen. A linear relationship exists between the ring diameter and the antigen concentration when plotted on

semi-log graph paper. This relationship was used to determine immunoglobulin concentration based on ring size.

Statistical Analyses

Cortisol levels in plasma and milk of experimental cows at 72, 48 and 24 hours prepartum and 3, 6, 12, 24 and 48 hours postpartum were statically analyzed using an Analysis of Variance (ANOVA) to compare variances within and between sampling periods. Calf cortisol and immunoglobulin levels in plasma at 3, 6, 12, 24 and 48 hours postpartum were also analyzed using an ANOVA to compare variances within and between sampling periods. After determining differences between cortisol in plasma and milk of cows and cortisol and immunoglobulin of calves, cows were split into low and high cortisol level groups based on their mean cortisol concentration over the entire experimental period. The variable “low” or “high” was then added to each statistical model for cows and calves to determine if low or high cow cortisol levels had an effect on calf cortisol concentration and/or calf IgG concentration. A P value of <0.05 was used to determine significant differences for this study. SAS version 9.1 was used to analyze each data set.

RESULTS

Since circulating cortisol in the dairy cow does not exhibit a diurnal variation (Hudson et al., 1974) and due to unpredictable calving times, the time of calving has been taken as time 0 for every cow, and the time of the other samples have been calculated with this reference point regardless of the time of day. Summaries of experimental cow and calf parameters are shown in Tables 4.1 and 4.2. Mean prepartum and postpartum weight in kilograms for Holstein cows were 668 ± 16 and 610 ± 17 , respectively. Mean gestation length was 279 ± 1 days and the mean time of day for parturition was 1175 ± 0106 hours. Mean calf birth weight in kilograms was 37.6 ± 1.2 . The results of cortisol concentrations in individual cow plasma, milk and calf plasma and calf IgG concentrations are presented in Appendix B.

The results of cow plasma and milk cortisol concentrations are presented in Figure 4.1 and Table 4.3. There was no difference in plasma cortisol between 72, 48, and 24 hours before parturition ($P > 0.05$). Overall milk cortisol in all cows for the entire experimental period was lower ($P < 0.0001$) compared with overall plasma cortisol (5.4 ng/ml vs. 10.2 ng/ml). Cortisol in plasma and milk at 24 hours prepartum (4.7 ng/ml and 4.2 ng/ml) was lower ($P < 0.05$) compared with plasma and milk cortisol at 3 hours postpartum (28.9 ng/ml and 11.2 ng/ml). By 6 hours after

parturition, there is a decrease in plasma cortisol (28.9 ng/ml to 9.5 ng/ml) ($P<0.05$). In addition, plasma cortisol in cows is higher ($P<0.05$) at 3 hours postpartum compared with 12, 24 and 48 hours postpartum (28.9 ng/ml vs. 7.6 ng/ml, 5.9 ng/ml and 8.3 ng/ml, respectively) (Appendix B1). There was no difference ($P>0.05$) in milk cortisol between 72, 48 and 24 hours before parturition (Appendix B1). There was no difference ($P>0.05$) in milk cortisol levels at 3 hours and 6 hours postpartum (11.2 ng/ml and 7.4 ng/ml, respectively). However, milk cortisol was higher ($P<0.05$) at 3 hours postpartum compared with 12, 24 and 48 hours postpartum (11.2 ng/ml vs. 5.3 ng/ml, 4.0 ng/ml and 3.7 ng/ml, respectively) (Appendix B2).

The results of calf plasma cortisol and calf immunoglobulin G concentration are presented in Figure 4.2 and Table 4.4. Calf plasma cortisol levels at 3, 6, 12, 24 and 48 hours after parturition were 258.1 ng/ml, 82.9 ng/ml, 71.9 ng/ml, 81.2 ng/ml and 60.6 ng/ml, respectively (Table 4.4 and Appendix B3). Plasma cortisol levels at 3 hours after parturition were higher ($P<0.001$) compared with 6, 12, 24 and 48 hours after parturition. There was no difference ($P>0.05$) between 6, 12, 24 and 48 hours after parturition. Calf immunoglobulin G concentration at 3, 6, 12, 24 and 48 hours after parturition were 134 mg/ml, 1,306 mg/ml, 2,324 mg/ml, 3,015 mg/ml and 2,715 mg/ml, respectively (Table 4.4 and Appendix B4). Immunoglobulin G concentration at 3 hours after parturition was lower ($P<0.05$) compared with 6, 12, 24 and 48 hours after parturition, as well as 6 hours after parturition being lower compared with 12, 24 and 48 hours. Immunoglobulin G concentration at 12 hours was lower ($P<0.05$) compared with 24 hours after parturition. There was no difference ($P=0.23$) in immunoglobulin G concentration between 12 hours and 48 hours after parturition. There was also no difference ($P=0.36$) in immunoglobulin G concentration between 24 and 48 hours after parturition.

Cows were divided into two groups based on plasma cortisol concentration throughout the periparturient period. Cortisol concentration for all cows throughout the sampling period was 10 ng/ml and cows with a mean cortisol concentration less than 10 ng/ml were designated as “low” and cows with a mean cortisol concentration greater than 10 ng/ml were designated as “high” (Table 4.5). Mean plasma and milk cortisol concentration for low cortisol cows was 5.0 ng/ml and 4.4 ng/ml, respectively (Table 4.6 and Figure 4.5). Mean plasma and milk cortisol concentration for high cortisol cows was 16.6 ng/ml and 6.6 ng/ml, respectively (Table 4.6 and Figure 4.6). Plasma cortisol concentrations did not differ ($P>0.05$) between low and high cortisol cows from 72 to 24 hours before parturition (Table 4.6). However, cortisol concentration in low cortisol cows was lower ($P<0.05$) compared with high cortisol cows at 3, 6 and 12 hours after parturition (20.5 ng/ml vs. 39.1 ng/ml, 1.6 ng/ml vs. 19.1 ng/ml and 2.9 ng/ml vs. 13.4 ng/ml,

respectively), but cortisol concentrations were not different ($P>0.05$) at 24 and 48 hours after parturition (Figure 4.3).

Calves were additionally separated into low and high cortisol groups based on mean cortisol concentration of their dam. Mean plasma cortisol concentration at 3 hours postpartum for the low and high cortisol calf group was 246.0 ng/ml and 279.4 ng/ml, respectively (Table 4.7 and Figure 4.5). There was no difference ($P>0.05$) in cortisol concentration for all sampling periods in the low cortisol calf group compared with the high cortisol calf group. Mean IgG concentration at 12 hours postpartum for the low and high cortisol calf group was 1,975 mg/ml and 2,667 mg/ml, respectively. Mean IgG concentration at 24 hours postpartum for the low and high cortisol calf group was 2,928 mg/ml and 3,079 mg/ml, respectively. There was no difference ($P>0.05$) in IgG concentration for all sampling periods in the low cortisol calf group compared with the high cortisol calf group.

DISCUSSION

The present results indicate that cortisol levels in cows 3 to 6 hours after parturition is significantly higher compared with 3 to 1 days before to 2 days after parturition. This is consistent with other reports on cortisol levels at parturition in the dairy cow (Adams and Wagner, 1970; Taverne et al., 1988; Comline et al., 1974). Concentrations of cortisol in the present study were elevated from 72 to 24 hours before parturition. Cortisol returned to basal concentrations by 24 hours after parturition, consistent with results of Taverne et al. (1988) and Patel et al. (1996). This is unlike the pattern in humans where cortisol levels begin to rise 2 to 3 months before parturition (Stewart et al., 1961).

Although the peak of cortisol in our study occurred 3 hours after parturition, a pattern consistent with previous reports, the values obtained in this study are greater than those values previously reported (Smith et al., 1973a; Eissa and El-Beleley, 1990). This may be, in part, due to differences in hormone determination techniques. A role of maternal cortisol during parturition remains poorly defined. Various groups have reported the maternal rise in cortisol to be related to the stress associated with labor and delivery (Adams and Wagner, 1970; Comline et al., 1974; Hoffman et al., 1977). It is accepted that secretion of cortisol from the bovine fetal adrenal gland during the periparturient period causes luteolysis (Wendorf et al., 1983; Königsson et al., 2001) and, since ACTH can depress corpus luteum formation in the cow (Brunner et al., 1969), it would seem that high levels of maternal cortisol could, at best, be attributed a supporting role in parturition as well as elevated cortisol resulting from the stress of labor.

The present results are consistent with previous reports (Johnston and Oxender, 1979; Takeishi et al., 1989; Hough et al., 1990) that calf cortisol is elevated above 120 ng/ml 3 hours after parturition and remains elevated up to 6 hours after parturition. Cortisol levels then decrease and reach less than 70 ng/ml by 48 hours after parturition. It is clear and widely accepted that the mechanism initiating parturition in ruminant species is the secretion of cortisol from the fetal adrenal gland in late gestation (Liggins, 1969; Kindahl et al., 2004). Flint et al. (1978) investigated this mechanism in sheep and found that intra-uterine fetal infusion of glucocorticoids results in a similar hormonal cascade that occurs at spontaneous parturition. Secretion of cortisol from the bovine fetal adrenal gland is required to activate the enzyme 17 α -hydroxylase in the placenta (Wendorf et al., 1983), which is necessary for the conversion of placental progesterone to estrogen, resulting in prostaglandin production and luteolysis (Königsson et al., 2001).

It has been proposed that maturation of the fetal adrenal gland is also necessary for these events to occur in cattle. This is evidence that no correlation between ACTH and cortisol secretion in calves is found between 5 and 7 months of gestation, but a positive correlation exists between 8 and 9 months and immediately after birth (Takeishi et al., 1989), suggesting that the fetal adrenal gland must be matured in order to respond to ACTH stimulation. Also, fetal injections of ACTH are more effective in initiating early calving compared with maternal injections of synthetic glucocorticoid (Comline et al., 1974). It appears that maturation of the fetal adrenal gland is necessary for parturition and maternal cortisol levels do not play a significant role in initiating parturition.

In the present study, milk cortisol concentrations paralleled plasma cortisol concentrations. To our knowledge, cortisol concentrations in milk 0 to 12 hours following parturition have not been reported. Milk cortisol parallels plasma cortisol (Bremel and Gangwer, 1978; Termeulen et al., 1981) and our results show a simultaneous rise and a peak in cortisol at 3 hours postpartum in both plasma and milk (Figure 4.1). Therefore, it can be concluded that mean cortisol concentrations in milk at 3 hours postpartum are in the range of 10 to 12 ng/ml. It has been reported that milk cortisol at 1 day postpartum is, on average, 4.4 ng/ml (Shutt and Fell, 1985), which is consistent with our present results.

In vitro data have shown that mammary cells possess a specific mechanism to bind cortisol, and this binding is inhibited by progesterone and dexamethasone (Collier and Tucker, 1974), but not by estradiol or testosterone (Tucker et al., 1971). This could explain why progesterone inhibits lactation throughout gestation by competitively binding glucocorticoid

receptors (Ray et al., 1986) and why dexamethasone injections in late gestation result in reduced immunoglobulin content in colostrum by decreasing cortisol uptake in mammary tissue (Field et al., 1989). Indeed, there are a greater proportion of secretory epithelial cells in the

Table 4.1. Experimental Holstein cow and heifer parameters around the time of parturition (n=20).

Cow ID	Parity ¹	Prepartum weight (kg)	Postpartum weight (kg)	Parturition time (hours)	Gestation length (days)	Colostrum count ²
29	1	737	653	1000	277	60
104	4	685	617	0500	280	80
401	4	665	637	0500	283	50
529	2	712	660	1500	276	60
604	2	719	676	1000	285	100
610	2	782	764	0800	274	50
622	2	612	540	0800	280	80
629	2	685	626	1400	286	140
635	2	683	669	1600	287	60
639	2	694	642	0700	285	60
709	1	708	671	1200	274	50
712	1	624	547	2200	277	50
720	1	640	574	1600	276	50
725	1	630	558	1600	278	30
726	1	583	517	0400	277	80
728	1	596	517	1700	277	30
731	1	644	578	1400	275	50
733	1	610	560	1000	279	100
737	6	839	737	1800	287	90
802	1	506	456	0800	276	50
Mean±SEM	2±0.3	668±16	610±17	1175±0106	279±1	66±6

¹Parity = number of live offspring born.

²Colostrum count = quantity of antibodies (g/L) present in colostrum as measured by a colostrometer.

Table 4.2. Experimental Holstein calf parameters at parturition (n=20).

Calf ID	Dam ID	Calf Weight (kg)	Calf Sex
2B	604	32	Bull
3B	104	37	Bull
4B	737	53	Bull
5B	635	45	Bull
6B	728	38	Bull
915	529	32	Heifer
917	610	42	Heifer
918	709	33	Heifer
919	726	32	Heifer
920	720	41	Heifer
925	401	33	Heifer
926	629	38	Heifer
929	731	37	Heifer
930	29	37	Heifer
931	712	38	Heifer
932	725	40	Heifer
937	622	34	Heifer
941	639	43	Heifer
942	802	37	Heifer
945	733	31	Heifer
Mean±SEM		37.6±1.2	

Table 4.3. Plasma and milk cortisol concentration (ng/ml) for experimental Holstein cows (n=20).

Time ¹ (hours)	Cortisol (ng/ml) (Mean±SEM)	
	Plasma	Milk
-72	6.5±2.6 ^a	3.3±0.3 ^a
-48	9.9±3.3 ^a	3.8±0.4 ^a
-24	4.7±1.1 ^a	4.2±0.5 ^a
+3	28.9±4.5 ^b	11.2±1.4 ^b
+6	9.5±4.6 ^a	7.4±1.2 ^b
+12	7.6±2.0 ^a	5.3±0.6 ^a
+24	5.9±1.8 ^a	4.0±0.5 ^a
+48	8.3±2.4 ^a	3.7±0.5 ^a
Mean±SEM	10.2±2.7 ^c	5.4±1.0 ^d

¹Time = hours before (-) and after (+) parturition.

^{a,b}Different superscripts within the same column are different ($P<0.05$).

^{c,d}Different superscripts within the last row are different ($P<0.05$).

