# EFFECTS OF DEXAMETHASONE ON ENDOCRINE AND OVARIAN FUNCTION IN DAIRY CATTLE

By

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## NOMENCLATURE

| | |
|---|---|
| ACTH | adrenocorticotropic hormone |
| BW | body weight |
| BCS | body condition score |
| CL | corpus luteum |
| CRH | corticotropin releasing hormone |
| CRF | corticotropin releasing factor |
| DF | dominant follicle |
| d | days |
| dl | deciliter |
| E2 | estradiol |
| FSH | follicle stimulating hormone |
| GH | growth hormone |
| GLM | general linear model |
| GnRH | gonadotropins releasing hormone |
| HDL | high density lipoproteins |
| HSD | hydroxysteroid dehydrogenase |
| HPAA | hypothalamic-pituitary-adrenal axis |
| IGF | insulin-like growth factor |
| IGFBP | insulin-like growth factor binding protein |

| | |
|---|---|
| kg | kilogram |
| kDa | kilodalton |
| LDL | low density lipoproteins |
| LLC | large luteal cells |
| mg | milligrams |
| ml | milliliter |
| mm | millimeters |
| mRNA | messenger RNA |
| ng | nanogram |
| pg | picograms |
| PGF | prostaglandins |
| P4 | progesterone |
| RIA | radioimmunoassay |
| SCC | side chain cleavage |
| SLC | small luteal cells |

# CHAPTER I

## INTRODUCTION

The ability of a dairy cow to have adequate (re) productive performance depends on a number of factors that somehow condition its capability to produce one calf per year and maintain milk production at the desired levels. In order to achieve this required performance, the dairy cow must have regular estrous cycles, which average 21 d, ranging from 14 to 29 d in length (Hafez and Jainudeen, 1993). Also, the postpartum period should not go beyond 100 d. Estrous cycles and, consequently, follicular development and ovulation are regulated by a series of internal factors such as gonadotropins and growth factors that act as an interdependent network conditioned by exogenous factors such as nutrition and environmental temperature.

One group of hormones that has been shown to prolong the length of the estrous cycle in cows is glucocorticoids (Li & Wagner, 1983; Roussel *et al.*, 1983). The predominant glucocorticoid, cortisol, is secreted by the adrenal gland in response to stress and has a physiologically important role in the organism. Very often high producing dairy cows have prolonged postpartum anestrous intervals, due to stress of lactation, negative energy balance and probably other environmental factors causing economic losses by decreasing its reproductive efficiency. However, very few studies evaluated the role of glucocorticoid on the reproductive and endocrine function of the dairy cow. Therefore,

this study attempts to clarify the function of glucocorticoids on endocrine and ovarian function of the non-lactating dairy cows.

# CHAPTER II

# REVIEW OF LITERATURE

## CHANGES IN OVARIAN FUNCTION DURING ESTROUS CYCLE

In cattle, each estrous cycle is characterized by a series of follicular waves. Each follicular wave comprises the growth of about 20 to 30 antral follicles with only one follicle ovulating at the end of each cycle. Although the remaining follicles normally undergo atresia, a relatively high percentage of these follicles have the capacity to ovulate and release a fertilizable oocyte (Fortune, 1993; Fortune *et al.*, 1991; D'Occhio *et al.*, 1999). In cows and mares, an estrous cycle comprises about 2 to 4 follicular waves (Roche *et al.*, 1998), with 3 waves in about 45.2% (10 to 80%) and 2 waves in 52.5% (15 – 90%) of the bovine cycles (Hamilton, 1995). The number of follicular waves depends on the life span of the corpus luteum with each follicular wave lasting about 5 to 10 d (Fortune, 1993).

A number of factors are related with alterations of follicular dynamics in cattle, such as negative energy balance during lactation, reproductive stage (postpartum and nonlactating), dietary fat and others. Genotype of cattle influences the number and size of follicles and corpus luteum (CL), apparently due to different endocrine patterns of hormone secretion such as follicle-stimulating hormone (FSH), insulin, insulin-like growth factor (IGF) -1 and growth hormone (GH), but not luteotropic hormone (LH),

IGF-II, or insulin-like growth factor binding protein (IGFBP) -2 or –3 (Alvarez *et al.*, 2000). Negative energy balance reduces luteal activity in cows with high milk yield (Spicer *et al.*, 1990; Lucy *et al.*, 1992) and might be associated with decreased IGF-I concentrations in serum (Spicer *et al.*, 1990). In beef heifers, the diameter and persistence of dominant follicles during an estrous cycle is reduced due to low dietary intake while the proportion of estrous cycles with 3 dominant follicles tend to increase (Murphy *et al.*, 1991).

This section of review will first describe the timing and later the morphological and endocrine changes that take place during follicular development in the estrous cycle of the cow.

**Growth and Development of Ovarian Follicles**

Follicular growth is a dynamic process that starts in the fetal ovary with the non-growing pools of primordial follicles that develop into primary follicles. This process is characterized by a change in the morphology of the granulosa cells, from flat cells to cuboidal shape, and an increase in the oocyte's size (Ireland and Roche, 1983; Fortune, 1994; Ginther *et al.*, 1996; Roche *et al.*, 1998).

Different physiological processes, comprised by the emergence of a wave, selection of a dominant follicle (DF), dominance phase and ovulation or atresia of the DF, are involved in the development of a follicular wave (Campbell *et al.*, 1995; Ginther *et al.*, 1996; Roche *et al.*, 1998). Just before selection of the dominant follicle takes place, there is a deviation period, characterized by the beginning of the greatest difference in

growth rates between the two largest follicles, or by the maximum diameter attained by the second-largest follicle (Ginther *et al.*, 1996). These stages of folliculogenesis are controlled by the hypothalamo-pituitary-ovarian feedback system, intrafollicular steroids, growth factors, extraovarian factors and a complex network system that establish the relationships among these factors (Campbell *et al.*, 1995).

In cattle, follicular waves begin before puberty and continue during postpartum anestrus and early pregnancy (Roche *et al.*, 1998). A follicular wave is characterized by a synchronous development of a group of large antral follicles (Knopf *et al.*, 1989), during which one is selected to continue growth and becomes dominant by inhibiting growth of the remaining follicles (Sirois and Fortune, 1988). The frequency of two- and three-follicular waves in the population is about 50%, while less than 10% of the population has a four-wave cycle (Sirois and Fortune, 1988; Savio *et al.*, 1988; Ginther *et al.*, 1989a; Knopf *et al.*, 1989). In a two-wave cycle, the first follicular wave begins on d 0 of a 21-d cycle and the second-wave on d 10 of the estrous cycle; in a three-wave cycle, the first wave occurs between d 0 and 4, the second on d 10 and the third on d 16 of the estrous cycle; in a four-wave cycle, the first wave begins on d 2, the second on d 8, the third on d 14 and the fourth on d 17 of a 23-d estrous cycle (Sirois and Fortune, 1988; Savio *et al.*, 1988; Ginther *et al.*, 1989a; Knopf *et al.*, 1989). The number of follicular waves is determined by the length of the luteal phase (Ginther *et al.*, 1989a), which in turn is influenced by a variety of factors such as lactation, energy balance and dietary fat (Lucy *et al.*, 1992).

The emergence of a follicular wave is associated with the presence of a cohort of growing follicles, between 2.5 and 5 mm in size, concomitantly with a peak in FSH

concentration (Adams *et al.*, 1992a, Sunderland *et al.*, 1994, Gong *et al.*, 1995, Bodensteiner *et al.*, 1996a; Roche *et al.*, 1998). Follicles < 3.5 mm size are low in estrogen activity with a high percentage of apoptotic granulosa cells, and follicles > 4 mm size are estrogen-active with low percentage of apoptotic granulosa cells (Roche *et al.*, 1998). Wave emergence, also designated as d 0, is defined as the last day that the dominant follicle was 4 mm (Ginther *et al.*, 1996). The follicle wave emergence is a dynamic process occurring over 2 to 4 d with progressive movement of only healthy follicles from smaller (3 mm) to larger size classes, involving an average of 24 growing follicles, until reaching the ovulatory quota, when selection ends (Bergfelt *et al.*, 1994; Roche *et al.*, 1998). These follicles continue their growth to 4, 5 and ≥ 6 mm size, meaning that the cohort emergence and subsequent selection is a continuous and dynamic process occurring very early in a wave (Bergfelt *et al.*, 1994; Roche *et al.*, 1998). Each wave is characterized by the presence of one or two dominant follicles attaining about 15 mm in diameter and several subordinate follicles, with the largest of these reaching 7 to 9 mm size (Ginther *et al.*, 1989b).