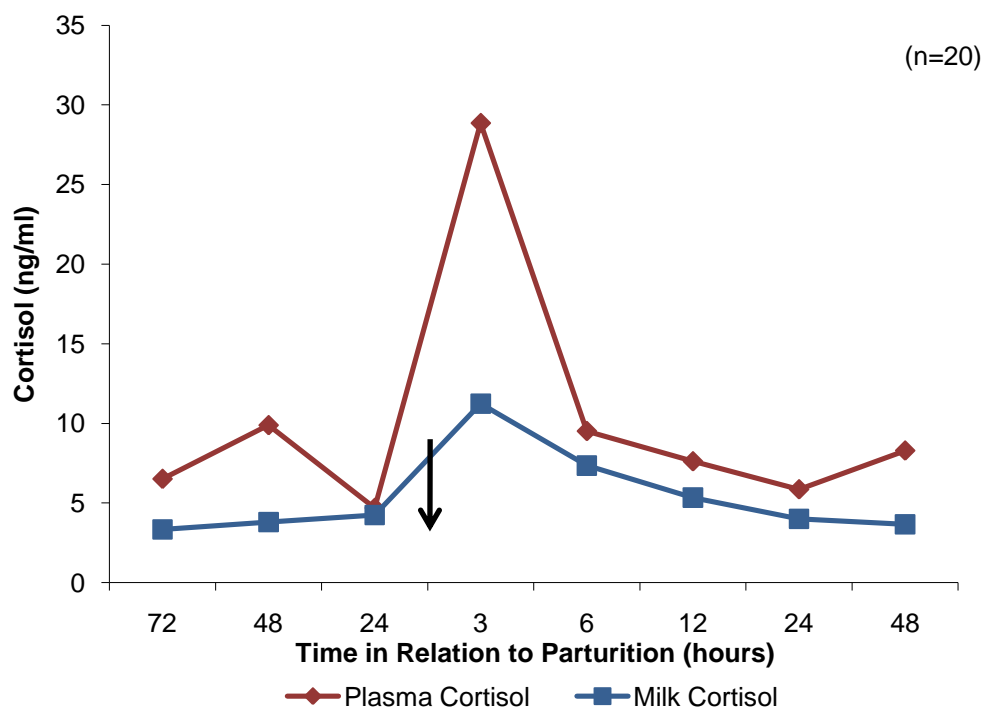


Figure 4.1. Cortisol concentration in plasma and milk before and after parturition in experimental Holstein cows. The arrow indicates time of parturition (0 hours).

Table 4.4. Plasma cortisol concentration and IgG concentration for experimental Holstein calves (n=20).

Hours postpartum	Cortisol (ng/ml) (Mean±SEM)	IgG (mg/ml) (Mean±SEM)
3	258.1±19.4 ^a	134.0±14.0 ^{a,c}
6	82.9±13.7 ^b	1,306.0±165.0 ^{b,c}
12	71.9±10.1 ^b	2,324.0±266.0 ^{b,d}
24	81.2±9.2 ^b	3,015.0±318.0 ^{a,d}
48	60.6±8.8 ^b	2,715.0±261.0 ^{b,d}
Mean±SEM	110.9±34.0	1,899.0±527

^{a,b,c,d} Different superscripts within the same column are different ($P<0.05$).

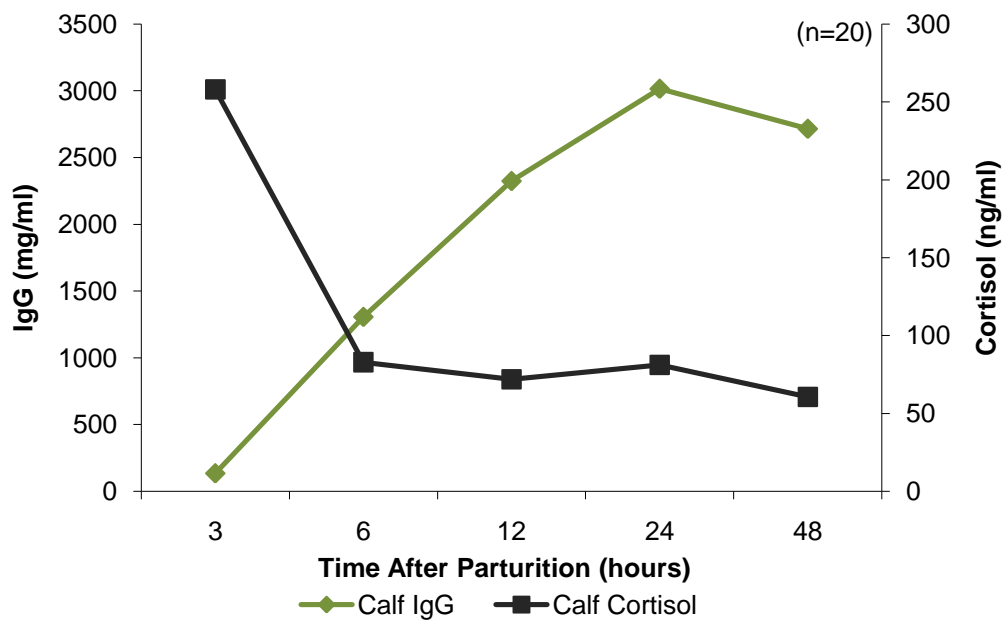


Figure 4.2. Calf plasma cortisol and immunoglobulin concentration in Holstein calves.

mammary gland in late pregnancy, which may account for the increased number of glucocorticoid receptors and increased cortisol concentration in milk in late pregnancy and the onset of lactation (Gorewit and Tucker, 1976a).

Mammary uptake of cortisol is 3 to 4% and mammary blood flow increases 70 to 120% 3 days before parturition, which is accompanied by an increase in glucose entry rate (Patterson and Linzell, 1974). It has been proposed that increased cortisol might be a factor in controlling glucose entry and uptake by the mammary gland, thereby increasing synthesis of milk components and milk production. This mechanism was also previously found to be similar in goat during early lactation (Patterson and Linzell, 1971). In addition, dairy cows induced to calve with dexamethasone at 270 days of gestation have significantly reduced immunoglobulin concentration in colostrum than cows spontaneously calving (Brandon et al., 1975; Field et al., 1989). This raises the question if the synthetic glucocorticoid itself inhibits transfer of immunoglobulins into the mammary gland or if the low concentration of immunoglobulin in colostrum is due to premature parturition. It seems most likely due to prematurity because selective transfer of immunoglobulins into mammary secretions does not increase until 2 weeks before parturition and declines abruptly 3 to 4 days before parturition (Brandon et al., 1971; Brandon and Lascelles, 1975), therefore, not allowing sufficient antibodies to be transferred into mammary secretions.

Our results of calf IgG concentration at 3 hours after birth with no colostrum feeding, as well as IgG concentration at 6, 12, 24 and 48 hours after birth, agree with the IgG values reported by others (Matte et al., 1982; Stott et al., 1979a). The present study, in addition to other reports (Stott et al., 1979a,b), indicates that IgG concentration reaches a maximum at 24 hours after birth and declines slightly by 48 hours after birth. Gut closure time, when the calf can no longer absorb colostral immunoglobulins, occurs at ~24 hours after birth, but increases by ~8 hours if colostrum feeding is delayed beyond 12 hours after birth (Stott and Fellah, 1983). Studies have shown that the volume of colostrum fed is only a minor factor affecting the amount of immunoglobulins absorbed, but rather the concentration of immunoglobulins in colostrum being more influential (Stott and Fellah, 1983; Jaster, 2005). Calves that are fed pooled colostrum and were from cows that were induced to calve prematurely or born at full-term have significantly different rates of IgG absorption (Johnston and Stewart, 1986). Calves born full-term had a higher rate of absorption and reached maximum absorption sooner than calves from cows induced to calve. However, increased calf cortisol secretion may have a stimulatory effect on immunoglobulin absorption and may be able to delay gut closure (Whitaker et al., 1996).

Based on these and previous studies, the role of cortisol in intestinal closure and immunoglobulin absorption remains unclear.

Calves that are postnatally treated with ACTH have increased IgG absorption compared with metyrapone-treated (a synthetic drug that lowers ACTH secretion) or control calves (Johnston and Oxender, 1979; Boyd and Hogg, 1981; Whitaker et al., 1996). Similar studies in pigs (Patt and Eberhart, 1976; Sangild et al., 1993) and lambs (Hough et al., 1990) have shown that postnatal metyrapone administration reduces immunoglobulin absorption and postnatal ACTH treatment enhances efficiency of passive immunity. This is in marked contrast to the pattern reported in rat pups in which injections of corticosterone or cortisone acetate reduces intestinal uptake of antibodies and causes precocious closure (Halliday, 1959; Daniels et al., 1973). This could be, in part, due to the fact that rat pups have the ability to absorb maternal antibodies up to 18 to 21 days after birth (Halliday, 1955) and cortisol concentrations increase at the time of closure (Daniels et al., 1972).

In the present study, as cortisol concentrations decrease 6 hours after birth, immunoglobulin concentrations increase (Figure 3.2). The reason for species differences in the mechanisms involving neonatal intestinal closure remains to be elucidated. Delaying colostrum feeding up to 24 hours after birth results in increased cortisol concentrations. Cortisol also increases after initial feeding, but as time elapses after feeding, cortisol concentrations decrease (Nightengale and Stott, 1981; Hammon and Blum, 1998). This could indicate that maximal absorption of antibodies does not take place unless cortisol in plasma is adequate, and the role of the adrenal gland responding to colostral ingestion could be both to mediate absorption and initiate closure.

Calves born prematurely and given intra-uterine ACTH treatment have improved organ weight parameters and IgG absorption compared with premature calves not given intra-uterine ACTH treatment (Schmidt et al., 2004). It would seem that full *in utero* maturation of the fetal adrenal gland is necessary for adequate immunoglobulin absorption and acquisition of passive immunity in neonatal calves. However, a study by Friedrich (1996) reported no correlation between ACTH and cortisol levels up to 8 days after birth. It was suggested that the feedback within the pituitary-adrenal axis is inhibited by reduced sensitivity of pituitary corticotropic cells and that normal feedback mechanisms of the pituitary-adrenal axis in neonatal calves is not fully functional until ~2 weeks after birth. Similar results were reported for neonatal rats and although the authors concluded that the pattern between ACTH and cortisol concentrations was difficult

Table 4.5. Mean plasma and milk cortisol concentration for low cortisol Holstein cows (n=11) and for high cortisol Holstein cows (n=9).

“Low” Cow ID (<10.0 ng/ml)	Cortisol (ng/ml)		“High” Cow ID (>10.0 ng/ml)	Cortisol (ng/ml)	
	Plasma	Milk		Plasma	Milk
104	6.6	4.4	29	21.4	7.3
610	8.5	3.9	401	15.6	8.6
635	4.3	5.1	529	27.0	6.9
709	7.8	2.2	604	10.7	7.1
720	3.1	2.4	622	17.1	4.9
725	2.1	3.2	629	15.1	4.7
726	3.2	4.0	639	20.8	8.7
728	6.8	6.3	712	11.0	4.6
731	3.6	5.2	802	10.3	6.6
733	3.1	5.3			
737	5.9	6.9			
Mean±SEM	5.0±0.7 ^a	4.4±0.5 ^a		16.6±1.9 ^b	6.6±0.5 ^a

^{a,b}Different superscripts indicate a significant difference ($P<0.05$).

Table 4.6. Cortisol concentration (ng/ml) in plasma and milk for low cortisol Holstein cows (n=11) and for high cortisol Holstein cows (n=9).

	Hours prepartum			Hours postpartum					Mean±SEM
	-72	-48	-24	3	6	12	24	48	
Low									
Plasma	1.0±0.7	2.4±1.3	1.9±1.2	20.5±4.4 ^a	1.6±0.8 ^a	2.9±1.2 ^a	4.7±2.7	3.7±2.0	4.8±2.3
Milk	3.2±0.6	2.7±0.3	3.5±0.6	11.4±2.3	4.4±0.6	3.9±0.4	3.0±0.4	3.0±0.4	4.4±1.0
High									
Plasma	12.8±4.6	19.1±6.1	8.0±1.5	39.1±7.4 ^b	19.1±9.4 ^b	13.4±3.3 ^b	7.2±2.3	13.8±4.2	16.6±6.6
Milk	3.5±0.4	5.1±0.6	0.5±0.1	11.0±1.7	10.9±2.1	7.1±1.1	5.2±0.8	4.5±0.8	6.0±1.3

^{a,b}Different superscripts within the same column are different ($P<0.05$).

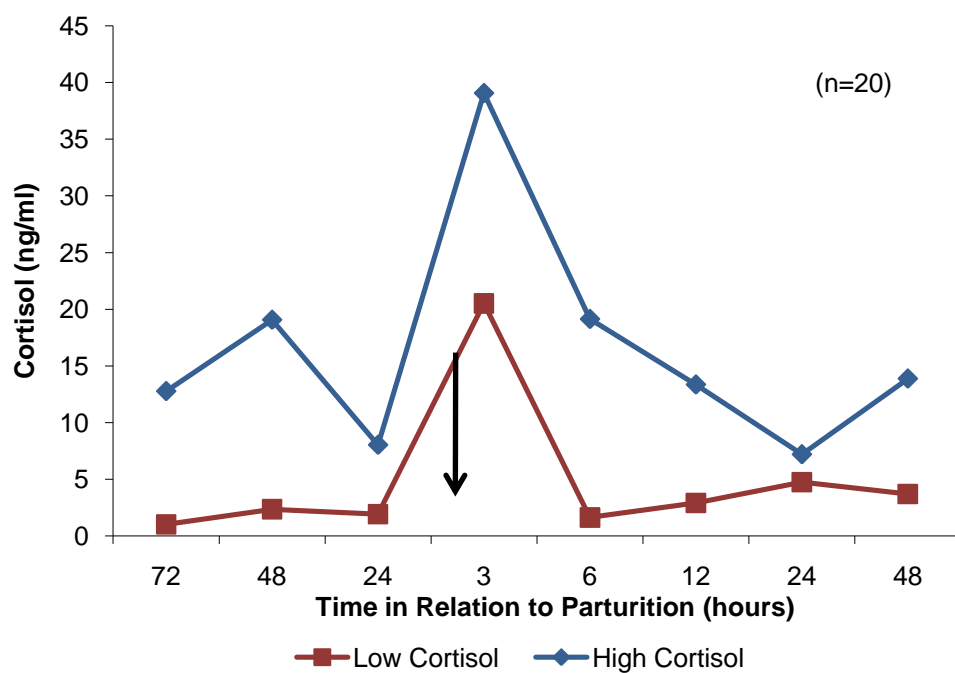


Figure 4.3. Plasma cortisol concentration in low cortisol and high cortisol Holstein cows. The arrow indicates time of parturition (0 hours).

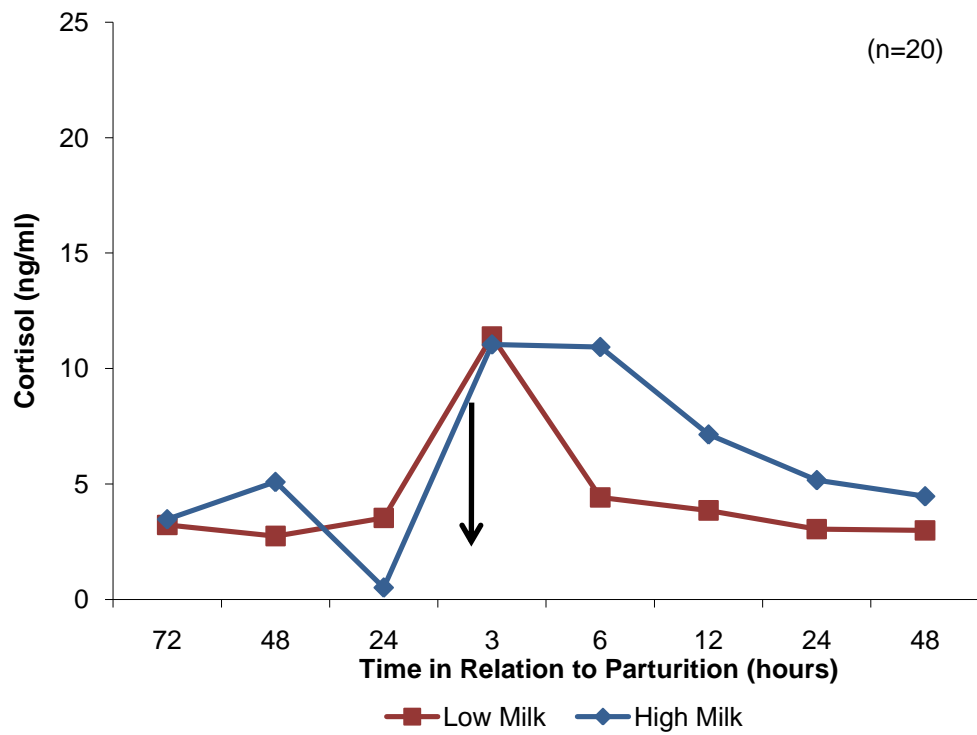


Figure 4.4. Milk cortisol concentration in low cortisol and high cortisol Holstein cows. The arrow indicates time of parturition (0 hours).

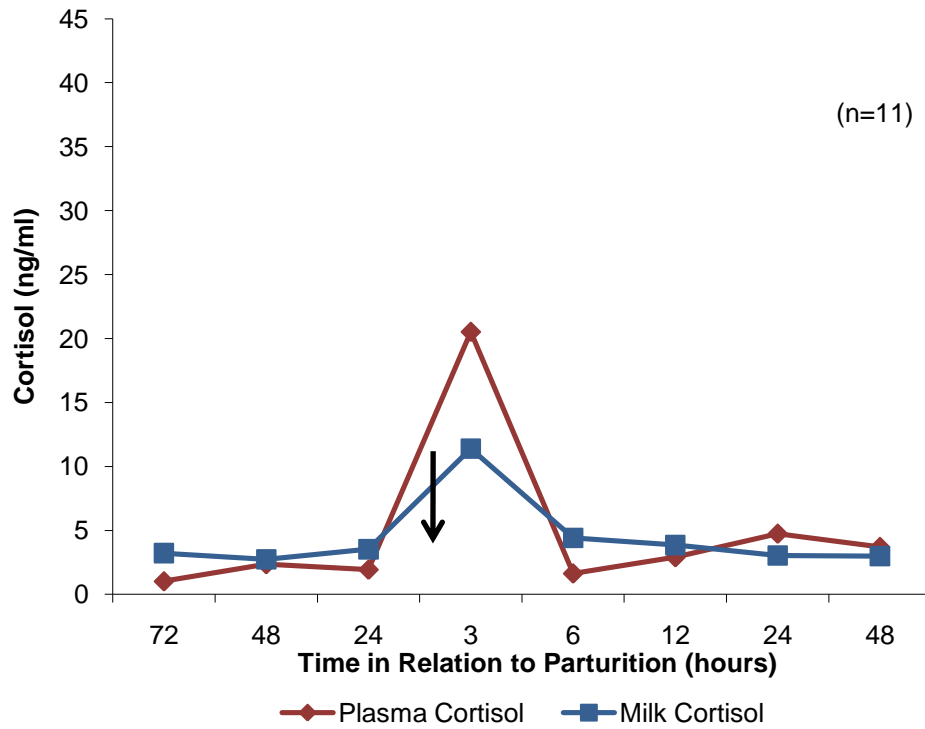


Figure 4.5. Plasma and milk cortisol concentration in low cortisol Holstein cows. The arrow indicates time of parturition (0 hours).

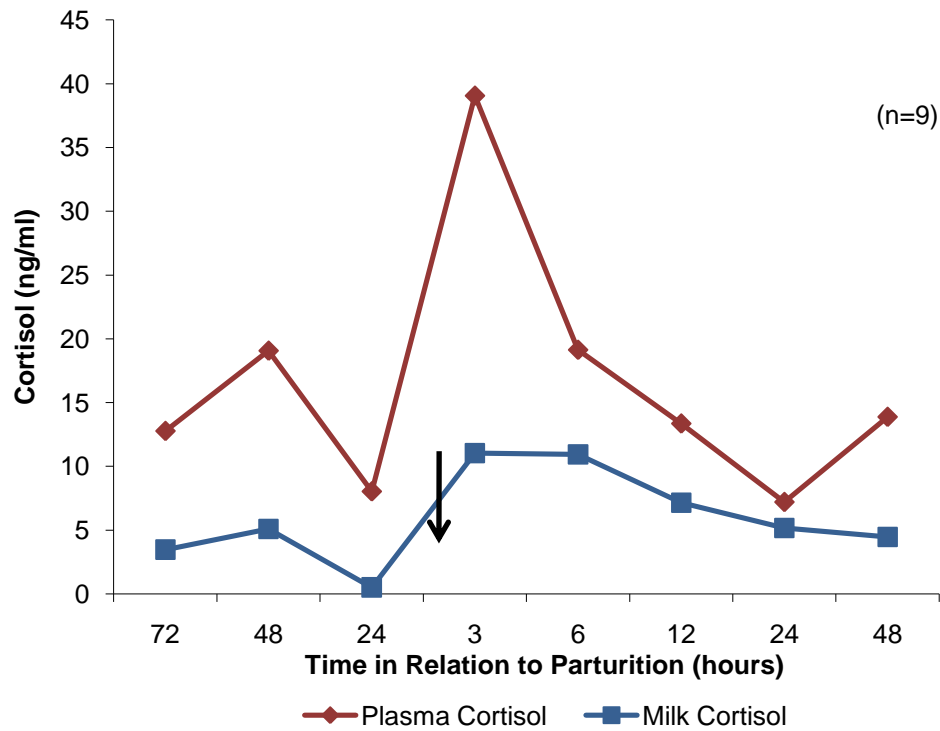


Figure 4.6. Plasma and milk cortisol concentration in high cortisol Holstein cows. The arrow indicates time of parturition (0 hours).

Table 4.7. Cortisol concentration (ng/ml) and IgG concentration (mg/ml) in low cortisol Holstein calves (n=11) and in high cortisol Holstein calves (n=9).

		Hours postpartum					Mean±SEM
		3	6	12	24	48	
Low							
Cortisol		246.0±27.6	95.2±21.5	78.1±13.0	72.7±8.3	62.3±10.6	110.9±34.2
IgG		145±25	1146±177	1975±281	2928±476	2555±353	1750±501
High							
Cortisol		279.4±23.7	74.3±14.3	67.1±15.6	92.5±17.6	57.3±15.6	114.1±41.7
IgG		120±0	1514±293	2667±487	3079±455	2956±446	2067±457

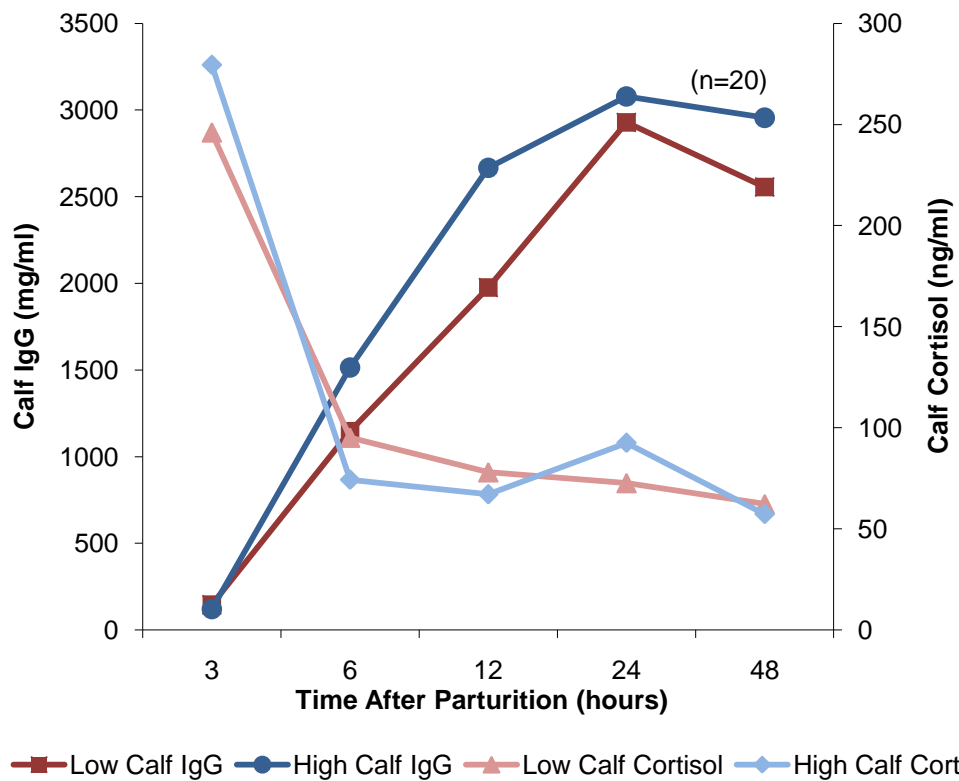


Figure 4.7. Immunoglobulin G and cortisol concentration in low cortisol and high cortisol Holstein calves.

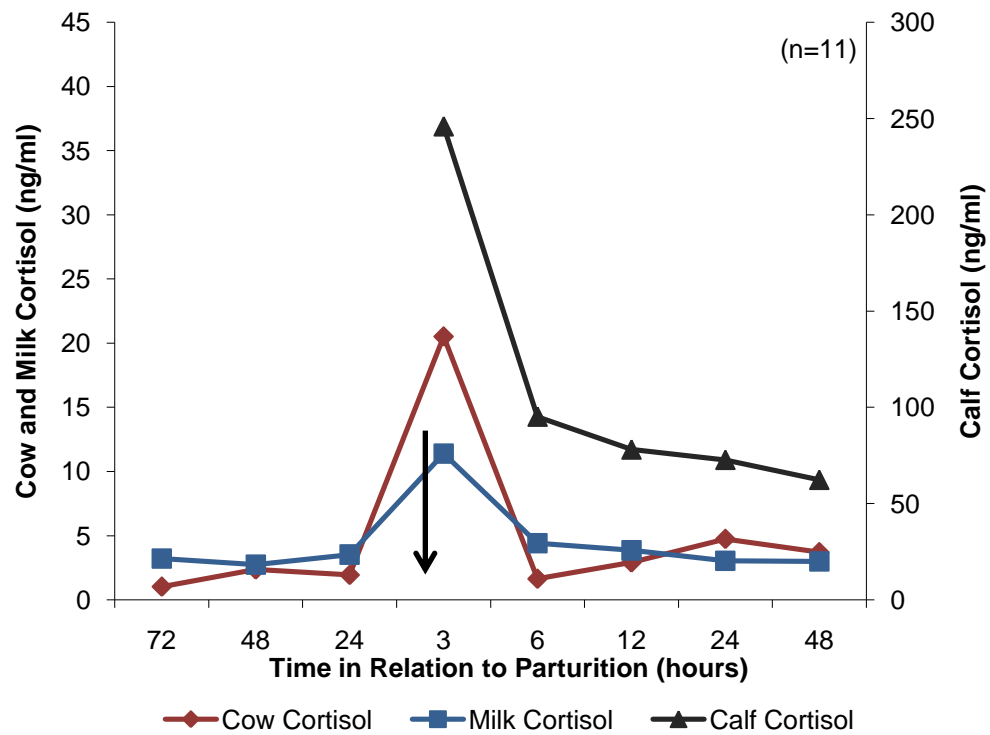


Figure 4.8. Cow plasma, milk and calf plasma cortisol concentration in low cortisol Holstein cows and calves. The arrow indicates time of parturition (0 hours).

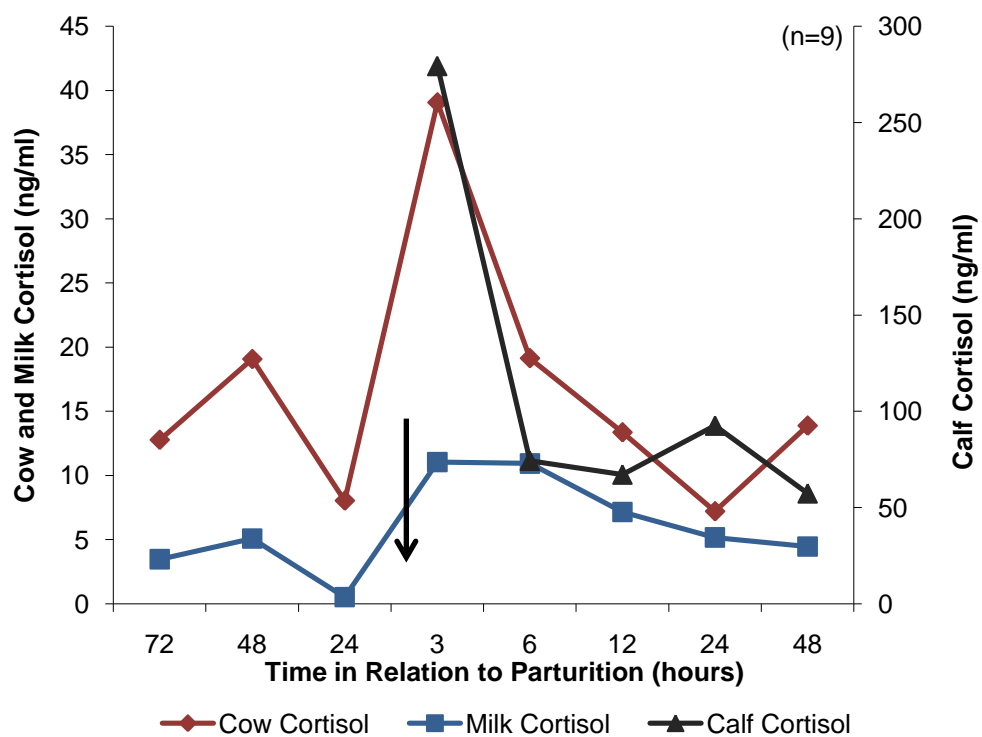


Figure 4.9. Cow plasma, milk and calf plasma cortisol concentration in high cortisol Holstein cows and calves. The arrow indicates time of parturition (0 hours).