**Follicular Selection and Dominance**

Selection is defined by a deviation of ≥ 2 mm in the growth of the selected DF concomitantly with the cessation of growth of the subordinate follicles in the cohort and a decrease in aromatase activity and intrafollicular estrogen: progesterone ratio (Sunderland *et al.*, 1994; Stewart *et al.*, 1996). The dominant follicle and the second largest follicle gradually begin differing in size between D 0 and 4; the period when they reach their maximum difference in growth rate is known as the time of deviation or

selection. Follicle deviation or selection is the time when the greatest difference between the two largest follicles begins or when the second-largest follicle reaches its maximum size (Ginther *et al.*, 1996) and when FSH reaches about its basal levels (Ginther *et al.*, 1997). In cattle, follicle deviation starts when the largest follicle reaches about 8.5 mm and it comprises the continued growth of the largest follicle in a follicular wave and a reduction or cessation of growth of the smaller follicles (Ginther *et al.*, 1999). During deviation, there is a several-fold increase in the DF intrafollicular estradiol concentrations, while it decreases several-fold in the subordinate follicles (Stewart *et al.*, 1996; Mihm *et al.*, 1997). Compared with preselection of follicles on d 3, subordinate follicles on d 5 and dominant follicles have similar in intrafollicular IGF-I concentrations, but have much lower total IGF-I binding activity due to decreased amounts of IGFBP -2, -4 and –5 (Stewart *et al.*, 1996; Mihm *et al.*, 1997; Roche *et al.*, 1998). After d 2 of the estrous cycle, within the cohort of the preselection follicles, or when the largest follicle reaches about ≥ 8.5 mm in diameter (Ginther *et al.*, 1999), there is a decline in FSH concentrations consequently causing alteration in growth factors dependent on FSH and other hormones, inducing growth and stimulating the estradiol-producing capacity of the DF and atresia of the subordinate follicles (Mihm *et al.*, 1997; Ginther *et al.*, 1999). Thus, the deviation mechanism in the selection process can be established in less than 8 h, or before the second largest follicle reaches a similar critical size (≥ 8.5 mm), by a close temporal coupling between change in FSH concentrations and the follicular response (Ginther *et al.*, 1999). Additionally, during the process of follicular selection LH receptor mRNA is expressed in the granulosa cells, contrary to the follicular recruitment phase

that is associated with mRNAs expression for $P450_{scc}$ and $P450_{arom}$ in granulosa cells of follicles (Bao *et al.*, 1997).

The selected dominant follicle has size advantage over the second largest follicle, which normally grows at a slower rate and is not the first reaching the decisive stage. This difference in growth rates and subsequent biologic selection seems to occur on or before the day of emergence, with no significant difference between growth rates until deviation begins (Ginther *et al.*, 1996). The size and persistence of dominant follicles during estrous cycles of beef heifers are decreased by low dietary intake while the proportion of estrous cycles with 3 dominant follicles tend to increase (Murphy *et al.*, 1991). The emergence of a new follicular wave with a transient increase in FSH concentrations is morphologically characterized by the loss in dominance (Adams *et al.*, 1992; Sunderland *et al.*, 1994) and subsequently atresia of the DF associated with the accumulation of IGFBP-2, -4 and –5 and a decrease in aromatase activity (Monget and Monniaux, 1995; Stewart *et al.*, 1996; Manikkam and Rajamahendran, 1997). The dominance phase is characterized by an increase in the bioavailability of IGFs with a marked reduction in the intrafollicular concentrations of IGFBP-2, -4 and –5 (Monget and Monniaux, 1995; Stewart *et al.*, 1996), and an increase in the number of LH receptors first in the thecal cells and then in the granulosa cells (Stewart *et al.*, 1996).

**Growth and Development of the Corpus Luteum (CL)**

The ovulatory follicle, originating from the last follicular wave, gives rise to the CL (Ginther *et al.*, 1996). The CL is formed as a result of morphological and functional

transformation of the cells from theca and granulosa follicle layers. Large luteal cells are derived from granulosa cells while small luteal cells are derived from theca interna cells (Leymarie and Martal, 1993; Senger, 1997; Niswender *et al.*, 2000). Both of these cells compose about 50% of the corpus luteum bearing steroidogenic activity (Spicer *et al.*, 1981), in which progesterone secretion is the primary steroid produced by both of these cells after luteinization through low-density lipoprotein (LDL), high-density lipoprotein (HDL) and hydrolysis of stored cholesterol esters (Niswender *et al.*, 2000). During early luteal development, an increase in numbers of LH receptors appears to be more associated with an increase in progesterone secretion, while adenylate cyclase activity seems to be more related to reduced function of the CL (Spicer *et al.*, 1981). Although the steroidogenic cells seldom multiply after ovulation, their luteal function depends on the number of luteal cells, the hormonal environment in which this ovulatory follicle developed and on the degree to which the CL becomes vascularized (Leymarie and Martal, 1993; Senger, 1997; Niswender *et al.*, 2000). In sheep, large luteal (LLC) derived from granulosa cells double their size without an increase in numbers, while numbers of small luteal cells (SLC) derived from theca cells increase 5-fold (Farin *et al.*, 1986; Niswender *et al.*, 2000). Steroidogenic individual capacity of these luteal cells and their ability to respond to LH increases throughout luteal development (Niswender, 2000). However in the bovine LLC, physiological levels of LH do not increase secretion of progesterone (Alila *et al.*, 1988). During the mid-luteal phase of the estrous cycle in the ewe, LLC are reported to produce > 80% of the total progesterone secretion (Niswender *et al.*, 1985). Ultrasonographic studies of the CL, report a maximum rate of luteal growth occurring between d 3 and 4, with an increase in the luteal tissue area from d 2 to 5 after

ovulation (Kot and Ginther, 1999). Energy balance (Spicer *et al.*, 1990) and the amount of dietary fat (Spicer *et al.*, 1993; Williams, 1996) can influence the amount of progesterone produced by the CL.

Normally the corpus luteum has a lifespan of 12 to 21 d depending on the species (Leymarie and Martal, 1993). In ruminants, the lifespan of the CL is about 15 to 17 d after which it undergoes luteolysis (Leymarie and Martal, 1993; Senger, 1997). Luteolysis occurs at the end of luteal phase during a 1- to 3-d period, with a decrease in the number and size of the LLC preceded by a decrease in the number of small steroidogenic luteal cells (Gemmell *et al.*, 1976; Braden *et al.*, 1988; Niswender *et al.*, 2000). Successful luteolysis requires communication between the CL and the uterine endometrium (Senger, 1997).

**Endocrine Modulation**

Several hormones play a role in the development of follicular waves (recruitment, selection and dominance of follicles); ovulatory follicles and subsequently the CL. Hormones responsible for the regulation of the cow's estrous cycle include gonadotropins and steroids. IGF-I and –II, and IGFBPs are also known to have a role in follicular growth of cattle. For example, greater plasma IGF-1 concentrations in Brahman versus Angus females are associated with greater numbers of antral follicles (Simpson *et al.*, 1994; Alvarez *et al.*, 2000).

**Changes in Gonadotropins**

Gonadotropins, FSH and LH, have a major role in regulating follicular development, by stimulating ovarian steroidogenesis in granulosa and theca cells through activation of cAMP (Hsueh *et al.*, 1984), which results in an increase in steroid concentrations, mainly estradiol-17β. Enhanced capacity of follicle estradiol production, essential in the process of differentiation and dominance, is a typical endocrine response from selected follicles (Fortune, 1994; Stewart *et al.*, 1996; Roche *et al.*, 1998). Gonadal steroids act at the hypothalamic and pituitary levels to modulate the release of FSH and LH through negative and positive feedback mechanisms (Farnworth, 1995; McCann *et al.*, 1998; Roche *et al.*, 1998).

As mentioned earlier, the new wave emergence and growth of antral follicles is closely related to a rise in plasma FSH concentrations (Ginther *et al.*, 1996; Bodensteiner *et al.*, 1996b; Gong *et al.*, 1995; Sunderland *et al.*, 1994; Roche *et al.*, 1998). In a typical follicular wave, the cohort of 3-mm growing follicles first appears during the incline of FSH and continues until its decline to basal levels (Ginther *et al.*, 1996). When the future dominant follicle (DF) reaches a 4 mm size there is a peak in the surge of FSH (Bergfeld *et al.*, 1996). FSH concentrations in follicular fluid, measured at 15 min intervals, increase from pre- to post-aspiration of the DF, with overall means increasing from 1.1 ± 0.7 to 1.8 ± 0.9, 1.7 ± 0.9 to 2.9 ± 0.7 and 0.8 ± 0.8 to 1.9 ± 0.9 ng/ml following aspiration during early-, mid- and late-luteal stages, respectively (Amiridis *et al.*, 1999). FSH concentrations increase about 8 h before and after follicular emergence, and decrease in 8 to 40 h after follicular emergence. In plasma, amplitude and frequency of LH pulses increase between the hour of emergence and 24 h after emergence, while the

average plasma LH concentration increases after 32 h of emergence. This means that FSH must be at minimal levels and LH must be already available to facilitate follicle deviation (Ginther *et al.*, 1998). Prior to and after DF aspiration, plasma LH does not vary with the cycle stage nor does DF aspiration have a significant effect on plasma LH; during early-, mid- and late-luteal stages plasma LH concentrations are 1.6 ± 0.4, 1.7 ± 0.4 and 1.8 ± 0.6 ng/ml, respectively (Amiridis *et al.*, 1999). During the period of follicle selection, between d 0 and 5, plasma LH concentrations vary from 5 to 9 ng/ml (Beck *et al.*, 1976). During the preovulatory period, pulses of LH increase from 1 or 2 pulses per 6 h during the mid luteal phase to 3 to 8 pulses per 6 h during late follicular phase, which may play a role in the selection of the ovulatory follicle (Spicer and Echternkamp, 1986). At ovulation in cattle, frequency of LH pulses increase to 1 pulse/h (Roche *et al.*, 1998). In support of the latter statement, during follicle deviation there is an increase in systemic concentrations of LH that starts between the beginning of deviation and 32 h after deviation (Kulick *et al.*, 1999).