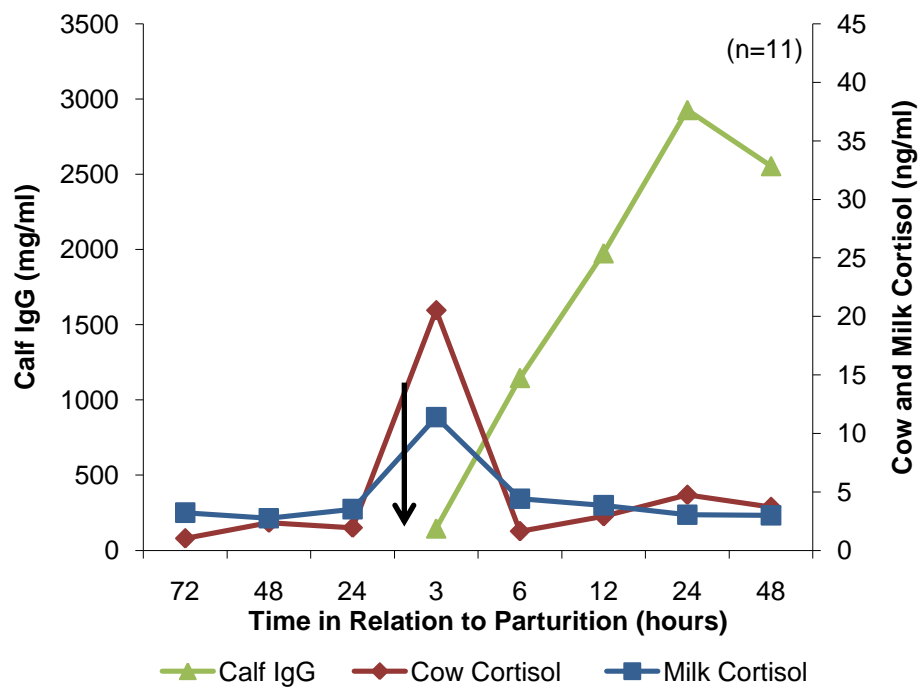


Figure 4.10. Cortisol concentration in plasma and milk for low cortisol Holstein cows and immunoglobulin G concentration in low cortisol Holstein calves. The arrow indicates time of parturition (0 hours).

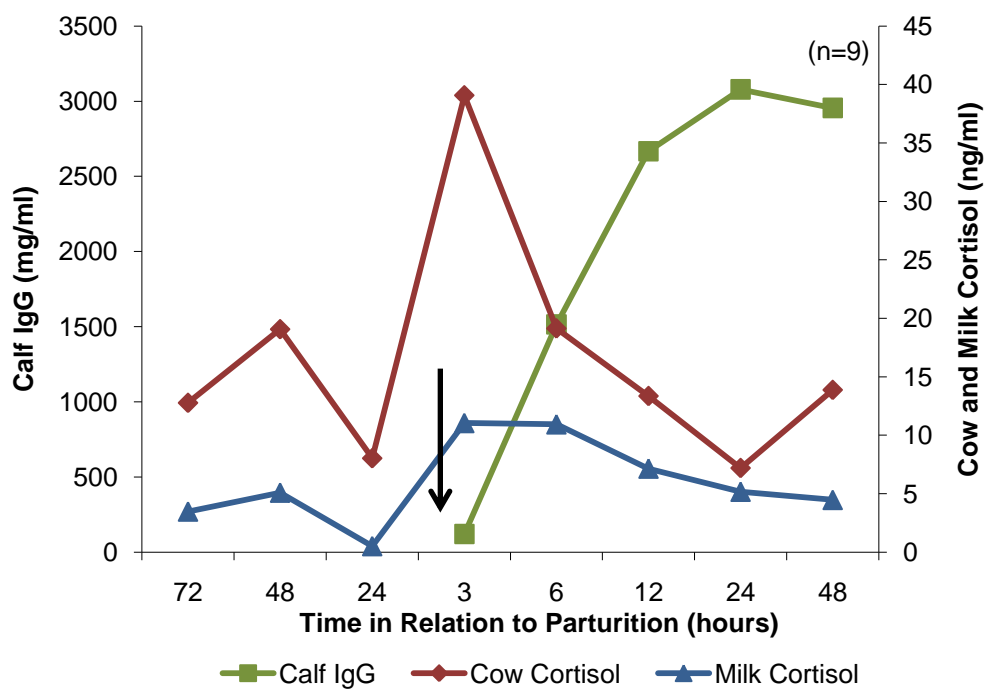


Figure 4.11. Cortisol concentration in plasma and milk for high cortisol Holstein cows and immunoglobulin G concentration in high cortisol Holstein calves. The arrow indicates time of parturition (0 hours).

to define, the normal pituitary-adrenal feedback mechanism was reported to emerge within 3 days after birth (Rosenfeld et al., 1991).

A study in goat kids investigating adrenal responsiveness between kids born with “low” or “high” cortisol revealed no difference between the two groups (Chen et al., 1999). However, kids born with high serum cortisol concentrations had similar mean serum immunoglobulin concentrations as the low cortisol kids prior to 12 hours of age, but reached a greater mean peak level around 18 hours of age and had a consistently greater concentration thereafter. In the present study, calves were divided into low and high cortisol groups based on the cortisol levels obtained from their dams at parturition. It was anticipated that IgG concentrations of high cortisol calves would exceed those of low cortisol calves, however, no difference was found between the two groups. It was concluded in previous studies (Hudson et al., 1976; Stott and Reinhard, 1978) and consistent with our present results, that cortisol concentrations in neonates are much higher at parturition than the dam. However, the fetal adrenal gland response to stimuli is not influenced by maternal cortisol concentrations. It can be concluded that calf cortisol concentrations at birth are not influenced by maternal or colostral cortisol at parturition, although elevated concentrations pose a net benefit on antibody absorption and passive immunity (Johnston and Oxender, 1979; Chen et al., 1999). Further studies are needed to determine mechanisms involving intestinal closure and how glucocorticoids, adrenal responsiveness, and prematurity affect these mechanisms.

CHAPTER V

SUMMARY AND CONCLUSIONS

The role of elevated maternal plasma cortisol at parturition in cows has been attributed to other physiological mechanisms (i.e., stress, lactation, colostrum formation), but little is known regarding its influence on neonatal calf cortisol and immunoglobulin absorption. Glucocorticoids have been shown to enhance passive immunity by exogenous administration of ACTH, but it is not known if calves with naturally higher plasma antibodies are influenced by maternal cortisol secretion. We investigated this hypothesis by measuring maternal plasma and milk cortisol concentration before and after parturition and calf plasma cortisol and immunoglobulin concentration after parturition.

Two preliminary experiments were conducted before the start of the primary experiment. Due to unpredictable calving times, it was necessary to eliminate diurnal variation in cortisol as a source of variation. Twenty-four Holstein cows in different stages of lactation were used to determine if a diurnal variation in cortisol exists in cows. Animals were brought into the milking parlor at two milkings in a 24-hour period (0200 and 1400) and a blood and milk sample was obtained before and after milking. Results indicated that no diurnal variation was present in plasma or milk cortisol secretion. There was, however, a significant difference in plasma and milk samples obtained before and after a single milking session ($P < 0.05$), with cortisol concentration being greatest after milking.

The objective of the second study was to evaluate concentrations of milk cortisol between two sampling techniques. A four-quarter milk sample rather than a composite sample may be necessary to avoid possible variation due to the reproductive status (pregnant, nonlactating) of the cow. Ten Holstein cows in different stages of lactation were brought into the milking parlor and a four-quarter milk sample was obtained and a composite sample from total milked removed during the milking session. Results showed no difference in cortisol concentration in four-quarter compared with composite milk samples.

In the final experiment, cows ($n=20$) were monitored for spontaneous parturition and blood and milk samples were obtained 72, 48 and 24 hours before parturition. Upon calving, blood and milk samples were obtained from cows and blood from calves at 3, 6, 12, 24 and 48 hours after calving. Cow plasma cortisol was significantly greater at 3 hours postpartum compared with 24 hours before and 6, 12, 24 and 48 hours after parturition. Calf cortisol was significantly greater at 3 hours compared with 6, 12, 24 and 48 hours after parturition. Calf immunoglobulin concentrations peaked at 24 hours postpartum. Cows were separated into "low"

and “high” cortisol groups based on their mean plasma cortisol concentrations throughout the experimental period. Calves were additionally separated into low and high groups based on their dams’ cortisol concentrations. Cows in the high cortisol group had significantly higher plasma and milk cortisol at 3, 6, 12 and 24 hours postpartum compared with cows in the low cortisol group. However, there was no significant difference in cortisol or IgG concentration in low and high cortisol calves.

Many have proposed that calves with impaired immunoglobulin absorption are a result of incomplete gastrointestinal tract or adrenal maturation, as evident by reduced adrenal responsiveness. It came into question if naturally occurring plasma and milk cortisol in cows around parturition had a significant influence or role in the amount of immunoglobulin absorbed in the neonatal calf. The results of the present study indicate that maternal cortisol concentrations in plasma or milk do not influence calf cortisol or immunoglobulin concentration. Furthermore, studies are needed to identify the role of glucocorticoids in the mechanisms involving intestinal closure in neonatal calves.

LITERATURE CITED

- Adams, W.M., Wagner, W.C. 1970. The role of corticoids in parturition. *Biol. Reprod.* 3: 223-228.
- Akers, R.M., Heald, C.W., Bibb, T.L., McGilliard, M.L. 1977. Effect of prepartum milk removal on quantitative morphology of bovine lactogenesis. *J. Dairy Sci.* 60: 1273-1282.
- Akers, R.M., Heald, C.W. 1978. Effect of removal of prepartum secretions on secretory cell differentiation in the bovine mammary gland. *J. Ultrastruct. Res.* 63: 316-322.
- Akers, R.M., Bauman, D.E., Capuco, A.V., Goodman, G.T., Tucker, H.A. 1981. Prolactin regulation of milk secretion and biochemical differentiation of mammary epithelial cells in periparturient cows. *Endocrinology* 109: 23-30.
- Akers, R.M., Nickerson, S.C. 1983. Effect of prepartum blockade of microtubule formation on milk production and biochemical differentiation of the mammary epithelium in Holstein heifers. *Int. J. Biochem.* 15: 771-775.
- Anderson, A.B.M., Flint, A.P.F., Turnbull, A.C. 1975. Mechanism of action of glucocorticoids in induction of ovine parturition: effect on placental steroid metabolism. *J. Endocrinol.* 66: 61-70.
- Arosh, J.A., Banu, S.K., Chapdelaine, P., Fortier, M.A. 2004. Temporal and tissue-specific expression of prostaglandin receptors EP2, EP3, EP4, FP, and cyclooxygenases 1 and 2 in uterus and fetal membranes during bovine pregnancy. *Endocrinology* 145: 407-417.
- Balfour, W.E., Comline, R.S. 1957. Secretion of progesterone by the adrenal gland. *Nature* 180: 1480-1481.
- Balfour, W.E., Comline, R.S., Short, R.V. 1957. Secretion of progesterone by the adrenal gland. *Nature* 180: 1480-1482.
- Ball, S., Polson, K., Emeny, J., Eyestone, W., Akers, R.M. 2000. Induced lactation in prepubertal Holstein heifers. *J. Dairy Sci.* 83: 2459-2463.
- Barnes, M.A., Kazmer, G.W., Akers, R.M., Pearson, R.E. 1985. Influence of selection for milk yield on endogenous hormones and metabolites in Holstein heifers and cows. *J. Anim. Sci.* 60: 271-284.
- Barrington, G.M., Besser, T.E., Davis, W.C., Gay, C.C., Reeves, J.J., McFadden, T.B. 1997a. Expression of immunoglobulin G₁ receptors by bovine mammary epithelial cells and mammary leukocytes. *J. Dairy Sci.* 80: 86-93.
- Barrington, G.M., Besser, T.E., Gay, C.C., Davis, W.C., Reeves, J.J., McFadden, T.B. 1997b. Effect of prolactin on in vitro expression of the bovine mammary immunoglobulin G₁ receptor. *J. Dairy Sci.* 80: 94-100.
- Barrington, G.M., Besser, T.E., Gay, C.C., Davis, W.C., Reeves, J.J., McFadden, T.B., Akers, R.M. 1999. Regulation of the immunoglobulin G₁ receptor: effect of prolactin on in vitro expression of the bovine mammary immunoglobulin G₁ receptor. *J. Endocrinol.* 163: 25-31.
- Beardsley, G.L., Muller, L.D., Owens, M.J., Ludens, F.C., Tucker, W.L. 1974. Initiation of parturition in dairy cows with dexamethasone. I. Cow response and performance. *J. Dairy Sci.* 57: 1061-1066.

- Beardsley, G.L., Muller, L.D., Garverick, H.A., Ludens, F.C., Tucker, W.L. 1976. Initiation of parturition in dairy cows with dexamethasone. II. Response to dexamethasone in combination with estradiol benzoate. *J. Dairy Sci.* 59: 241-247.
- Bell, F.R. 1960. The electroencephalogram of goats during somnolence and rumination. *Anim. Behav.* 8: 39-46.
- Belvedere, P., Gabai, G., Dalla Valle, L., Accorsi, P., Trivioletti, M., Colombo, L., Bono, G. 1996. Occurrence of steroidogenic enzymes in the bovine mammary gland at different functional stages. *J. Steroid Biochem. Molec. Biol.* 59: 339-347.
- Bequette, B.J., Blackwell, F.R.C., Crompton, L.A. 1998. Current concepts of amino acid and protein metabolism in the mammary gland of the lactating ruminant. *J. Dairy Sci.* 81: 2540-2559.
- Berry, S.D., McFadden, T.B., Pearson, R.E., Akers, R.M. 2001. A local increase in the mammary IGF-I: IGFBP-3 ratio mediates the mammary effects of estrogen and growth hormone. *Dom. Anim. Endocrinol.* 21: 39-53.
- Bittrich, S., Philipona, C., Hammon, H.M., Romé, V., Guilloteau, P., Blum, J.W. 2004. Preterm as compared with full term neonatal calves are characterized by morphological and functional immaturity of the small intestine. *J. Dairy Sci.* 87: 1786-1795.
- Blättler, U., Hammon, H.M., Morel, C., Philipona, C., Rauprich, A., Romé, V., Le Huërou-Luron, I., Guilloteau, P., Blum, J.W. 2001. Feeding colostrum, its composition and feeding duration variably modify proliferation and morphology of the intestine and digestive enzyme activities of neonatal calves. *J. Nutr.* 131: 1256-1263.
- Blum, J.W., Hammon, H. 2000. Colostrum effects on the gastrointestinal tract, and on nutritional, endocrine and metabolic parameters in neonatal calves. *Livest. Prod. Sci.* 66: 151-159.
- Boos, A., Kohtes, J., Steljies, A., Zerbe, H., Thole, H.H. 2000. Immunohistochemical assessment of progesterone, estrogen, and glucocorticoid receptors in bovine placentomes during pregnancy, induced parturition, and after birth with or without retention of fetal membranes. *J. Reprod. Fertil.* 120: 351-360.
- Bottoms, G., D., Roessel, O.F., Rausch, F.D., Atkins, E.L. 1972. Circadian variation in plasma cortisol and corticosterone in pigs and mares. *Amer. J. Vet. Res.* 33: 785-792.
- Boyd, J.W., Hogg, R.A. 1981. Field investigations on colostrum composition and serum thyroxine, cortisol, and immunoglobulin in naturally suckled dairy calves. *J. Comp. Path.* 91: 193-203.
- Brandon, M.R., Watson, D.L., Lascelles, A.K. 1971. The mechanism of transfer of immunoglobulin into mammary secretion of cows. *Aust. J. Exp. Biol. Med. Sci.* 49: 613-623.
- Brandon, M.R., Husband, A.J., Lascelles, A.K. 1975. The effect of glucocorticoid on immunoglobulin secretion into colostrum in cows. *Aust. J. Exp. Biol. Med. Sci.* 53: 43-48.
- Brandon, M.R., Lascelles, A.K. 1975. The effect of prepartum milking on the transfer of immunoglobulins into mammary secretions of cows. *Aust. J. Exp. Biol. Med. Sci.* 53: 197-204.
- Bremel, R.D., Gangwer, M.I. 1978. Effect of adrenocorticotropin injection and stress on milk cortisol content. *J. Dairy Sci.* 61: 1103-1108.

- Brignole, T.J., Stott, G.H. 1980. Effect of suckling followed by bottle feeding colostrum on immunoglobulin absorption and calf survival. *J. Dairy Sci.* 63: 451-456.
- Brunner, M.A., Donaldson, L.E., Hansel, W. 1969. Exogenous hormones and luteal function in hysterectomized and intact heifers. *J. Dairy Sci.* 52: 1849-1854.
- Burton, J.L., Kehrli Jr., M.E., Kapil, S., Horst, R.L. 1995. Regulation of L-selectin and CD18 on bovine neutrophils by glucocorticoids: effects of cortisol and dexamethasone. *J. Leuk. Biol.* 57: 317-325.
- Butler, W.R., Des Bordes, C.K. 1980. Radioimmunoassay technique for measuring cortisol in milk. *J. Dairy Sci.* 63: 474-477.
- Chassagne, M., Barnouin, J. 1992. Circulating PGF_{2α} and nutritional parameters at parturition in dairy cows with and without retained placenta: relation to prepartum diet. *Theriogenology* 38: 407-418.
- Chen, J.C., Chang, C.J., Peh, H.C., Lee, S.L. 1999. Perinatal adrenocortical function in relation to the growth rate and immunoglobulin acquisition of goat kids. *Sm. Rumin. Res.* 33: 255-262.
- Chew, B.P., Erb, R.E., Randel, R.D., Rouquette, F.M. 1978. Effect of corticoid induced parturition on lactation and on prepartum profiles of serum progesterone and the estrogens among cows retaining and not retaining fetal membranes. *Theriogenology* 10: 13-25.
- Chew, B.P., Erb, R.E., Fessler, J.F., Callahan, C.J., Malven, P.V. 1979. Effects of ovariectomy during pregnancy and of prematurely induced parturition on progesterone, estrogens, and calving traits. *J. Dairy Sci.* 62: 557-566.
- Collier, R.J., Croom, W.J., Bauman, D.E., Hays, R.L., Nelson, D.R. 1976. Cellular studies of mammary tissue from cows hormonally induced into lactation: lactose and fatty acid synthesis. *J. Dairy Sci.* 59: 1226-1231.
- Collier, R.J., Bauman, D.E., Hays, R.L. 1977. Lactogenesis in explant cultures for mammary tissue from pregnant cows. *Endocrinology* 100: 1192-1197.
- Collier, R.J., Tucker, H.A. 1978. Regulation of cortisol uptake in mammary tissue of cows. *J. Dairy Sci.* 61: 1709-1714.
- Comline, R.S., Hall, L.W., Lavelle, R.B., Nathanielsz, P.W., Silver, M. 1974. Parturition in the cow: endocrine changes in animals with chronically implanted catheters in the fetal and maternal circulations. *J. Endocrinol.* 63: 451-472.
- Croom, W.J., Collier, R.J., Bauman, D.E., Hays, R.L. 1976. Cellular studies of mammary tissue from cows hormonally induced into lactation: histology and ultrastructure. *J. Dairy Sci.* 59: 1232-1246.
- Daniels, V.G., Hardy, R.N., Malinowska, K.W., Nathanielsz, P.W. 1972. Adrenocortical hormones and the absorption of macromolecules by the small intestine of the young rat. *J. Endocrinol.* 52: 405-406.
- Daniels, V.G., Hardy, R.N., Malinowska, K.W., Nathanielsz, P.W. 1973. The influence of exogenous steroids on macromolecule uptake by the small intestine of the new-born rat. *J. Physiol.* 229: 681-695.

- Deayton, J.M., Young, I.R., Hollingworth, S.A., White, A., Crosby, S.R., Thorburn, G.D. 1994. Effect of late hypothalamic-pituitary disconnection on the development of the HPA axis in the ovine fetus and the initiation of parturition. *J. Neuroendocrinol.* 6: 25-31.
- Detilleux, J.C., Kehrli Jr., M.E., Stabel, J.R., Freeman, A.E., Kelley, D.H. 1995. Study of immunological dysfunction in periparturient Holstein cattle selected for high and average milk production. *Vet. Immun. Immunopath.* 44: 251-267.
- Devery-Pocius, J.E., Larson, B.L. 1983. Age and previous lactations as factors in the amount of bovine colostrum immunoglobulins. *J. Dairy Sci.* 66: 221-226.
- Dijane, J., Delouis, C., Denamur, R. 1975. Lactogenesis in organ cultures of heifer mammary tissue. *J. Endocrinol.* 65: 453-460.
- Donovan, G.A., Badinga, L., Collier, R.J., Wilcox, C.J., Braun, R.K. 1986. Factors influencing passive transfer in dairy calves. *J. Dairy Sci.* 69: 754-759.
- Edqvist, L., Kindahl, H., Stabenfeldt, G. 1978. Release of prostaglandin $F_{2\alpha}$ during the bovine periparturient period. *Prostaglandins* 16: 111-117.
- Eissa, H.M., El-Belely, M.S. 1990. Sequential changes in plasma progesterone, total estrogens, and corticosteroids in the cow throughout pregnancy and around parturition. *Brit. Vet. J.* 146: 24-29.
- Erb, R.E., Estergreen Jr., V.L., Gomes, W.R., Plotka, E.D., Frost, O.L. 1968. Progesterone levels in corpora lutea and progesterone in ovarian venous and jugular vein blood plasma of the pregnant bovine. *J. Dairy Sci.* 51: 401-410.
- Estergreen Jr., V.L., Frost, O.L., Gomes, W.R., Erb, R.E., Bullard, J.F. 1968. Effect of ovariectomy on pregnancy maintenance and parturition in dairy cows. *J. Dairy Sci.* 50: 1293-1295.
- Fairclough, R.J., Kaltenbach, C.C., Peterson, A.J., Welch, R.A.S., Cox, R.I., Wong, M.S.F. 1984. Failure of exogenous progestagens to block dexamethasone-induced prostaglandin $F_{2\alpha}$ release from the uterus of the late-pregnant cow. *Biol. Reprod.* 30: 112-118.
- Ferrell, C.L., Ford, S.P., Prior, R.L., Christenson, R.K. 1983. Blood flow, steroid secretion and nutrient uptake of the gravid bovine uterus and fetus. *J. Anim. Sci.* 56: 656-667.
- Field, R.W., Bretzlaff, K.N., Elmore, R.G., Rupp, G.P. 1989. Effect of induction of parturition on immunoglobulin content of colostrum and calf serum. *Theriogenology* 32: 501-506.
- Filep, R., Akers, R.M. 2000. Casein secretion and cytological differentiation in mammary tissue from bulls of high or low genetic merit. *J. Dairy Sci.* 83: 2261-2268.
- Fingerle-Rowson, G., Koch, P., Bikoff, R., Lin, X., Metz, C.N., Dhabhar, F.S., Meinhardt, A., Bucala, R. 2003. Regulation of macrophage migration inhibitory factor expression by glucocorticoids in vivo. *Amer. J. Pathol.* 162: 47-56.
- Fleet, I.R., Goode, J.A., Hamon, M.H., Laurie, M.S., Linzell, J.L., Peaker, M. 1975. Secretory activity of goat mammary glands during pregnancy and the onset of lactation. *J. Physiol.* 251: 763-773.

- Flint, A.P.F., Kingston, E.J., Robinson, J.S., Thorburn, G.D. 1978. Initiation of parturition in the goat: evidence for control by fetal glucocorticoids through activation of placental C21-steroid 17 α -hydroxylase. *J. Endocrinol.* 78: 367-378.
- Fox, L., Butler, W.R., Everett, R.W., Natzke, R.P. 1981. Effect of adrenocorticotropin on milk and plasma cortisol and prolactin concentrations. *J. Dairy Sci.* 64: 1794-1803.
- Friedrich, M. 1996. Influence of exogenous and endogenous ACTH on adrenal cortex activity in calves during the early postnatal period. *Brit. Vet. J.* 152: 315-320.
- Fuchs, A.R., Helmer, H., Behrens, O., Liu, H., Antonian, L., Chang, S.M., Fields, M.J. 1992. Oxytocin and bovine parturition: a steep rise in endometrial oxytocin receptors precedes onset of labor. *Biol. Reprod.* 47: 937-944.
- Fuchs, A.R., Helmer, H., Chang, S.M., Fields, M.J. 1992. Concentration of oxytocin receptors in the placenta and fetal membranes of cows during pregnancy and labor. *J. Reprod. Fertil.* 96: 775-783.
- Fuchs, A.R., Rollyson, M.K., Meyer, M., Fields, M.J., Minix, J.M., Randel, R.D. 1996. Oxytocin induces prostaglandin F_{2 α} release in pregnant cows: influence of gestational age and oxytocin receptor concentration. *Biol. Reprod.* 54: 647-653.
- Fuchs, A.R., Rollyson, M.K., Meyer, M., Fields, M.J., Minix, J.M., Randel, R.D. 1996. Oxytocin induces prostaglandin F_{2 α} release in pregnant cows: influence of gestational age and oxytocin receptor concentrations. *Biol. Reprod.* 54: 647-653.
- Fuchs, A.R., Rust, W., Fields, M.J. 1999. Accumulation of cyclooxygenase-2 gene transcripts in uterine tissues of pregnant and parturient cows: stimulation by oxytocin. *Biol. Reprod.* 60: 341-348.
- Fukasawa, M., Tsukada, H., Kosako, T., Yamada, A. 2008. Effect of lactation stage, season, and parity on milk cortisol concentration in Holstein cows. *Livest. Sci.* 113: 280-284.
- Gilbert, R.O., Gröhn, Y.T., Miller, P.M., Hoffman, D.J. 1993. Effect of parity on periparturient neutrophil function in dairy cows. *Vet. Immun. Immunopath.* 36: 75-82.
- Goodman, G.T., Akers, R.M., Friderici, K.H., Tucker, H.A. 1983. Hormonal regulation of α -lactalbumin secretion from bovine mammary tissue cultured in vitro. *Endocrinology* 112: 1324-1330.
- Gorewit, R.C., Tucker, H.A. 1976a. Corticoid binding in mammary tissue slices from lactating cows. *J. Dairy Sci.* 59: 232-240.
- Gorewit, R.C., Tucker, H.A. 1976b. Glucocorticoid binding in mammary tissue slices of cattle in various reproductive states. *J. Dairy Sci.* 59: 1890-1896.
- Gorewit, R.C., Tucker, H.A. 1976b. Lactational events related to glucocorticoid binding in bovine mammary tissue. *J. Dairy Sci.* 60: 889-895.
- Gorewit, R.C., Tucker, H.A. 1976c. Comparison of binding proteins of glucocorticoids in mammary tissue and blood sera from lactating cows. *J. Dairy Sci.* 59: 1247-1253.
- Gorewit, R.C., Svennersten, K., Butler, W.R., Unväs-Moberg, K. 1992. Endocrine responses in cows milked by hand and machine. *J. Dairy Sci.* 75: 443-448.

- Gorski, J., Erd, R.E., Dickson, W.M., Butler, H.C. 1958. Sources of progesterone in the pregnant cow. *J. Dairy Sci.* 41: 1380-1386.
- Gross, T.S., Williams, W.F. 1988a. In-vitro steroid conversion by the placenta of cows in late gestation and at parturition. *J. Reprod. Fertil.* 83: 565-573.
- Gross, T.S., Williams, W.F. 1988b. Bovine placental prostaglandin synthesis: principal cell synthesis as modulated by the binucleate cell. *Biol. Reprod.* 38: 1027-1034.
- Grunert, E., Ahlers, D., Heuwieser, W. 1989. The role of endogenous estrogens in the maturation process of the bovine placenta. *Theriogenology* 31: 1081-1091.
- Guidry, A.J., Paape, M.J., Pearson, R.E. 1976. Effects of parturition and lactation on blood and milk cell concentrations, corticosteroids, and neutrophil phagocytosis in the cow. *Amer. J. Vet. Res.* 37: 1195-2000.
- Guilleman, R., Dear, W.E., Liebelt, L.A. 1959. Nychthermal variations in plasma free corticosteroid levels of the rat. *Proc. Soc. Exp. Biol. Med.* 101: 394-395.
- Guy, M.A., McFadden, T.B., Cockrell, D.C., Besser, T.E. 1994a. Effects of unilateral prepartum milking on concentrations of immunoglobulin G₁ and prolactin in colostrum. *J. Dairy Sci.* 77: 3584-3591.
- Guy, M.A., McFadden, T.B., Cockrell, D.C., Besser, T.E. 1994b. Regulation of colostrum formation in beef and dairy cows. *J. Dairy Sci.* 77: 3002-3007.
- Hadorn, U., Hammon, H., Bruckmaier, R.M., Blum, J. 1997. Delaying colostrum intake by one day has important effects on metabolic traits and on gastrointestinal and metabolic hormones in neonatal calves. *J. Nutr.* 127: 2011-2023.
- Halliday, R. 1955. The absorption of antibodies from immune sera by the gut of the young rat. *Proc. R. Soc. B.* 143: 408-413.
- Halliday, R. 1959. The effect of steroid hormones on the absorption of antibody by the young rat. *J. Endocrinol.* 18: 56-66.
- Hammon, H.M., Blum, J.W. 1998. Metabolic and endocrine traits of neonatal calves are influenced by feeding colostrum of different durations or only milk replacer. *J. Nutr.* 128: 624-632.
- Hardy, R.N. 1969. The influence of specific chemical factors in the solvent on the absorption of macromolecular substances from the small intestine of the newborn calf. *J. Physiol.* 204: 607-632.
- Haslam, S.Z., Shyamala, G. 1979. Effect of estradiol on progesterone receptors in normal mammary glands and its relationship with lactation. *Biochem. J.* 182: 127-131.
- Heald, C.W., Saacke, R.G. 1972. Cytological comparison of milk protein synthesis of rat mammary tissue in vivo and in vitro. *J. Dairy Sci.* 55: 621-628.
- Henricks, D.M., Rawlings, N.C., Ellicott, A.R. 1977a. Plasma hormone levels in beef heifers during prostaglandin-induced parturition. *Theriogenology* 7: 17-27.
- Henricks, D.M., Rawlings, N.C., Ellicott, A.R., Dickey, J.F., Hill, J.R. 1977b. Use of prostaglandin F₂{alpha} to induce parturition in beef heifers. *J. Anim. Sci.* 44: 438-441.