At the cellular level, ovine follicles begin expressing FSH receptor mRNA within their just 2 layers of granulosa cells (Tisdall *et al.*, 1995). In the bovine preantral follicles, FSH receptors also present within the follicles with just 2 layers granulosa cells increase their numbers at early antral stage (Wandji *et al.*, 1992), do not change during the deviation period in the dominant follicle (Xu *et al.*, 1995; Bodensteiner *et al.*, 1996a,b). However in the granulosa cells of the dominant follicles expression of mRNA for FSH receptors is highest on d 3 of the follicular wave (Evans and Fortune, 1997). Between d 2 and 4 of the follicular wave, during follicular deviation there is a great increase in LH receptor mRNA and binding of LH in granulosa and thecal cells of dominant follicles

(Ireland and Roche, 1983; Xu *et al.*, 1995; Bodensteiner *et al.*, 1996b; Bao *et al.*, 1997; Evans and Fortune, 1997), although granulosa cells LH receptors do not increase in dominant follicles until after d 5 (Stewart *et al.*, 1996). Since FSH receptors do not change at the deviation period, this means that the future dominant follicle might be the first one to reach a decisive stage at which LH receptors are expressed in its theca cells (Stewart *et al.*, 1996).

Therefore, the development of the dominant follicle is, in part, driven by an increase in LH and decrease in FSH concentrations, processes that are influenced by estradiol (Ginther, 2000) and inhibin secreted by the dominant follicle (Amiridis *et al.*, 1999). The dominant follicle, which already has receptors for FSH and LH on its granulosa cells, apparently switches from FSH to LH dependency and the frequency of LH pulses determine its final fate, by acquiring or not the estrogen producing capacity (Roche *et al.*, 1998). Loss of dominance is characterized by loss of FSH and LH receptors and estrogen producing capacity (Ireland and Roche, 1983; Badinga *et al.*, 1992) accompanied by the progesterone-induced decrease in LH pulse frequency (Rahe *et al.*, 1980; Savio *et al.*, 1993; Cupp *et al.*, 1995).

**Changes in Steroid**

Estradiol concentrations in plasma increase 2- to 6-fold above the mid-luteal phase levels at the time of estrus in cattle (Wettemann *et al.*, 1972; Echternkamp and Hansel, 1973). Around the time of deviation (d 0 to 5 of the 1$^{st}$ follicular wave of the estrous cycle) and during its subsequent growing phase (d 6 to 9 of the first follicular wave of the estrous cycle), the dominant follicle produces and releases estradiol, which

might be involved in the deviation mechanism (Ginther *et al.*, 1996; Ginther *et al.*, 1997; Ginther *et al.*, 1998; Amiridis *et al.*, 1999). The deviation mechanism occurs within 8 h, during which systemic concentrations of LH are increased, and plasma estradiol concentrations are unchanged (Kulick, *et al.*, 1999). In heifers, between d 2 and 3 of the estrous cycle there is a decrease in FSH parallel to an increase in intrafollicular estradiol concentrations of > 5-fold in the DF, which causes alterations in FSH-dependent growth factors; this induces growth and further enhances the estradiol-producing capacity of the DF causing atresia of the subordinate follicles (Mihm *et al.*, 1997).

Healthy follicles have high expression of StAR mRNA (steroidogenic acute regulatory protein) in their theca cells (Bao *et al.*, 1998). This factor increases with the advance of the follicular wave and with the size of follicles indicating that other factors, such as $P450_{scc}$ and $P450_{arom}$, LH receptor mRNA might be primarily associated with follicular recruitment and selection, respectively (Bao *et al.*, 1997; Bao *et al.*, 1998).

Plasma progesterone concentrations are at minimal levels during the first 3 d of the cycle, increasing afterward until reaching a maximum of 3 to 6 ng/ml between the $11^{th}$ and $18^{th}$ d of the cycle (Robertson, 1972; Wettemann *et al.*, 1972; Amiridis *et al.*, 1999). High progesterone levels suppress LH pulses, which would otherwise prevent the dominant follicle to ovulate (Sirois and Fortune, 1990; Fortune *et al.*, 1991; Adams *et al.*, 1992b; Savio *et al.*, 1993; Smith and Stevenson, 1995). Progesterone, secreted by the CL, is derived from free esterified cholesterol present in the form of LDL and HDL. By binding to its membrane receptor, LH activates adenylate cyclase which then induces a rise in cAMP and stimulates steroidogenesis by converting cholesterol into pregnenolone and subsequently, to progesterone (Leymarie and Martal, 1993; Norman and Litwack,

1997). Increases in total cholesterol concentrations in plasma enhances luteal function in dairy cows, between 5 and 12 weeks postpartum fed with dietary inert fat (Spicer *et al.*, 1993). Processing of LDL / HDL by luteal cells is stimulated by LH and IGF-I (May *et al.*, 1990; Rajapaksha *et al.*, 1997).

## ROLE OF GLUCOCORTICOIDS IN CATTLE

Glucocorticoids increase gluconeogenesis, decrease protein synthesis and increase lipolysis releasing glycerol and free fatty acid (Ferguson and Hoenig, 1995; McKay and Cidlowski, 1999). The main role of glucocorticoids is the maintenance of fluid homeostasis through modulating the volume and composition of body fluid, and regulation of essential cellular metabolism (Ferguson and Hoenig, 1995). Glucocorticoids also regulate immune cell apoptosis (Compton and Cidlowski, 1986). Besides these effects, glucocorticoids also have a strong action on fetal development (specifically, fetal lung maturation) and induction of parturition in several species (McKay and Cidlowski, 1999). This section of review will describe changes in systemic glucocorticoids during physiologic events.

### Physiological control of glucocorticoid secretion

Synthesis of glucocorticoids takes place in the zona fasciculata /reticularis of the cortical layer of the adrenal gland. Cortisol is the main glucocorticoid in most domestic animals and humans. Cortisol, like other steroid hormones, is dependent on cholesterol biosynthesis, which in turn is regulated by HMG-coA reductase, the rate-limiting enzyme

for *de novo* cholesterol synthesis (Norman and Litwack, 1997). Another rate limiting step in adrenal steroidogenesis is the conversion of cholesterol to pregnenolone, which occurs via the enzyme P450sc involving two hydroxylations and a side-chain cleavage of cholesterol (Ferguson and Hoenig, 1995). Responding to a variety of stressful situations, such as starvation, pain, trauma, emotional stress, environmental extremes of temperature, sudden changes of management practices and cellular damage, glucocorticoids are released into the circulation through the hypothalamic-pituitary-adrenal axis (HPAA), where hypothalamic corticotropin-releasing hormone (CRH) acts on the pituitary causing the release of ACTH, which stimulates the adrenal gland to release glucocorticoids (Ferguson and Hoenig, 1995; McKay and Cidlowski, 1999). Secretion of CRH and ACTH are controlled by long-loop negative feedback mechanism of glucocorticoids (Ferguson and Hoenig, 1995).

Glucocorticoids exert two types of negative feedback on CRH and ACTH secretion, respectively at the levels of hypothalamus and anterior pituitary: fast and slow. The fast-feedback system occurs without interacting with nuclear steroid receptors and is sensitive to a change in cortisol levels. In this system, ACTH inhibits its own production at the pituitary gland; this is an important system to consider when administering exogenous glucocorticoid, since the recovery of the HPAA requires withdrawal of glucocorticoid and is not enhanced by exogenous ACTH (Ferguson and Hoenig, 1995). The slow-feedback mechanism mediated by nuclear receptors, results in a decrease of ACTH synthesis that is sensitive to the absolute cortisol concentrations (Ferguson and Hoenig, 1995). The dexamethasone suppression test mediated by this slow-feedback system is used for clinical diagnosis of spontaneous hyperadrenocorticism (Keller-Wood,

1990; Tyrrell *et al.*, 1994). Dexamethasone a long-acting glucocorticoid, which means that the duration of its action lasts about 48 h, its suppressing effects on HPAA may be longer and more pronounced (Kemppainen and Sartin, 1984; Kemppainen, 1986).