- Henry, C., Kabbaj, M., Simon, H., LeMoal, M., Maccari, S. 1994. Prenatal stress increases the hypothalamo-pituitary-adrenal axis response in young and adult rats. *J. Neuroendocrinol.* 6: 341-345.
- Hoedemaker, M., Weston, P.G., Wagner, W.C. 1990. Influence of cortisol and different steroidogenic pathways on estrogen synthesis by the bovine placenta. *Amer. J. Vet. Res.* 51: 1012-1015.
- Hoffman, B., Wagner, W.C., Giménez, T. 1976. Free and conjugated steroids in the maternal and fetal plasma in the cow near term. *Biol. Reprod.* 15: 126-133.
- Hoffman, B., Wagner, W.C., Hixon, J.E., Bahr, J. 1979. Observations concerning the functional status of the corpus luteum and the placenta around parturition in the cow. *Anim. Reprod. Sci.* 2: 253-266.
- Hoffman, B., Schuler, G. 2002. The bovine placenta; a source and target of steroid hormones: observations during the second half of gestation. *Dom. Anim. Endocrinol.* 23: 309-320.
- Hough, R.L., McCarthy, F.D., Thatcher, C.D., Kent, H.D., Eversole, D.E. 1990. Influence of glucocorticoid on macromolecular absorption and passive immunity in neonatal lambs. *J. Anim. Sci.* 68: 2459-2464.
- Hudson, S., Mullord, M., Whittlestone, W.G., Payne, E. 1974. Diurnal variations in blood cortisol in the dairy cow. *J. Dairy Sci.* 58: 30-33.
- Hudson, S., Mullford, M., Whittlestone, W.G., Payne, E. 1976. Bovine plasma corticoids during parturition. *J. Dairy Sci.* 19: 744-746.
- Hunter, D.L., Erb, R.E., Randel, R.D., Garverick, H.A., Callahan, C.J., Harrington, R.B. 1970. Reproductive steroids in the bovine. I. relationships during late gestation. *J. Anim. Sci.* 30: 47-59.
- Husband, A.J., Brandon, M.R., Lascelles, A.K. 1973. The effect of corticosteroid on absorption and endogenous production of immunoglobulins in calves. *Aust. J. Exp. Biol. Med. Sci.* 51: 707-710.
- Hydbring, E., Madej, A., MacDonald, E., Drugge-Boholm, G., Berglund, B., Olsson, K. 1999. Hormonal changes during parturition in heifers and goats are related to the phases and severity of labor. *J. Endocrinol.* 160: 75-85.
- Ingalls, W.G., Convey, E.M., Hafs, H.D. 1973. Bovine serum LH, GH, and prolactin during late pregnancy, parturition, and early lactation. *Proc. Soc. Exp. Biol. Med.* 143: 161-164.
- Ishikawa, H. 1987. Observation of lymphocyte function in perinatal cows and neonatal calves. *Jpn. J. Vet. Sci.* 49: 469-475.
- Janowski, T., Zduńczyk, S., Raś, A., Okrasa, S. 1988. Mammary secretion of estrogens and prostaglandin F_{2α} in cows near parturition. *Anim. Reprod. Sci.* 17: 297-302.
- Jaster, E.H. 2005. Evaluation of quality, quantity, and timing of colostrum feeding on immunoglobulin G₁ absorption in jersey calves. *J. Dairy Sci.* 88: 296-302.
- Johnson, K.R., Erb, R.E. 1962. Maintenance of pregnancy in ovariectomized cattle with progestin compounds and their effect on progestin levels in the corpus luteum. *J. Dairy Sci.* 45: 633-639.

- Johnston, N.E., Oxender, W.D. 1979. Effect of altered serum glucocorticoid concentrations on the ability of the newborn calf to absorb colostral immunoglobulin. *Amer. J. Vet. Res.* 40: 32-34.
- Johnston, N.E., Stewart, J.A. 1986. The effect of glucocorticoids and prematurity on absorption of colostral immunoglobulin in the calf. *Aust. Vet. J.* 63: 191-192.
- Judd, A.M., MacLeod, R.M. 1992. Adrenocorticotropin increases interleukin-6 release from rat adrenal zona glomerulosa cells. *Endocrinology* 130: 1245-1254.
- Kann, G., Denamur, R. 1974. Possible role of prolactin during the estrous cycle and gestation in the ewe. *J. Reprod. Fertil.* 39: 473-483.
- Kask, K., Gustafsson, H., Gunnarsson, A., Kindahl, H. 2000. Induction of parturition with prostaglandin F_{2α} as a possible model to study impaired reproductive performance in the dairy cow. *Anim. Reprod. Sci.* 59: 129-139.
- Kehrli Jr., M.E., Nonnecke, B.J., Roth, J.A. 1989a. Alterations in bovine lymphocyte function during the periparturient period. *Amer. J. Vet. Res.* 50: 215-220.
- Kehrli Jr., M.E., Nonnecke, B.J., Roth, J.A. 1989b. Alterations in bovine neutrophil function during the periparturient period. *Amer. J. Vet. Res.* 50: 207-214.
- Kensinger, R.S., Bauman, D.E., Collier, R.J. 1979. Season and treatment effects on serum prolactin and milk yield during induced lactation. *J. Dairy Sci.* 62: 1880-1888.
- Kimura, K., Goff, J.P., Kehrli Jr., M.E., Harp, J.A. 1999. Phenotype analysis of peripheral blood mononuclear cells in periparturient dairy cows. *J. Dairy Sci.* 82: 315-319.
- Kindahl, H., Kornmatitsuk, B., Gustafsson, H. 2004. The cow in endocrine focus before and after calving. *Reprod. Dom. Anim.* 39: 217-221.
- Koets, A.P., de Schwartz, N., Tooten, P., Kankofer, M., Broekhuijsen-Davies, J.M., Rutten, V.P.M.G., van Leengoed, L.A.M.G., Taverne, M.A.M., Gruys, E. 1998. Release of proinflammatory cytokines related to luteolysis and the periparturient acute phase response in prostaglandin-induced parturition in cows. *Theriogenology* 49: 797-812.
- Königsson, K., Kask, K., Gustafsson, H., Kindahl, H., Parvizi, N. 2001. 15-ketodihydro-PGF_{2α}, progesterone, and cortisol profiles in heifers after induction of parturition by injection of dexamethasone. *Acta. Vet. Scand.* 42: 151-159.
- Kornmatitsuk, B., Veronesi, M.C., Madej, A., Dahl, E., Ropstad, E., Beckers, J.F., Forsberg, M., Gustafsson, H., Kindahl, H. 2002. Hormonal measurements in late pregnancy and parturition in dairy cows – Possible tools to monitor fetal well being. *Anim. Reprod. Sci.* 72: 153-164.
- Krieger, D.T., Silverberg, A.I., Rizzo, F., Krieger, M.P. 1968. Abolition of circadian periodicity of plasma 17-OHCS levels in the cat. *Amer. J. Physiol.* 215: 959-964.
- Lay, D.C. Jr., Friend, T.H., Randel, R.D., Jenkins, O.C., Neuendorff, D.A., Kapp, G.M., Bushong, D.M. 1996. ACTH dose response and some physiological effects of transportation on pregnant Brahman cattle. *J. Anim. Sci.* 74: 1806-1811.
- Lay Jr., D.C., Randel, R.D., Friend, T.H., Carroll, J.A., Welsh Jr., T.H., Jenkins, O.C., Neuendorff, D.A., Bushong, D.M., Kapp, G.M. 1997. Effects of prenatal stress on the fetal calf. *Dom. Anim. Endocrinol.* 14: 73-80.

- Leece, J.G., Morgan, D.O. 1962. Effect of dietary regimen on cessation of intestinal absorption of large molecules (closure) in the neonatal pig and lamb. *J. Nutr.* 78: 263-266.
- Le Huerou-Luron, I., Guilloteau, P., Wicker-Planquart, C., Chayvialle, J., Burton, J., Mouats, A., Toullec, R., Puigserver, A. 1992. Gastric and pancreatic enzyme activities and their relationship with some gut regulatory peptides during postnatal development and weaning in calves. *J. Nutr.* 122: 1434-1445.
- Li, Y., Perezgrovas, R., Gazal, O.S., Schwabe, C., Anderson, L.L. 1991. Antiprogestosterone, RU 486, facilitates parturition in cattle. *Endocrinology* 129: 765- 770.
- Liggins, G.C. 1969. Premature delivery of fetal lambs infused with glucocorticoids. *J. Endocrinol.* 45: 515-523.
- Liggins, G.C., Grieves, S. 1971. Possible role for prostaglandin $F_{2\alpha}$ in parturition in the sheep. *Nature* 232: 62-64.
- MacAdam, W.R., Eberhart, R.J. 1972. Diurnal variation in plasma corticosteroid concentration in dairy cattle. *J. Dairy Sci.* 55: 1792-1795.
- Mallard, B.A., Watger, L.C., Ireland, M.J., Dekkers, J.C.M. 1997. Effects of growth hormone, insulin-like growth factor-I, and cortisol on periparturient antibody response profiles of dairy cattle. *Vet. Immun. Immunopath.* 60: 61-76.
- Mallard, B.A., Dekkers, J.C., Ireland, M.J., Leslie, K.E., Sharif, S., Vankampen, C.L., Wagter, L., Wilkie, B.N. 1998. Alteration in immune responsiveness during the peripartum period and its ramification on dairy cow and calf health. *J. Dairy Sci.* 81: 585-595.
- Matte, J.J., Girard, C.L., Seoane, J.R., Brisson, G.J. 1982. Absorption of colostral immunoglobulin G in the newborn dairy calf. *J. Dairy Sci.* 65: 1765-1770.
- McCoy, G.C., Reneau, J.K., Hunter, A.G., Williams, J.B. 1970. Effects of diet and time on blood serum proteins in the newborn calf. *J. Dairy Sci.* 53: 358-362.
- McDonald, L.E., McNutt, S.H., Nichols, R.E. 1953. On the essentiality of the bovine corpus luteum of pregnancy. *Amer. J. Vet. Res.* 14: 539-541.
- McDonald, L.E., Hays, R.L. 1958. The effects of prepartum administration of progesterone to the cow. *Amer. J. Vet. Res.* 19: 97-98.
- Mehrzad, J., Duchateau, L., Pyörälä, S., Burvenich, C. 2002. Blood and milk neutrophil chemiluminescence and viability in primiparous and pluriparous dairy cows during late pregnancy, around parturition, and early lactation. *J. Dairy Sci.* 85: 3268-3276.
- Muller, L.D., Beardsley, G.L., Ellis, R.P., Reeds, D.E., Owens, M.J. 1975. Calf response to the initiation of parturition in dairy cows with dexamethasone or dexamethasone with estradiol benzoate. *J. Anim. Sci.* 41: 1711-1716.
- Nagahata, H., Ogawa, A., Sanada, Y., Noda, H., Yamamoto, S. 1992. Peripartum changes in antibody producing capability of lymphocytes from dairy cows. *Vet. Quart.* 14: 39-40.
- Nathanielsz, P.W., Abel, M.H., Bass, F.G., Krane, E.J., Thomas, A.L., Liggins, G.C. 1978. Pituitary stalk-section and some of its effects on endocrine function in the fetal lamb. *Quart. J. Exp. Physiol.* 63: 211-219.

- Negrão, J.A., Porcionato, M.A., de Passillé, A.M., Rushen, J. 2004. Cortisol in saliva and plasma of cattle after ACTH administration and milking. *J. Dairy Sci.* 87: 1713-1718.
- Nickerson, S.C., Akers, R.M. 1984. Biochemical and ultrastructural aspects of milk synthesis and secretion. *Int. J. Biochem.* 16: 855-865.
- Nightengale, G.T., Stott, G.H. 1981. Adrenal response of the newborn calf to acute inanition and colostrum feeding. *J. Dairy Sci.* 64: 236-240.
- Norman, L.M., Hohenboken, W.D. 1981. Genetic differences in concentration of immunoglobulins G(1) and M in serum and colostrum of cows and in serum of neonatal calves. *J. Anim. Sci.* 53: 1465-1471.
- Oxender, W.D., Hafs, H.D., Edgerton, L.A. 1972. Serum growth hormone, LH, and prolactin in the pregnant cow. *J. Anim. Sci.* 35: 51-55.
- Patel, O.V., Takahashi, T., Takenouchi, N., Hirako, M., Sasaki, N., Domeki, I. 1996. Peripheral cortisol levels throughout gestation in the cow: Effect of stage of gestation and fetal number. *Brit. Vet. J.* 152: 425-432.
- Paterson, J.Y.F., Linzell, J.L. 1971. The secretion of cortisol and its mammary uptake in the goat. *J. Endocrinol.* 50: 493-499.
- Paterson, J.Y.F., Linzell, J.L. 1974. Cortisol secretion rate, glucose entry rate, and the mammary uptake of cortisol and glucose during pregnancy and lactation in dairy cows. *J. Endocrinol.* 62: 371-383.
- Patt, J.A., Eberhart, R.J. 1976. Effects of metyrapone and ACTH on intestinal absorption of immunoreactive bovine IgG in cesarean-derived pigs. *Amer. J. Vet. Res.* 37: 1409-1413.
- Pepe, G.J., Burch, M.G., Albrecht, E.D. 2001. Estrogen regulates 11 β -hydroxysteroid dehydrogenase-1 and -2 localization in placental syncytiotrophoblast in the second half of primate pregnancy. *Endocrinology* 142: 4496-4503.
- Peter, A.T., Bosu, W.T.K. 1987. Periparturient endocrine changes associated with retained placenta in dairy cows. *Theriogenology* 28: 383-394.
- Peterson, A.J., Hunter, J.T., Welch, R.A.S., Fairclough, R.J. 1975. Estrogens in bovine fetal and maternal plasma near term. *J. Reprod. Fertil.* 43: 179-181.
- Pimentel, S.M., Pimentel, C.A., Weston, P.G., Hixon, J.E., Wagner, W.C. 1986. Progesterone secretion by the bovine fetoplacental unit and responsiveness of corpora lutea to steroidogenic stimuli at two stages of gestation. *Amer. J. Vet. Res.* 47: 1967-1961.
- Pimentel, S., Evans, G., Wagner, W.C. 1987. Placental synthesis of estrogens at parturition and during placental retention in the cow. *Theriogenology* 28: 755-766.
- Plaut, K., Bauman, D.E., Agergaard, N., Akers, R.M. 1987. Effect of exogenous prolactin administration on lactational performance of dairy cows. *Dom. Anim. Endocrinol.* 4: 279-290.
- Poore, K.R., Young, I.R., Canny, B.J., Thorburn, G.D. 1998. Studies on the role of ACTH in the regulation of adrenal responsiveness and the timing of parturition in the ovine fetus. *J. Endocrinol.* 158: 161-171.

- Pope, G.S., Gupta, S.K., Munro, I.B. 1969. Progesterone levels in the systemic plasma of pregnant, cycling, and ovariectomized cows. *J. Reprod. Fertil.* 20: 369-381.
- Preisler, M.T., Weber, P.S.D., Tempelman, R.J., Erskine, R.J., Hunt, H., Burton, J.L. 2000a. Glucocorticoid receptor expression profiles in mononuclear leukocytes of periparturient Holstein cows. *J. Dairy Sci.* 83: 38-47.
- Priesler, M.T., Weber, P.S.D., Tempelman, R.J., Erskine, R.J., Hunt, H., Burton, J.L. 2000b. Glucocorticoid receptor down-regulation in neutrophils of periparturient cows. *Amer. J. Vet. Res.* 61: 14-19.
- Randel, R.D., Erb, R.E. 1971. Reproductive steroids in the bovine. VI. Changes and interrelationships from 0 to 260 days of pregnancy. *J. Anim. Sci.* 33: 115-123.
- Ray, D.B., Jansen, R.W., Horst, I.A., Mills, N.C., Kowal, J. 1986. Complex noncoordinate regulation of α -lactalbumin and 25 K β -casein by corticosterone, prolactin, and insulin in long term cultures of normal rat mammary cells. *Endocrinology* 118: 393-407.
- Reber, A.J., Hippen, A.R., Hurley, D.J. 2005. Effects of the ingestion of whole colostrum or cell-free colostrum on the capacity of leukocytes in newborn calves to stimulate or respond in one-way mixed leukocyte cultures. *Amer. J. Vet. Res.* 66: 1854-1860.
- Reber, A.J., Donovan, D.C., Gabbard, J., Galland, K., Aceves-Avila, M., Holbert, K.A., Marshall, L., Hurley, D.J. 2008a. Transfer of maternal colostrum leukocytes promotes development of the neonatal immune system. I. Effects on monocyte lineage cells. *Vet. Immun. Immunopath.* 123: 186-196.
- Reber, A.J., Donovan, D.C., Gabbard, J., Galland, K., Aceves-Avila, M., Holbert, K.A., Marshall, L., Hurley, D.J. 2008b. Transfer of maternal colostrum leukocytes promotes development of the neonatal immune system. II. Effects on neonatal lymphocytes. *Vet. Immun. Immunopath.* 123: 305-313.
- Ricketts, A.P., Gaili, A.K.A., Ackland, N., Heap, R.B., Flint, A.P.F. 1980. Activation by corticosteroids of steroid metabolizing enzymes in ovine placental explants in vitro. *J. Endocrinol.* 85: 457-469.
- Riedel-Caspari, G., Schmidt, F.W. 1991a. The influence of colostrum leukocytes on the immune system of the neonatal calf. I. Effects on lymphocyte responses. *Dtsch. Tierarzti. Wochenschr.* 98: 102-107.
- Riedel-Caspari, G., Schmidt, F.W. 1991b. The influence of colostrum leukocytes on the immune system of the neonatal calf. II. Effects on passive and active immunization. *Dtsch. Tierarzti. Wochenschr.* 98: 190-194.
- Riedel-Caspari, G. 1993. The influence of colostrum leukocytes on the course of an experimental *Escherichia coli* infection and serum antibodies in neonatal calves. *Vet. Immun. Immunopath.* 35: 275-288.
- Robertson, H.A. 1974. Changes in the concentrations of unconjugated estrone, estradiol-17 α and estradiol-17 β in the maternal plasma of the pregnant cow in relation to the initiation of parturition and lactation. *J. Reprod. Fertil.* 36: 1-7.
- Robertson, H.A., King, G.J. 1979. Conjugated and unconjugated estrogens in fetal and maternal fluids of the cow throughout pregnancy. *J. Reprod. Fertil.* 55: 463-470.

- Robinson, P.M., Rowe, E.J., Wintour, E.M. 1979. The histogenesis of the adrenal cortex in the fetal sheep. *Acta. Endocrinol.* 91: 134-149.
- Robinson, P.M., Comline, R.S., Fowden, A.L., Silver, M. 1983. Adrenal cortex of fetal lamb: changes after hypophysectomy and effects of synacthen on cytoarchitecture and secretory activity. *Quart. J. Exp. Physiol.* 68: 15-27.
- Robison, J.D., Stott, G.H., DeNise, S.K. 1988. Effects of passive immunity on growth and survival in the dairy heifer. *J. Dairy Sci.* 71: 1283-1287.
- Rose, R.M., Krueze, L.E., Holaday, J.W., Sulak, K.J., Johnson, C.E. 1972. Diurnal variation of plasma testosterone and cortisol. *J. Endocrinol.* 54: 177-181.
- Rosen, J.M., Wyszomierski, S.L., Hadsell, D. 1999. Regulation of milk protein gene expression. *Annu. Rev. Nutr.* 19: 407-436.
- Rosenfeld, P., Gutierrez, Y.A., Martin, A.M., Mallett, H.A., Alleva, E., Levine, S. 1991. Maternal regulation of the adrenocortical response in prevailing rats. *Physiol. Behav.* 4: 661-671.
- Rushen, J., Munksgaard, L., Marnet, P.G., DePassillé, A.M. 2001. Human contact and the effects of acute stress on cows at milking. *Appl. Anim. Behav. Sci.* 73: 1-14.
- Saad, A.M., Concha, C., Aström, G. 1989. Alterations in neutrophil phagocytosis and lymphocyte blastogenesis in dairy cows around parturition. *Zentralbl. Veterinarmed. B.* 36: 337-345.
- Salas, M.A., Evans, S.W., Levell, M.J., Whicher, J.T. 1990. Interleukin-6 and ACTH act synergistically to stimulate the release of corticosterone from adrenal gland cells. *Clin. Exp. Immunol.* 79: 470-473.
- Sangild, P.T., Diernæs, L., Christiansen, I.J., Skadhauge, E. 1993. Intestinal transport of sodium, glucose, and immunoglobulin in neonatal pigs. Effects of glucocorticoids. *Exp. Physiol.* 78: 485-497.
- Sartin, J.L., Kemppaninen, R.J., Cummins, K.A., Williams, J.C. 1988. Plasma concentrations of metabolic hormones in high and low producing dairy cows. *J. Dairy Sci.* 71: 650-657.
- Sasaki, M., Davis, C.L., Larson, B.L. 1977. Production and turnover of IgG1 and IgG2 immunoglobulins in the bovine around parturition. *J. Dairy Sci.* 59: 2046-2055.
- Schanbacher, F.L., Talhouk, R.S., Murray, F.A. 1997. Biology and origin of bioactive peptides in milk. *Livest. Prod. Sci.* 50: 105-123.
- Schäubli, M., Ritter, N., Hässig, M., Zerbe, H., Bleul, U., Boos, A. 2008. Progesterone receptors, estrogen receptor α and glucocorticoid receptors in the bovine intercaruncular wall around parturition. *Anim. Reprod. Sci.* 103: 215-227.
- Schmidt, M., Sangild, P.T., Blum, J.W., Andersen, J.B., Greve, T. 2004. Combined ACTH and glucocorticoid treatment improves survival and organ maturation in premature newborn calves. *Theriogenology* 61: 1729-1744.
- Schuler, G., Hartung, F., Hoffman, B. 1994. Investigations on the use of C-21 steroids as precursors for placental estrogen synthesis in the cow. *Exp. Clin. Endocrinol.* 102: 169-174.

Schuler, G., Özalp, G.R., Hoffman, B., Harada, N., Browne, P., Conley, A.J. 2006a. Reciprocal expression of 17 α -hydroxylase-C17,20-lyase and aromatase cytochrome P450 during bovine trophoblast differentiation: a two-cell system drives placental estrogen synthesis. *Reproduction* 131: 669-679.

Schuler, G., Tiechmann, U., Kowalewski, M.P., Hoffman, B., Madore, E., Fortier, M.A., Klisch, K. 2006b. Expression of cyclooxygenase-II (COX-II) and 20 α -hydroxysteroid dehydrogenase (20 α -HSD)/prostaglandin F-synthase (PGFS) in bovine placentomes: implications for the initiation of parturition in cattle. *Placenta* 27: 1022-1029.

Schwalm, J.W., Tucker, H.A. 1978. Glucocorticoids in mammary secretion and blood serum during reproduction and lactation and distributions of glucocorticoids, progesterone, and estrogens in fractions of milk. *J. Dairy Sci.* 61: 550-560.

Shafer-Weaver, K. A., Sordillo, L.M. 1997. Bovine CD8+ suppressor lymphocytes alter immune responsiveness during the postpartum period. *Vet. Immun. Immunopath.* 56: 53-64.

Shirley, J.E., Emery, R.S., Convey, E.M., Oxender, W.D. 1973. Enzymatic changes in bovine adipose and mammary tissue, serum and mammary tissue hormonal changes with initiation of lactation. *J. Dairy Sci.* 56: 569-574.

Shun, K. and Myatt, L. 2003. Enhancement of glucocorticoid induced 11 β -hydroxysteroid dehydrogenase type 1 expression in by proinflammatory cytokines in cultured human amnion fibroblasts. *Endocrinology* 144: 5568-5577.

Shutt, D.A., Fell, L.R. 1985. Comparison of total and free cortisol in bovine serum and milk or colostrum. *J. Dairy Sci.* 68: 1832-1834.

Simmons, C.R., Bergen, W.G., VandeHaar, M.J., Sprecher, D.J., Sniffen, C.J., Stanisiewski, E.P., Tucker, H.A. 1994. Protein and fat metabolism in cows given somavubove before parturition. *J. Dairy Sci.* 77: 1835-1847.

Sinowatz, F., Schams, D., Kölle, S., Plath, A., Lincoln, D. 2000. Cellular localization of GH receptor in the bovine mammary gland during mammogenesis, lactation, and involution. *J. Endocrinol.* 166: 503-510.

Smith, V.R., Erwin, E.S. 1959. Absorption of colostrum globulins introduced directly into the duodenum. *J. Dairy Sci.* 42: 364-365.

Smith, K.L., Muir, L.A., Ferguson, L.C., Conrad, H.R. 1971. Selective transport of IgG1 into the mammary gland: Role of estrogen and progesterone. *J. Dairy Sci.* 54: 1886-1894.

Smith, V.G., Edgerton, L.A., Hafs, H.D., Convey, E.M. 1973. Bovine serum estrogens, progestin's, and glucocorticoids during late pregnancy, parturition, and early lactation. *J. Anim. Sci.* 36: 391-396.

Smith, K.L., Schanbacher, F.L. 1973. Hormone induced lactation in the bovine. I. Lactational performance following injections of 17 β -estradiol and progesterone. *J. Dairy Sci.* 56: 738-743.

Sordillo, L.M., Redmond, M.J., Campos, M., Warren, L., Babiuk, L.A. 1991. Cytokine activity in bovine mammary gland secretions during the periparturient period. *Can. J. Vet. Res.* 55: 298-301.

Sordillo, L.M., Pighetti, G.M., Davis, M.R. 1995. Enhanced production of bovine tumor necrosis factor- α during the periparturient period. *Vet. Immun. Immunopath.* 49: 263-270.

- Staley, T.E., Corley, L.D., Bush, L.J., Jones, E.W. 2004. The ultrastructure of the neonatal calf intestine and absorption of heterologous proteins. *Anat. Rec.* 172: 559-579.
- Staples, R.E., Hansel, W. 1961. Luteal function and embryo survival in the bovine. *J. Dairy Sci.* 44: 2040-2048.
- Steele, P.A., Flint, A.P.F., Turnbull, A.C. 1976. Activity of steroid C-17,20 lyase in the ovine fetal placenta: effect of exposure to fetal glucocorticoid. *J. Endocrinol.* 69: 239-246.
- Stelwagen, K., Verkerk, G.A., Phipps, A.H., Matthews, L.R. 1997. Effect of cortisol on mammary tight junction (TJ) permeability in lactating dairy cows. *Livest. Prod. Sci.* 50: 39-40.
- Stewart, C.P., Albert-Recht, F., Osman, L.M. 1961. The simultaneous fluorimetric microdetermination of cortisol and corticosterone in plasma. *Clin. Chim. Acta* 6: 696-701.
- Stewart, H.J. 1983. Progesterone uptake and metabolism by the goat mammary gland during lactogenesis. *Brit. Vet. J.* 139: 61-67.
- Stormshak, F., Erd, R.E. 1960. Progestins in bovine corpora lutea, ovaries, and adrenals during pregnancy. *J. Dairy Sci.* 43: 310-320.
- Stott, G.H., Reinhard, E.J. 1978. Adrenal function and passive immunity in the dystocial calf. *J. Dairy Sci.* 61: 1457-1461.
- Stott, G.H., Marx, D.B., Menefee, B.E., Nightengale, G.T. 1979a. Colostral immunoglobulin transfer in calves. I. Period of absorption. *J. Dairy Sci.* 62: 1632-1638.
- Stott, G.H., Marx, D.B., Menefee, B.E., Nightengale, G.T. 1979b. Colostral immunoglobulin transfer in calves. II. The rate of absorption. *J. Dairy Sci.* 62: 1766-1773.
- Stott, G.H., Fleenor, W.A., Kleese, W.C. 1981. Colostral immunoglobulin concentration in two fractions of first milking postpartum and five additional milkings. *J. Dairy Sci.* 64: 459-465.
- Stott, G.H., Fella, A. 1983. Colostral immunoglobulin absorption linearly related to concentration for calves. *J. Dairy Sci.* 66: 1319-1328.
- Sud, S.C., Tucker, H.A., Meites, J. 1968. Estrogen-progesterone requirements for udder development in ovariectomized heifers. *J. Dairy Sci.* 51: 210-214.
- Takagi, M., Fujimoto, S., Ohtani, M., Miyamoto, A., Wijagunawardane, P.B., Acosta, T.J., Miyazawa, K., Sato, K. 2002. Bovine retained placenta: hormonal concentrations in fetal and maternal placenta. *Placenta* 23: 429-437.
- Takeishi, M., Shibata, S., Tsumagari, S. 1989. Adrenocorticotropin and cortisol levels in the plasma of bovine fetuses and neonates. *Jpn. J. Vet. Sci.* 51: 975-980.
- Taverne, M.A.M., Bevers, M.M., van der Weyden, G.C., Dieleman, S.J., Fontijne, P. 1988. Concentration of growth hormone, prolactin, and cortisol in fetal and maternal blood and amniotic fluid during late pregnancy and parturition in cows with cannulated fetuses. *Anim. Reprod. Sci.* 17: 51-59.
- Taverne, M.A.M., de Schwartz, N.C.M., Kankofer, M., Bevers, M.M., van Oord, H.A., Schams, D., St. Gutzjahr, van der Weijden, G.C. 2001. Uterine responses to exogenous oxytocin before and after parturition luteolysis in the cow. *Reprod. Dom. Anim.* 36: 267-272.