Activation of glucocorticoid receptors (GR) induce changes in many genes' expression especially the ones involved in immune and inflammatory responses such as proinflammatory cytokines and cell adhesion molecules, where glucocorticoid suppress their activity (McKay and Cidlowski, 1999). Two types of glucocorticoid receptors were identified; the type I receptor is tonically stimulated, while the type II receptor is stimulated only if glucocorticoid concentrations are high, in situations after stress or at the peak of the circadian release of corticosterone (Tempel and Leibowitz, 1994). Studies on the relative binding affinities ($IC_{50}$) of cortisol and dexamethasone for type I and II receptors in the hamster found that dexamethasone is 75% and 64-fold as effective as cortisol in binding to type I and II receptors, respectively (Sutanto and Kloet, 1987), while dexamethasone is 1.4-fold more potent than cortisol in reducing specifically bound [$^3$H] cortisol to 50% (Norris and Kohler, 1977).

**Changes during stress and disease**

Stress is a systemic response of the organism to any changes in its environment or within itself. Stress is defined as trauma, infection, intense heat or cold, surgery, among many others. Almost any type of stress, neurogenic or physical, is characterized by an increase in cortisol secretion following the ACTH increase from the hypothalamus (Guyton and Hall, 1996). This increase in ACTH or in adrenal steroids directly stimulates renal cell gluthatione (GSH) by activating the defense mechanism in the kidney, which

might be the connection between the endocrine stress response and the cellular defense mechanism (Ogasawara *et al.*, 1999).

Cortisol secretion is in the form of irregular pulses, with transient increases, although more sustained increases are normally associated with stress and with its effects on the immune system (Rhind *et al.*, 1999). Increases in circulating cortisol concentrations might induce a reduction (Munck *et al.*, 1984) or an increase (Croiset *et al.*, 1987) in immune function. Large and rather infrequent pulses of cortisol might stimulate the basal cell mediated immune activity, whereas frequent or continuous smaller pulses of cortisol even at high concentrations do not alter the immune system response (Rhind, *et al.*, 1999). The magnitude of the increase in cortisol concentrations of an animal during handling may be influenced by the experience in handling animals and on individual differences among animals (Hopster *et al.*, 1999).

Effects of dystocia were measured in neonatal calves, regarding serum concentrations of glucose and cortisol, and their tolerance to cold temperatures (Bellows and Lammoglia, 1999). Severe dystocia, such as the required use of a mechanical calf puller, lowers calf rectal temperature (38.3 ± 0.02°C), reduces serum cortisol (58.2 ± 2.0 ng/ml) and increases serum glucose (110.1 ± 1.6 mg/dl) while calves born from natural calving have higher serum cortisol (80.0 ± 1.7 ng/ml) levels and rectal temperature (39.0 ± 0.2 °C), and lower serum glucose concentrations (77.2 ± 1.3 mg/dl). This means that a calf's ability to withstand low environmental temperatures is probably altered by severe dystocia and involves stress-induced releases of maternal cortisol (Bellows and Lammoglia, 1999).

Lay *et al.* (1997a) found that repeated activation of the HPAA during pregnancy, induced by stress of transportation, causes larger fetal body weight at 266 d of gestation and heavier fetal pituitary gland, but does not change plasma ACTH or cortisol concentrations. Nevertheless, this might result in alterations of many body functions such as growth, stress coping, and reproduction by altering the endocrine system related to these functions (Lay *et al.*, 1997a). Prenatal exposure to mild stress in calves results in maintenance of high levels of cortisol (50 ± 7 ng/ml versus 25 ± 7 ng/ml in transported and "walked through" at 150 d of age, respectively) for a longer post-natal period, which might enable the calf to better respond to mild stress. However, in severe exposure to stress, plasma cortisol levels may remain high, causing deleterious effects on metabolism and immune system (Lay *et al.*, 1997b). Based on these studies, the divergent responses to stress, either increasing or decreasing plasma cortisol concentrations, are probably dependent on the type of stress an animal is exposed to, age, genotype and other factors. As suggested by Lay *et al.* (1997a), plasma cortisol or ACTH concentrations might not even change, but body functions might be altered.

Comparing calves fed *ad libitum* and restricted fed, it is found that during the 20 min restraint in the squeeze chute, plasma cortisol concentrations are increased in both groups, but they are higher in restricted fed versus *ad libitum* fed calves at 15 (30 to 35 ng/ml) and 20 min (25 to 28 ng/ml). Heart rates are greater in restricted fed calves (79 ± 0.7 beats/min) than in *ad libitum* calves (74 ± 0.7 beats/min). Together these results indicate that the HPAA of the restricted fed calves respond better to restrain stress (Lay *et al.*, 1998). In chronically stressed gilts, the ones isolated from social and visual contact had a more pronounced increase in ACTH-induced cortisol response than gilts not

subjected to these environmental conditions, indicating that adaptation changes in tethered gilts occur at the adrenal cortex and affect the ACTH-induced adrenocortical response (Janssens *et al.*, 1994).

**Changes during various reproductive states**

As stated above, glucocorticoids are essential to life and without them an organism would not be able to cope with different types of stressors. Because exposure to a stressful environment may result in alterations of endocrine glands associated with growth, reproduction and tolerance to biological stressors (Lay *et al.*, 1997a), an understanding of how glucocorticoids change during normal physiologic states is important. Therefore, a review on the changes of glucocorticoids during different reproductive stages will be presented.

*Growth/puberty*

Calves at 10 d of age have greater heart rates and plasma cortisol concentrations than calves at the age of 150 and 180 d. Clearance rate of ACTH was faster for calves at 150 d of age than at 10 d of age. All the calves (10, 150 and 180 d of age) subjected to ACTH treatment had greater cortisol levels than controls. At 10 d of age HPAA might still be immature but by the150 d of age it is fully capable of responding to ACTH with high amounts of cortisol (Lay *et al.*, 1997b). Plasma cortisol concentrations in calves increase after weaning, reaching a peak of about 18 ng/ml on d 0 and decreasing afterward to basal concentrations of 8 to 10 ng/ml by d 5 after weaning (Lay *et al.*, 1998).

Plasma cortisol concentrations differ between bulls and heifers. Between 7 and 12 mo of age cortisol remains at 1.6 to 1.8 ng/ml in bulls, but in heifers it increases from 2.8 ± 0.3 to 5.7 ± 0.4 ng/ml, with significant correlations between the age or BW and cortisol concentrations for heifers but not for bulls (Henricks *et al.*, 1984). Lower plasma cortisol concentrations in bulls, compared with heifers, are related to concurrent greater growth (Henricks *et al.*, 1984). When the period of exposure to light decreases from 15.7 to 8 h, there is a 118% increase in plasma glucocorticoid in prepubertal bulls; conversely light increase in the same range brings a decrease in 58% of plasma glucocorticoid, meaning that reduced serum glucocorticoid might be associated with light-induced growth of prepubertal bulls and heifers (Leining *et al.*, 1980).

A study correlating steroid hormones from first estrus to breeding size ($7^{th}$ estrous cycle) in Holstein heifers was carried out (Swanson *et al.*, 1972). In heifers serum cortisol levels are high (> 10 ng/ml) at estrus (d 0), decrease to about 7 ng/ml at d 2 after estrus and remain low (< 4 ng/ml) throughout midcycle (d 11). Corticosterone concentrations in serum decrease from 0.5 ± 0.1 ng/ml during the first estrus cycle to an average of 0.2 ± 0.1 ng/ml during the fourth and seventh cycles; serum cortisol concentrations also decrease from 9.9 ± 1.3 ng/ml in first estrous cycle to an average of 6.5 ± 0.8 ng/ml in the fourth and seventh estrous cycles (Swanson *et al.*, 1972). These decreasing levels of corticosterone and cortisol, during the first seven estrous cycles are associated with an increased length of standing heat, increased follicle size and decreased luteal size and are probably related to continuous reproductive maturation after the first estrous cycle (Swanson *et al.*, 1972). Interestingly, a congenital defect in the glucocorticoid and mineralocorticoid steroidogenic pathways is characterized by a late-onset 21-hydroxylase

deficiency, with symptoms including hirsutism and infertility at puberty or young adulthood in humans; these symptoms are attenuated by the administration of glucocorticoid therapy, resulting in induction of ovulation (Laohaprasitiporn, 1996).

In bulls between the age of 6 and 15 mo, there is an effect of age on plasma cortisol levels. Verkerk and Macmillan (1997) demonstrated that, plasma cortisol concentrations after ACTH challenge decrease with increasing age. At 50 min, plasma cortisol drops from 52 ± 4 ng/ml at 6 mo of age to 33 ± 3 ng/ml at 15 mo, at 120 min, plasma cortisol concentrations decrease from 29 ± 4 ng/ml at 6 mo of age to 3 ± 1 ng/ml at 15 mo. When compared to steers, responses to exogenous ACTH are similar for bulls and steers at 6 mo of age, but by 8 mo of age bulls decrease their response to ACTH remaining less than those of steers; at 50 min and at peak, plasma cortisol concentrations in steers are 1.5 times greater than in bulls at the same dose rates (mg/kg BW) of ACTH. This indicates that after puberty onset, testicular androgens influence the adrenocortical function in bulls (Verkerk and Macmillan, 1997).

*Pregnancy*

Plasma glucocorticoids remain low during most of gestation, increasing just before parturition and reaching a peak at the time of delivery (Edgerton and Hafs, 1973). Plasma corticoids measured 7 d before and after calving show that in the bovine, maternal cortisol are at 8 ng/ml on d 7 prior to calving, increase on d 4 reaching about 14 ng/ml and remain high until d 0 of calving (about 15 ng/ml), after which plasma cortisol decrease (Adams and Wagner, 1970; Challis *et al.*, 1977). Parallel to an increase in fetal corticosteroid during the last 3 d of pregnancy in cows there is a rise in maternal estrogen

and prostaglandin and a decrease in maternal progesterone, suggesting that the fetal pituitary-adrenal axis is the organ responsible for the initiation of parturition (Thorburn *et al.*, 1977; Fairclough *et al.*, 1981; Senger, 1997). For example, in sheep, during the last 20 d of pregnancy, fetal adrenal weight doubles (Comline and Silver, 1961) accompanied by a gradual increase in concentrations of fetal plasma cortisol during the last 10 to 15 d prior to lambing and a rapid increase during the last 2 to 3 d of gestation (Basset and Thorburn, 1969).

In cows with normal calving the highest concentration of cortisol (55 to 65 ng/ml) is observed during calving, after the rupture of the amnion whereas in cows with dystocia, the highest concentration of cortisol (75 to 105 ng/ml) is observed after fetal expulsion (Osawa *et al.*, 1998). Concentrations of cortisol, from 24 h after calving until the first ovulation, do not differ between cows with dystocia and cows with normal calving (Osawa *et al.*, 1998). Whether this higher concentration of cortisol in cows with dystocia alters postpartum endocrine and reproductive function is unknown.

*Postpartum and estrous cycle*

On d 1 postpartum, glucocorticoids start decreasing from 10 ng/ml until they reach minimum levels of about 2 ng/ml on d 5 to d 7 after calving (Adams and Wagner, 1970). Between d 7 and 56 postpartum, serum cortisol concentrations did not change in suckled beef cows (Spicer *et al.*, 1986). On d 12 postpartum, cortisol concentrations in serum do not differ between primiparous and multiparous beef cows (Stewart and Stevenson, 1987). In suckling beef cows, cortisol concentrations are greatest (25 ng/ml at min 0) at the reunion of cow-calf decreasing gradually during the remaining hour

(Stevenson *et al.*, 1994), which in turn may prolong the anovulatory interval postpartum by inhibiting gonadotropin secretion (Padmanabhan *et al.*, 1983). The suckling-induced inhibition of LH secretion during the latter part of the postpartum period might be due to endogenous opioid rather than cortisol (Whisnant *et al.*, 1986). Correlations of cortisol with immuno-reactive β-endorphin in dairy cows show that both hormones have similar changes in plasma concentrations during the periparturient period, but in the postpartum period increases in β-endorphin and cortisol are not always concomitant (Osawa *et al.*, 1998). These latter results suggest that a cow's response to stress may differ between opioid and non-opioid mechanisms (Osawa *et al.*, 1998).

**Effects of Glucocorticoid on the reproductive system**

Glucocorticoids are essential for the regulatory processes in the organism, but when in excess they appear to be detrimental. Prolonged anestrus, or chronic anovulatory state appears to be associated with high levels of glucocorticoids or hyperactivity of the adrenal gland (Irahara *et al.*, 1999). In the following paragraphs, evidence for direct glucocorticoid action at the level of the hypothalamus, pituitary, ovary and mammary gland will be reviewed. The studies summarized below indicate that high levels of glucocorticoid may act on the pituitary to prevent the preovulatory rise LH as well as interrupt follicular development before the preovulatory surge of gonadotropins.

of the brain. In the rat, dexamethasone acts on the pituitary rather than on the hypothalamus to induce suppression of LH secretion (Kloet *et al.*, 1974). Further evidence in the rat suggests that the negative effects of glucocorticoids on reproduction are limited to a slight suppression of basal LH secretion, whereas they stimulate the pituitary by directly increasing FSH synthesis (Suter and Schwartz, 1985). Similarly, in humans and other primates, glucocorticoids reduce GnRH-induced release of LH from the pituitary in both *in vivo* and *in vitro* systems (Sapolsky *et al.*, 2000). The effects of glucocorticoids on FSH secretion in cattle are unknown.

**Effects on ovarian function**

Glucocorticoids appear to act directly on the ovary by inhibiting FSH-dependent granulosa cell differentiation in rats (Schoonmaker and Erickson, 1983) and IGF-I-induced progesterone production in cattle (Spicer and Chamberlain, 1998). Since changes in estradiol concentrations of follicular fluid are not accompanied by changes in cortisol concentrations it is suggested that normal fluctuating concentrations of cortisol in follicular fluid of cattle do not regulate follicular estradiol production (Spicer and Zinn, 1987). In ovariectomized estradiol-treated heifers, different doses of cortisol (0 to 200 mg/d) have no effect on inhibition of estrus, while 4 mg/d of dexamethasone inhibits estrus behavior (Cook *et al.*, 1987). In agreement with this, Spicer and Chamberlain (1998) found that 1 d of cortisol treatment does not affect insulin-induced cell numbers or estradiol production by granulosa cells from small follicles. However, in porcine granulosa cells, dexamethasone reduced IGF-I production, IGF-stimulated progesterone and estrogen synthesis indicating that high levels of cortisol can alter IGF-I synthesis and

IGF-mediated actions with specific actions dependent on follicle stage in pigs (Viveiros and Liptrap, 1999). Furthermore, glucocorticoids have a direct inhibitory effect on meiotic maturation of pig oocytes (Yang *et al.*, 1999). Thus, species differences may exist with regard to glucocorticoid effects at the level of the ovary.

Although ovarian cells are unable to synthesize glucocorticoid *de novo*, due to lack in 21-hydroxylase (Omura and Morohashi, 1995), they undertake glucocorticoid metabolism catalyzed by 11β-HSD1, which converts cortisone to its more active metabolite, cortisol, and 11β-HSD2 which converts cortisol to its less active metabolite, cortisone (Hillier and Tetsuka, 1998). In human granulosa cells, high expression of 11β-HSD2 mRNA is found in non-luteinizing granulosa cells during late follicular stage while 11β-HSD1 mRNA is highly expressed in luteinizing granulosa cells of periovulatory follicles (Hillier and Tetsuka, 1998). This suggests a developmental mechanism differentially regulated in the expression of 11β-HSD2 during the preovulatory phase to increase intrafollicular cortisol levels (Hillier and Tetsuka, 1998). Because glucocorticoids are anti-inflammatory and ovulation is an inflammatory process, it appears that a local rise of cortisol within the ovary might minimize the inflammatory process of ovulation, and aid in the rapid healing of tissue damage preparing the ovary for the next estrous cycle (Hillier and Tetsuka, 1998). Changing levels of cortisol in follicular fluid may alter follicular development and oocyte maturation (Fateh *et al.*, 1989) by stimulating androstenedione production in thecal cells (Spicer and Chamberlain, 1998). In follicular fluid of both large and small follicles, cortisol concentrations are highest in early luteal phase, with small follicles having 11% higher cortisol concentrations than large follicles in all developmental stages (Spicer and Zinn, 1987).

**Effects on mammary gland function**

Many hormones, including cortisol, are implicated in the regulation of the maturation process of the mammary gland (Tucker, 2000). Two stages can be distinguished during mammary development: the proliferative phase in which stem cells are divided into secretory cells, regulated by insulin, EGF and GH; and the differentiative phase, mediated by glucocorticoids, prolactin and insulin. In the latter stage, glucocorticoids appear to be required by prolactin to increase the mRNAs for milk proteins (Norman and Litwack, 1997). The major function of cortisol in the mammary gland is to induce differentiation of the lobule-alveolar system in order to later enable prolactin to induce synthesis of milk proteins (Tucker, 2000). Glucocorticoids are important for the maintenance of the tight junction integrity in the epithelium of the mammary gland (Stelwagen *et al.*, 1997).

During stress, cortisol has a negative effect on the mammary gland by exerting a negative feedback on the mammotrope inhibiting release of prolactin (Norman and Litwack, 1997) and, probably due to an increase in epinephrine secretion there is an increase in potassium overriding the beneficial effects of cortisol in the tight junctions (Stelwagen *et al.*, 1997). In dairy cows deprived from feed for 12 to 60 h, milk production decreases and this decrease is associated with an increase in cortisol and GH, and reduction in somatostatin, which might be a mechanism to meet the nutrients demand for both the cow and the calf (Samuelsson *et al.*, 1996a). Feeding and milking separately results in a decrease in plasma cortisol and GH levels, simultaneously with an increase in

somatostatin, gastrin and prolactin, which might be important for improving the management practices in dairy herd, since these hormones have a direct or indirect effect on milk production (Samuelsson *et al.*, 1996b).