- Termeulen, S.B., Butler, W.R., Natzke, R.P. 1981. Rapidity of cortisol transfer between blood and milk following adrenocorticotropin injection. *J. Dairy Sci.* 64: 2197-2200.
- Toofanian, F. Hill, F.W.G., Kidder, D.E. 1973. The mucosal disaccharidases in the small intestine of the calf. *Ann. Rech. Vétér.* 4: 57-69.
- Trahair, J.F., Robinson, P.M. 1989. Enterocyte ultrastructure and uptake of immunoglobulins in the small intestine of the neonatal lamb. *J. Anat.* 166: 103-111.
- Tuboly, S., Bernáth, S., Glávits, R., Medveczky, I. 1988. Intestinal absorption of colostral lymphoid cells in newborn piglets. *Vet. Immun. Immunopath.* 20: 75-85.
- Tucker, H.A., Larson, B.L., Gorski, J. 1971. Cortisol binding in cultured bovine mammary cells. *Endocrinology* 89: 152-159.
- Tucker, H.A. 1981. Physiological control of mammary growth, lactogenesis, and lactation. *J. Dairy Sci.* 64: 1403-1421.
- Van Kampen, C., Mallard, B.A. 1997. Effects of peripartum stress and health on circulating bovine lymphocyte subsets. *Vet. Immun. Immunopath.* 59: 79-91.
- Van Reenen, C.G., Van der Werf, J.T.N., Bruckmaier, R.M., Hopster, H., Engel, B., Noordhuizen, J.P.T.M., Blokhuis, H.J. 2002. Individual differences in behavioral and physiological responsiveness of primiparous dairy cows to machine milking. *J. Dairy Sci.* 85: 2551-2561.
- Wagner, W.C., Oxenreider, S.L. 1972. Adrenal function in the cow. Diurnal changes and the effects of lactation and neurohypophyseal hormones. *J. Anim. Sci.* 34: 630-635.
- Warner, R.G., Flatt, W.P., Lossli, J.K. 1956. Dietary factors influencing the development of the ruminant stomach. *Agric. Food Chem.* 4:788-801.
- Watson, D.L., Brandon, M.R., Lascelles, A.K. 1972. Concentrations of immunoglobulin in mammary secretion of ruminants during involution with particular reference to selective transport of IgG1. *Aust. J. Exp. Biol. Med. Sci.* 50: 535-539.
- Weber, P.S.D., Toelboell, T., Chang, L., Tirrell, J.D., Saama, P.M., Smith, G.W., Burton, J.L. 2004. Mechanisms of glucocorticoid-induced down-regulation of neutrophil L-selectin in cattle: Evidence for effects at the gene-expression level and primarily on blood neutrophils. *J. Leuk. Biol.* 75: 815-827.
- Wendorf, G.L., Lawyer, M.S., First, N.L. 1983. Role of the adrenals in the maintenance of pregnancy in cows. *J. Reprod. Fertil.* 68: 281-287.
- Wesselink, R., Stafford, K.J., Mellor, D.J., Todd, S., Gregory, N.G. 1999. Colostrum intake by dairy calves. *New Zeal. Vet. J.* 47: 31-34.
- Whitaker, S.M., Jeffrey, S.L., Willett, L.B., Borger, D.C, Neiswander, R.L., Schanbacher, F.L., Weiss, W.P. 1996. The effect of cortisol and time of first feeding on immunoglobulin absorption in Holstein calves. *Anim. Sci. Res. Rev.* 156: 87-92.
- Willett, L.B., Erb, R.E. 1972. Short term changes in plasma corticoids in dairy cattle. *J. Anim. Sci.* 34: 103-111.

- Williams, P. 1993. Immunomodulating effects of intestinal absorbed maternal colostral leukocytes by neonatal pigs. *Can. J. Vet. Res.* 57: 1-8.
- Winger, K., Gay, C.C. 1995. Immunoglobulin G₁ transfer into induced mammary secretions: the effect of dexamethasone. *J. Dairy Sci.* 78: 1306-1309.
- Wischrall, A., Verreschi, I.T.N., Lima, S.B., Hayashi, L.F., Barnabe, R.C. 2001. Pre-parturition profile of steroids and prostaglandin in cows with or without fetal membrane retention. *Anim. Reprod. Sci.* 67: 181-188.
- Woodward, T.L., Beal, W.E., Akers, R.M. 1993. Cell interactions in initiation of mammary epithelial proliferation by estradiol and progesterone in prepubertal heifers. *J. Endocrinol.* 136: 149-157.
- Yang, K., Fraser, M., Yu, M., Krkosek, M., Challis, J.R.G., Lamming, G.E., Campbell, L.E., Darnel, A. 1996. Pattern of 11 β -hydroxysteroid dehydrogenase type 1 messenger ribonucleic acid expression in the ovine uterus during the estrous cycle and pregnancy. *Biol. Reprod.* 55: 1231-1236.
- Zhou, D., Kusnecov, A.W., Shurin, M.R., DePaoli, M., Rabin, B.S. 1993. Exposure to physical and psychological stressors elevates plasma interleukin-6: relationship to the activation of hypothalamic-pituitary-adrenal axis. *Endocrinology* 133: 2523-2530.

APPENDIX A
SUPPLEMENTAL TABLES FOR CHAPTER III

Table A.1. Plasma cortisol concentration (ng/ml) in Holstein cows before and after AM and PM milkings¹.

Cow ID	AM Before	AM After	PM Before	PM After
401	42.6	56.7	60.6	68.2
402	47.3	31.7	44.9	72.6
403	123.2	97.1	113.1	63.8
513	73.7	85.1	65.0	75.3
514	19.9	59.8	29.5	70.8
520	62.6	52.9	33.6	40.2
524	22.0	46.1	24.1	51.1
528	77.4	37.5	59.5	40.2
604	57.5	84.3	49.7	113.3
607	87.2	110.1	58.2	118.6
610	51.1	125.8	98.3	55.4
611	80.4	65.6	63.6	-
613	65.5	84.4	35.0	57.3
618	66.6	60.1	43.5	134.5
622	112.4	125.6	120.1	185.1
624	69.8	55.9	65.0	91.1
627	80.0	101.9	32.3	57.6
629	34.6	53.4	38.8	39.7
633	69.4	68.5	94.4	106.9
635	103.1	96.2	64.8	71.9
639	51.3	103.3	44.0	66.2
718	39.4	57.4	45.2	74.7
723	37.0	55.1	319.6	252.1
767	72.1	102.1	51.5	81.1
Mean±SEM	64.4±5.4 ^a	75.7±5.5 ^b	69.0±12.1 ^a	86.4±10.4 ^b

¹The AM and PM milkings were conducted at 0200 and 1400 hours.

^{a,b}Different superscripts indicate a significant difference ($P<0.05$).

Table A.2. Milk cortisol concentration (ng/ml) in Holstein cows before and after AM and PM milkings¹.

Cow ID	AM Before	AM After	PM Before	PM After
401	3.5	6.5	4.9	4.2
402	16.5	6.3	8.4	6.6
403	8.6	16.5	4.8	9.2
513	10.8	7.5	7.5	17.4
514	5.7	4.8	9.7	5.4
520	1.1	7.1	6.1	5.7
524	10.7	6.1	12.5	2.9
528	12.3	13.8	2.9	9.8
604	11.6	9.7	9.2	5.7
607	3.7	19.6	17.4	18.3
610	25.1	12.9	6.7	8.0
611	8.8	11.7	11.3	12.2
613	8.8	13.8	7.6	8.1
618	9.4	17.8	12.6	17.8
622	16.3	20.3	7.4	19.3
624	10.4	22.6	16.4	10.4
627	15.2	21.8	11.3	17.9
629	16.7	17.0	20.7	12.8
633	25.3	23.1	11.0	21.9
635	13.4	23.5	13.4	14.3
639	28.3	33.1	15.1	14.2
718	20.4	26.7	10.3	18.2
723	9.7	15.5	40.1	33.0
767	11.8	12.5	4.3	4.0
Mean±SEM	12.7±1.4	15.4±1.5	11.3±1.5	12.4±1.5

¹The AM and PM milkings were conducted at 0200 and 1400 hours.

APPENDIX B

SUPPLEMENTAL TABLES FOR CHAPTER IV

Table B.1. Plasma cortisol concentration (ng/ml) in Holstein cows before and after parturition.

Cow ID	Hours prepartum			Hours postpartum				
	72	48	24	3	6	12	24	48
29	30.4	11.3	11.9	26.2	30.1	24.8	14.8	22.0
104	0.0	1.6	5.4	28.3	3.1	5.6	4.8	3.6
401	0.0	18.1	5.5	69.5	12.5	17.0	1.8	0.0
529	9.4	32.7	15.6	24.8	89.7	16.6	0.0	26.8
604	0.0	18.1	11.3	17.1	0.0	16.5	8.9	13.9
610	-	4.9	1.8	47.2	5.6	0.0	0.0	0.0
622	9.5	5.7	8.6	11.6	21.9	27.0	18.0	34.8
629	-	21.9	6.5	45.9	7.7	2.7	12.3	8.9
635	5.3	0.0	0.0	0.0	0.0	0.0	10.6	18.1
639	12.7	59.6	5.1	66.8	2.8	1.2	0.0	18.5
709	2.9	13.6	12.3	20.1	7.3	0.8	2.6	3.1
712	27.4	0.0	0.0	29.4	7.5	14.4	9.0	0.0
720	0.0	0.0	0.0	19.6	0.0	4.8	0.0	0.0
725	0.0	0.0	0.0	17.1	0.0	0.0	0.0	0.0
726	0.0	0.0	0.0	13.8	0.0	11.8	0.0	0.0
728	0.0	4.8	0.0	0.3	0.0	3.3	29.9	16.0
731	-	1.1	0.0	20.8	0.0	3.3	0.0	0.0
733	-	0.0	1.8	15.9	0.0	0.0	4.3	0.0
737	0.0	0.0	0.0	42.6	2.0	2.4	0.0	0.0
802	-	4.2	7.8	60.3	0.0	0.0	0.0	0.0
Mean±SEM	6.5±2.6 ^a	9.9±3.3 ^a	4.7±1.1 ^a	28.9±4.5 ^b	9.5±4.6 ^a	7.6±2.0 ^a	5.9±1.8 ^a	8.3±2.4 ^a

^{a,b}Different superscripts indicate a significant difference ($P<0.05$).

Table B.2. Milk cortisol concentration (ng/ml) in Holstein cows before and after parturition.

Cow ID	Hours prepartum			Hours postpartum				
	72	48	24	3	6	12	24	48
29	3.9	6.3	7.0	11.6	12.6	7.5	3.9	5.7
104	2.9	2.0	7.5	10.2	2.9	5.3	1.7	2.8
401	2.2	7.2	9.6	19.3	16.7	6.8	2.5	4.7
529	2.8	3.9	5.1	11.5	11.1	9.3	8.5	3.6
604	4.2	2.8	2.7	13.9	6.5	8.3	8.0	10.3
610	-	2.8	5.2	8.7	3.6	3.8	2.0	1.2
622	3.1	5.0	7.5	7.1	5.5	3.7	3.0	4.5
629	-	3.2	3.7	8.3	5.8	5.7	4.5	1.6
635	6.4	4.2	4.1	9.3	3.0	2.7	5.2	6.2
639	5.0	7.2	1.0	8.5	23.5	14.1	7.5	2.4
709	1.1	2.6	1.2	1.7	3.6	2.8	2.4	2.4
712	3.1	3.9	4.3	2.3	12.4	4.4	3.4	3.3
720	1.6	2.5	1.9	1.9	5.2	2.1	2.6	1.5
725	4.1	1.3	2.4	7.8	2.9	2.6	1.8	2.3
726	2.8	1.4	2.7	11.3	3.4	4.6	2.4	3.4
728	3.2	2.6	4.3	18.8	6.2	6.4	4.8	4.0
731	-	2.7	1.9	15.8	4.2	5.5	2.8	3.6
733	-	4.9	5.5	11.2	4.6	4.3	3.5	2.9
737	3.7	3.2	2.1	28.6	9.0	2.3	4.3	2.6
802	-	6.3	5.2	16.9	4.3	4.5	5.2	4.1
Mean±SE	3.3±0.3	3.8±0.4	4.2±0.5	11.2±1.4	7.4±1.2	5.3±0.6	4.0±0.5	3.7±0.5
M	a	a	a	b	b	a	a	a

^{a,b}Different superscripts indicate a significant difference ($P<0.05$).

Table B.3. Plasma cortisol concentration (ng/ml) in Holstein calves after parturition.

Calf ID	Hours postpartum				
	3	6	12	24	48
2B	276.8	41.8	24.2	89.7	51.3
3B	154.1	78.4	76.3	82.4	67.6
4B	232.5	141.2	83.0	31.6	134.1
5B	395.0	270.5	164.0	127.9	89.1
6B	204.6	48.9	58.2	51.6	34.7
915	247.5	0.0	24.7	114.3	28.6
917	243.9	40.0	57.8	69.4	45.8
918	124.9	25.1	15.1	65.4	47.7
919	97.1	22.1	20.9	37.6	54.6
920	280.6	120.4	61.9	97.4	88.9
925	294.6	135.9	170.2	159.8	51.6
926	401.2	48.6	78.0	13.0	6.1
929	279.6	38.4	105.0	60.6	80.1
930	219.0	119.6	42.8	35.0	68.2
931	314.7	64.1	43.8	54.5	40.1
932	236.9	63.1	52.0	77.3	52.7
937	360.6	110.6	98.5	152.8	172.2
941	216.0	65.5	81.8	139.8	61.3
942	184.5	82.3	39.6	73.6	36.1
945	397.6	141.5	140.0	90.4	1.9
Mean±SEM	258.1±19.4 ^a	82.9±13.7 ^b	71.9±10.1 ^b	81.2±9.2 ^b	60.6±8.8 ^b

^{a,b}Different superscripts indicate a significant difference ($P<0.05$).

Table B.4. Plasma IgG concentration (ng/ml) in Holstein calves after parturition.

Calf ID	Hours postpartum				
	3	6	12	24	48
2B	120	2600	5000	5000	5000
3B	120	1600	3200	5000	3700
4B	120	960	1900	4400	2250
5B	120	800	1600	2600	2250
6B	120	1150	2600	4400	2600
915	120	1350	5000	5000	5000
917	120	2250	3700	5000	4400
918	120	680	1900	1350	1350
919	120	1900	2600	3200	3200
920	120	570	680	800	960
925	120	1150	2250	2600	2250
926	120	680	1600	2250	3200
929	120	1350	1350	2250	1900
930	120	1900	3200	3700	2600
931	120	1150	1600	2250	1600
932	400	960	1350	1350	1900
937	120	800	1600	2250	3200
941	120	3200	2600	3700	2600
942	120	800	1150	960	1150
945	120	270	1600	2250	3200
Mean±SEM	134±14 ^{a,c}	1306±165 ^{b,c}	2324±266 ^{b,d}	3015±318 ^{a,d}	2715±261 ^{b,d}

^{a,b,c,d} Different superscripts indicate a significant difference ($P<0.05$).

VITA

Dana Nadine Wooley was born in February 1986, in Shreveport, Louisiana, to parents David Wooley and Kathern Davis. Dana is an only child and she was raised in Many, Louisiana. Dana graduated from Many High School in May of 2004. She then attended Louisiana State University in Baton Rouge, Louisiana, and graduated in May of 2008 with a bachelor's degree in animal science. After completing Dr. Robert Godke's undergraduate course in reproductive physiology, she acquired a passion for research in assisted reproductive technologies. She immediately enrolled in August of 2008 into the master's program within the School of Animal Science under the direction of Professor Robert A. Godke. She will earn her master's degree in reproductive physiology in December of 2010.