In cows with mastitis during early lactation, there is an increase in plasma cortisol concentrations in the luteal phase of the estrous cycle, which might be associated with ovulation failure due to insufficient LH and/or estradiol concentrations or even due to high levels of $PGF_{2\alpha}$, thereby inducing alterations in the interestrous intervals (Hockett *et al.*, 2000). Increased susceptibility of dairy cows to mastitis and other infectious diseases after calving might be associated with increased cortisol concentrations, as well as other factors, which then might induce changes in numbers, differentiation and maturity of circulating leukocytes (Hoeben *et al.*, 1999; Kehrli, *et al.*, 1999; Tucker, 2000).

**Clinical use of glucocorticoids**

Synthetic glucocorticoids in humans are widely used as anti-inflammatory and immune-modulatory drugs, are considered as standard therapy for the management of various types of diseases such as arthritis, asthma, inflammatory bowel disease, systemic lupus erythematosus, prevention of transplant rejection (Pfeilschifter and Mühl, 1999) and in the alleviation of the inflammatory symptoms associated with infectious diseases. In dogs and cats, hypoadrenocorticism or Addison's disease resulting from the destruction of the adrenal cortices or deficiency in ACTH, is often managed throughout life with the administration of glucocorticoids (Monroe, 1997).

Ketosis is a metabolic disorder often occurring in dairy cattle with high levels of milk production, due to glucose demand by the mammary gland that exceed the available energy sources. Dexamethasone (0.04 mg/kg BW) or betamethasone is often used in order to prolong the hyperglycemic effect by reducing both the tissue uptake of glucose and milk production for up to 3 d, during the peak of lactation (Smith, 1996). In cattle, dexamethasone suppresses γ-IFN in the infection with *Mycobacterium bovis*, the bacteria responsible for tuberculosis in cattle (Goff, 1996). In cattle with herpesvirus 1, glucocorticoid treatment inhibits the re-excretion or transmission of the AgE-negative herpesvirus (Mars *et al.*, 2000). Due to its immunosuppressive properties, glucocorticoids are also used in viral infections, which might enhance the pathogenic effects of some viruses such as the feline immunodeficiency virus (FIV), a lentivirus (Barr *et al.*, 2000). In horses, exogenous corticosteroids are used as in respiratory diseases together with bronchodilators (Foreman, 1999). Glucocorticoids are used in diverse situations in order to alleviate the inflammation symptoms and aid the healing process.

**Induction of parturition**

Exogenous glucocorticoids are effective in parturition induction in the cow, sheep and goat (Adams and Wagner, 1970; Thorburn *et al.*, 1977; Fairclough *et al.*, 1981), but seem to be ineffective in the mare, sow and bitch (Thorburn *et al.*, 1977). In cows during the final month of gestation, calving is induced through administration of exogenous glucocorticoids, which increase estradiol and $PGF_{2\alpha}$ by acting on the fetoplacental unit (Jöchle, 1971; Comline *et al.*, 1974; Garverick *et al.*, 1974; Thorburn *et al.*, 1977). A dose of 3.3 mg/d of dexamethasone injection to the fetal calf induces calving within 72 ±

19 h, with a rise in estrogen and fall in progesterone in a similar way as the normal calving (Comline *et al.*, 1974), although there is also an increase in the incidence of retained placenta, calf mortality and delay in the onset of milk production (Thorburn *et al.*, 1977). Using dexamethasone at a dose of 20 mg during different periods of gestation (< 245 to > 275 d) in cows, indicated that parturition can be successfully induced if used as early as 255 to 264 d of pregnancy, although within an average of ≤ 254 d can raise the number of failures, including stillborn calves and retention of fetal membranes (Adams and Wagner, 1970). The administration of an increasing dose of dexamethasone, from 0.1 to 10 mg/d at 240 d of gestation into fetal calves, resulted in a reduction of induction time for calving from 12 to 3 d (Fairclough *et al.*, 1981). Treatment of cows with 20 to 30 mg of dexamethasone alone or in combination with 25 mg $PGF_{2\alpha}$ within 14 d previous to the anticipated calving date, results in good calf viability with reduced birth weight (Smith, 1996).

Glucocorticoids stimulate the production of surfactant phospholipid by alveolar type II cells, enhance the expression of surfactant-associated proteins, reduce microvascular permeability, and accelerate overall structural maturation of the lungs (Challis *et al.*, 1977; Robertson, 1993; Smith, 1996; Zaremba *et al.*, 1997), therefore decreasing the incidence of respiratory distress syndrome in calves (Zaremba *et al.*, 1997). In women at risk of premature birth, the use of glucocorticoids to aid in the fetal lung maturation reduces neonatal mortality and morbidity from respiratory distress syndrome (Sloboda *et al.*, 2000).

**Treatment of inflammation**

Glucocorticoids have the ability to inhibit inflammatory mediators, including the ones released by mast cells, as well as inhibit chemotaxis of inflammatory cells and diminish the edema and vasodilation characteristics of allergy symptoms. Allergy problems, commonly seen in humans, dogs and cats, and other pathological conditions leading to inflammation, are often alleviated by the administration of glucocorticoids, besides the use of antihistamines (Noxon, 1997). They act by inducing alterations in the recruitment and activation of monocytes/macrophages, eosinophils or lymphocytes as well as inhibiting the generation of inflammatory mediators such as prostaglandins, thromboxanes, leukotrienes and nitric oxide (Pfeilschifter and Mühl, 1999). At therapeutic levels glucocorticoids regulate the catabolism of prostaglandins, which are pro-inflammatory (Tong and Tai, 2000).

The mechanisms of glucocorticoids actions include transcriptional, post-transcriptional, translational and post-translational by simultaneously inhibiting the expression of proinflammatory cytokines (interleukin-1 to -8), TNF-$\alpha$ and interferon-$\gamma$ and enhancing the expression of IL-1 receptor antagonist (Pfeilschifter and Mühl, 1999). IL-1 consists of three ligands (IL-1 $\alpha$, $\beta$, and $\gamma$) and two receptors (IL-1RI and IL-1RII) and plays a key role in inflammation and immune response. In dexamethasone-treated cattle, an early increase in the expression of IL-1RII mRNA suggests that the early upregulation of IL-1RII may be part of the anti-inflammatory mechanism of glucocorticoids (Yu *et al.*, 1998).

In lactating dairy cows the use of dexamethasone should be considered when mastitis is present, especially in cases exhibiting "toxic-shock" due to infection with

coliforms, which require additional therapy besides an antimicrobial one (Smith, 1996). During early stages of acute inflammation in mastitic cows there is release of inflammatory mediators, such as eicosanoids, histamine and serotonin as well as $PGF_{2\alpha}$, $PGE_2$ and thromboxane $B_2$ (Smith, 1996). In these cases corticosteroids are used to help reduce inflammation, but they concomitantly induce bacteremia, requiring the simultaneous administration of systemic antimicrobials effective against coliforms (Smith, 1996). The use of corticosteroid together with dimercapto-succinic acid (DMSA) in the treatment of pyelonephritis reduces renal scarring in severely affected kidneys in pigs (Pohl *et al.*, 1999). Short-systemic glucocorticoids are also recommended for cases of tendonitis in order to restrain swelling of the tendon in horses (Smith, 1996).

## CONCLUSIONS

Ovarian folliculogenesis occurs in waves, during the estrous cycles of cattle. Each follicular wave is comprised of wave emergence, selection, follicle dominance, and ovulation or atresia of the dominant follicle. A follicular wave lasts about 5 to 10 d, with the DF in the last wave producing the ovulatory follicle. Follicular development is regulated by gonadotropins (FSH, LH) and the production of steroid hormones by the ovary, estradiol and progesterone. Recent evidence indicates that other hormones, such as IGF-I and cortisol, can directly alter ovarian activity. Cortisol, which belongs to the glucocorticoid family of steroid hormones, is required for the maintenance of fluid homeostasis, metabolic, immunologic, and other physiological processes. In response to stressful conditions, such as heat, food deprivation, diseases and other abnormal

conditions, plasma cortisol concentrations are increased suppressing the HPAA, and consequently the GH-IGF axis. These mechanisms may be physiologic processes used to adapt to the new environmental conditions. However, in cattle it is not yet known by which *in vivo* mechanisms glucocorticoid regulate the whole process of follicular development and thus, the reproductive function in the dairy cow.

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# CHAPTER III

## EFFECTS OF DEXAMETHASONE ON ENDOCRINE AND OVARIAN FUNCTION IN DAIRY CATTLE

### Abstract

Multiparous non-lactating Holstein cows were used to determine the effect of dexamethasone on ovarian follicular development and plasma hormone concentrations. Animals were randomly divided into two groups, control (C; n = 5) and treatment (T; n = 6), but managed as one group. Both groups were synchronized with 2 injections of $PGF_{2\alpha}$ at a dose of 25 mg each (i. m.), 11 d apart. One day after ovulation (d 0) the T group received a daily injection of dexamethasone (44 μg/ kg BW; i. m.) until the first dominant follicle stopped growing or up to d 12 post-ovulation. The C group received vehicle injections. Concentrations of LH and FSH did not differ ($P > 0.10$) between the C and T cows. Treatment ($P < 0.10$) and day ($P < 0.05$) effects were observed for plasma progesterone concentrations. Progesterone concentrations were lower in T than in C cows from d 4 onward ($P < 0.10$). Concentrations of E2 were greater ($P < 0.05$) in C (3.32 ± 1.05 pg/ml) and T (1.07 ± 0.45 pg/ml) cows on d 0 and 1. Treatment × day interaction influenced ($P < 0.05$) plasma insulin concentrations such that T cows had 2.9- to 6.0-fold greater ($P < 0.05$) insulin concentrations than C cows between d 2 and 9. Dexamethasone decreased ($P < 0.05$) IGF-I and –II concentrations from d 5 onwards. None of the IGFBPs were affected by dexamethasone ($P > 0.10$). Numbers of small follicles (< 10 mm) were 46% to 48% lower ($P < 0.10$) in T than in C cows on d 2 and 4, whereas numbers of large follicles (> 10 mm) were increased 2.0- to 2.9-fold in dexamethasone-

treated cows on d 2 and 8 ($P < 0.05$). Growth rate of the dominant follicles and maximum diameter of the dominant and subordinate follicles were not affected ($P > 0.10$) by treatment. Diameter of the CL was 21% to 39% larger ($P < 0.01$) in T than in C cows between d 6 and 10. Plasma leptin and cholesterol concentrations did not differ ($P > 0.10$) between C and T cows. Plasma glucose concentrations were greater ($P < 0.05$) in T than in C cows between d 1 and 10. In summary, dexamethasone suppressed luteal function by inhibiting the IGF-system through suppression of IGF-I and –II, which subsequently might have suppressed steroidogenesis without affecting secretion of gonadotropins.

## Introduction

Corticosteroids are mainly synthesized from cholesterol, by the adrenal cortex in the zona fasciculata. Secretion of glucocorticoid is regulated by the adrenocorticotropic hormone (ACTH), from the anterior pituitary, which in turn is regulated by corticotropin releasing hormone (CRH) from the hypothalamus (Hafez and Jainudeen, 1993). Gluconeogenesis (formation of glycogen) as well as lipid and protein metabolism are promoted by corticoid (Marfaing *et al.*, 1991; Johansen *et al.*, 1999; Sapolsky *et al.*, 2000). The well-established stress-induced release of glucocorticoid is known to inhibit the immune system (Liptrap, 1993; Stryer, 1995), reproductive system (Norman and Littwack, 1997; Viveiros and Liptrap, 1999) and the endocrine system (Norman and Littwack, 1997; Tan *et al.*, 1998) in humans. In particular, glucocorticoids have recently been reported to stimulate leptin secretion in humans (Larsson and Ahren, 1996; Dagogo-Jack *et al.*, 1997; Janssen *et al.*, 1998). Glucocorticoid effects on these systems in cattle are not well defined, however.

Exogenous glucocorticoids appeared to have an inhibitory effect on the reproductive cycle of the cow and rats, by inhibiting LH secretion on the anterior pituitary (Li and Wagner, 1983; Padmanabhan *et al.*, 1983; Suter and Schwartz, 1985) and by directly inhibiting the follicular function (Hsueh and Erickson, 1978; Spicer and Chamberlain, 1998). Cortisol reduced the number of IGF-I binding sites in thecal cells, but had no effect on the number of granulosa cell IGF-I binding sites (Spicer and Chamberlain, 1998). In porcine granulosa cells, high concentrations of cortisol decreased the synthesis of IGF-I and IGF-I mediated steroid production (Viveiros and Liptrap,

1999). Alhtough these studies report an effect of glucocorticoid at the cellular level of the ovary, little has been done to understand the systemic mechanisms by which glucocorticoids act to suppress reproductive function in cattle, particularly its action on the leptin, insulin and insulin-like growth factor (IGF) system. Therefore the objective of this study was to determine the influence of dexamethasone on follicular development and plasma hormones of dairy cows.

## Material and Methods

*Management of animals and estrous synchronization*

Eleven non-lactating multiparous Holstein cows from the Dairy Cattle Center at Oklahoma State University, were randomly divided into two groups: dexamethasone-treated (n = 6) and control (n = 5) cows. Both groups were synchronized with prostaglandin $F_{2\alpha}$ (PGF; Lutalyse®, The Upjohn Company, Kalamazoo, MI) using two injections of 25 mg each (I.M.), 11d apart.

One day after ovulation (d 0), the cows from the Treatment group were injected daily with dexamethasone (Azium, Schering-Plough Animal Health Corp., NJ) until the first dominant follicle stopped growing or up to the d 12 post-ovulation. Each cow was injected with 44 μg/kg BW of dexamethasone per day (I.M.) in a volume of 1 ml/100 kg BW/ d. The Control group received 1 ml/100 kg BW/d of a vehicle solution containing ethanol, polyethylene glycol and sterile-filtered millipore water at pH 4.9.

All animals were managed as one group, and housed in a dry lot with access to shelter and water. This study was carried out in December 1998. During the trial, feeding

consisted of as fed basis, 2 kg of grain, 7 kg sorghum silage and free choice of Bermuda grass, containing 16%, 8% and 13% (% DM) crude protein, respectively.

Cows were weighed daily. At the beginning of the experiment, average body weight for the control group was 646.3 ± 29.6 kg and 648.2 ± 27.02 kg for the treatment group. The average age of the cows (range from 3 to 9 yr), did not differ between control and treatment groups. Milk production (305 d ME) during the previous lactation averaged 11037 ± 1976 kg for the control animals and 10171 ± 1869 kg for the treatment animals.

*Ovarian ultrasonography*

Ovarian follicular development was monitored via transrectal ultrasonography using an Aloka 510 V ultrasound scanner with a 7.5 mHz probe (Corometrics Medical Systems, Inc., Wallingford, CT). Each ovary was recorded on videotape using a VCR (Mitsubishi HS-U510, Mitsubishi Electronics America, Inc. Norcross, GA). Ovarian ultrasonography was started 5 d before the initiation of treatment.

Videotapes were then projected on a monitor and a diagram of all follicles with ≥ 5 mm and the corpus luteum (CL) indicating their relative location on the ovary were recorded for each ovary. Diameters of follicles and CL were calculated as the mean of the largest and the smallest diameters. Follicles were classified as small (< 10 mm) and large (≥ 10 mm) based on their average diameter. Determination of the dominant follicle was based on the maximum diameter achieved by the largest follicle on or after d 5. For the determination of the growth rate of the dominant follicle, its minimum diameter within the previous 6 d was subtracted from its maximum diameter achieved after d 5.

Determination of ovulation was based on the disappearance of the previous dominant follicle and the subsequent formation of a CL at the same location in the ovary.

*Blood collection and hormone analysis*

Daily blood collection via tail venipuncture started 5 d prior to the beginning of the study. Blood samples were collected into 9-ml tubes containing EDTA (Monovette® Sarstedt, Newton NC), and immediately placed on ice. Samples were then centrifuged at 4°C, 300 x g for 5 min and plasma collected and stored at -20°C until hormonal analyses were performed. For most of the hormones and metabolite assays, samples were randomly separated into two groups to be run in two assays, with samples from about the same number of cows from the control and treatment groups in each assay. All plasma samples for hormonal analyses were run in duplicate with samples thawed at room temperature.

Solid-phase RIA kits (ICN Pharmaceuticals Inc., Costa Mesa, CA) were used to measure insulin concentrations in plasma as previously described (Simpson et al., 1994). Inter- and intra- assays coefficients of variation for the two insulin assays were 8.6% and 7.6%, respectively. Sensitivity of the assay, defined as 80% of total binding, was 0.071 ng/ml.

Double-antibody radioimmunoassay (RIA) was used to determine IGF-I concentrations in plasma after acid-ethanol extraction (16 h at 4° C) as previously described by Echternkamp et al. (1990). Inter- and intra-assay coefficients of variation for the IGF-I assays were 19.0% and 18.0%, respectively. Sensitivity of the assay, defined as 90% of total binding, was 0.665 ng/ml.

Concentrations of IGF-II in plasma were determined by a double-antibody RIA after acid-ethanol extraction as previously described (Spicer et al., 1995). Inter- and intra-assays coefficients of variation for the IGF-II assays were 22.7% and 7.2%, respectively. Sensitivity of the assay, defined as 80% of total binding, was 5.2 ng/ml.

Plasma estradiol concentrations were measured using Serono Estradiol MAIA assay kit (Polymedco Inc., Cortlandt Manor, NY) after extraction with ethyl acetate as previously described (Vizcarra et al., 1997). Inter- and intra-assay coefficients of variation for the estradiol assays were 14.5% and 9.5%, respectively. Sensitivity of the assay, defined as 90% of total binding, was 0.86 pg/ml.

A double-antibody RIA was used to determine FSH concentrations in plasma as previously described (Vizcarra *et al.*, 1997). The intra-assay coefficient of variation was 11.8%. The sensitivity of the assay, defined as 90% of binding, was 0.03 ng/ml.

A double-antibody RIA was used to determine LH concentrations in plasma as previously described (Bishop and Wettemann, 1993). Intra-assay coefficient of variation was 9.3%. Sensitivity of the assay, defined as 90% of total binding, was 0.68 ng/ml.

Progesterone concentrations in plasma were determined using a solid-phase progesterone assay RIA kit (Coat-a-Count, Diagnostic Products Corporation, Los Angeles, CA) as previously described (Stewart et al., 1996). Inter- and intra-assay coefficients of variation for the two progesterone assays were 0.1% and 9.5%, respectively. Sensitivity of the assay, defined as 95% of total binding, was 0.015 ng/ml.

Leptin concentrations in plasma were determined using a Multi-species RIA kit assay (LINCO Research, Inc., St. Charles, MO). Bovine plasma produced curves parallel to standard curves when added at increasing volumes. Samples (100 µl each) were

assayed in duplicate and after the addition of the 1$^{st}$ antibody (100 µl multi-species leptin) to all tubes, with the exception of the total counts (TC) and non-specific binding (NSB) tubes, samples were vortexed, covered and incubated for 24 h at 4°C. On the next day, 100 µl of the 2$^{nd}$ antibody ($^{125}$I-human leptin) was added to all tubes, which were incubated for another 24 h at 4°C. On the 2$^{nd}$ d, 1.0 ml of precipitating reagent (at 4°C) was added to all tubes, excepting to the total count ones, and tubes were incubated at 4°C for 20 min. Tubes were centrifuged for 30 min at 3,000 X g, supernatant from each tube was decanted, and tubes were inverted one time on a paper towel to remove any excess liquid. The precipitate was then counted in a Gamma counter. Inter- and intra-assays coefficients of variation for two assays were 6.0% and 6.6%, respectively. Sensitivity of the assay, defined as 90% of total binding, was 0.68 ng/ml human leptin equivalent.

Glucose concentrations in plasma were determined using an enzymatic colorimetric procedure (n° 510, Sigma Chemical, Co., St. Louis, MO), and the intra-assay coefficient of variation was 2.75%. Sensitivity of the glucose assay, defined as the lowest value at 95% confidence interval of the lowest point on the standard curve, was 23.7 mg/dl.

Plasma cholesterol concentrations were determined using an enzymatic colorimetric procedure (n° 352, Sigma Chemical, Co., St. Louis, MO). The intra-assay coefficient of variation was 1.26%. Sensitivity of the cholesterol assay, defined as the lowest value at 95% confidence interval of the lowest point on the standard curve, was 44.9 mg/dl.

*Ligand Blots*

One-dimensional SDS-PAGE was used to determine the IGFBPs in plasma samples collected on D 0 and 10 of treatment, as previously described (Echternkamp et al. 1994; Stewart et al., 1996). Briefly, 4 µl of sample was mixed with 21 µl of Laemmili sample buffer (BIORAD, Hercules, CA). Heat treatment was used to denature proteins (3 min at 100°C) and then samples were centrifuged at 4657 X g for 3 min and added to the 12-lane SDS-PAGE. Control lanes were composed of a 25 µl wide range color marker (MW 6,500-205,000, Sigma, St.Louis, MO) and a mixture of 4 µl of bovine follicular fluid with 21 µl of the sample buffer. Samples were subjected to electrophoresis on a 12% polyacrylamide gel for about 18 to 20 h, at constant current and varying voltage. Gels were then electrophoretically transferred to nitrocellulose paper (Midwest Scientific, St. Louis, MO) for 2.5 to 3.0 h. $^{125}$I-IGF II (about 15,000 cpm/0.1 ml; total volume = 6 ml) was incubated with each nitrocellulose paper at 4°C for 12 h, which was then placed on a platform shaker. The following day, Tris-buffered saline (TBS) with 0.1% Tween was used to wash the nitrocellulose blots, followed by additional washings with TBS only. After this, nitrocellulose blots were dried and placed on an X-ray film for about 14 d at -80°C. X-ray films were then developed and individual band were densitometrically analyzed using Molecular Analyst (BIORAD, Hercules, CA).

*Statistical Analysis*

This experiment was a completely randomized design with repeated measures. The data was analyzed using PROC MIXED (Littell et al., 1996), with sources of variation being treatment, cow within treatment (error term for treatment), day, treatment

by day interaction, and residual. An autoregressive with lag equal to one model was used to model the covariance structure of the repeated measurements. If the treatment by day interaction was significant, simple effects of treatment were analyzed using the SLICE option for the LSMEANS statement (SAS, 1996). Degrees of freedom of the pooled error term were calculated by using the Satterthwait's approximation. If the treatment by day interaction was not significant, the main effects, if significant, were analyzed using the LSMEANS with the PDIFF option. For certain data (follicle and CL diameter), the maximum value during the experiment was analyzed using a completely randomized design and PROC GLM (SAS, 1996). Data are presented as least squares means ± SEM.

## Results

*Body Weight and Body Condition Score (BCS)*

Treatment, day, and treatment × day interaction ($P > 0.5$) did not affect body weight. Averaged over the 10-d treatment, control and dexamethasone-treated cows weighed 663.5 ± 28.4 kg and 648.7 ± 25.8 kg, respectively.

BCS was measured on d 7 of treatment. There was no treatment effect ($P > 0.10$) on BCS, which averaged 3.65 ± 0.21 and 3.15 ± 0.23 in dexamethasone-treated and control cows, respectively.

*Follicular dynamics and function*

However, there was a trend ($P < 0.10$) for both treatment and day effects on the number of small (< 10 mm) follicles. There was no treatment × day interaction ($P > 0.50$)

for the number of small follicles. On d 2 and 4, numbers of small follicles were lower ($P < 0.05$) in dexamethasone-treated than in control cows (Figure 1A). Numbers of small follicles decreased ($P < 0.05$) 45% between d 2 and 7 in control cows and 64% between d 1 and 8 in dexamethasone-treated cows (Figure 1 A).

Treatment × day interaction affected ($P < 0.05$) the number of large follicles (≥ 10 mm) (Figure 2). Dexamethasone-treated cows had more large follicles on d 2 (2.33 ± 0.3 versus 0.80 ± 0.35) and on d 8 (2.00 ± 0.32 versus 1.00 ± 0.35) than control cows. Numbers of large follicles increased ($P < 0.05$) 3-fold between d 3 and 5 in control cows and 3.4-fold between d 0 and 2 in dexamethasone-treated cows (Figure 1B).

Treatment did not affect ($P > 0.10$) maximum diameter of the dominant follicle, which averaged 15.8 ± 1.01 mm in control cows and 15.3 ± 0.92 mm in dexamethasone-treated cows (Table 1). Growth rate of the dominant follicle was not affected ($P > 0.10$) by dexamethasone treatment (Table 1). Treatment also did not affect ($P > 0.10$) maximum diameter of the subordinate follicles (Table 1).

*Luteal development*

Day and treatment ($P < 0.10$), but not treatment × day interaction ($P > 0.10$) affected the diameter of CL. CL diameter increased ($P < 0.05$) 52% between d 0 and 8 in control cows and 73% between d 0 and 8 in dexamethasone-treated cows (Figure 3A). Between d 6 and 10, CL diameter was 21% to 39% greater ($P < 0.10$) in dexamethasone-treated than control cows (Figure 3A). Growth rate of the CL did not differ ($P > 0.05$) between control and dexamethasone-treated cows (Table 1).

*Endocrine profiles*

There was no treatment or day effect ($P > 0.10$) or treatment × day interaction ($P > 0.10$) on plasma concentrations of FSH. Concentrations of FSH averaged 0.20 ± 0.020 ng/ml and 0.19 ± 0.02 ng/ml in dexamethasone-treated and control cows, respectively.

There was no treatment effect or day effect ($P > 0.10$) or treatment × day interaction ($P > 0.10$) on plasma concentration of LH. Plasma concentrations of LH averaged 2.8 ± 0.11 ng/ml and 2.63 ± 0.11 ng/ml in dexamethasone-treated and control cows, respectively.

Treatment ($P < 0.01$) but not day or treatment × day interaction ($P > 0.10$) affected plasma concentrations of estradiol. On d 0 and 1, plasma concentrations of estradiol were lower ($P < 0.05$) in dexamethasone-treated cows (1.07 ± 0.45 pg/ml) than in control cows (3.32 ± 1.05 pg/ml) (Figure 1B). After d 1, plasma estradiol concentrations did not differ between control and treated cows (Figure 2).

Day ($P < 0.0001$) and treatment ($P < 0.10$) but not treatment × day interaction ($P > 0.10$) affected plasma progesterone (P4) concentrations. Between d 4 and 10, P4 concentrations tended to be greater ($P < 0.10$) by 15 to 40% in control versus dexamethasone-treated cows (Figure 3B). Between d 0 and 10, plasma P4 concentrations increased ($P < 0.05$) in both groups by 40 to 50% (Figure 3B).

There was a treatment × day ($P < 0.05$) interaction for plasma insulin concentrations. Between d 2 and 9, plasma insulin concentrations were 2.9- to 6.8-fold greater ($P < 0.05$) in dexamethasone-treated cows versus control cows (Figure 4B). Between d 0 and 3, plasma insulin concentrations increased ($P < 0.05$) in treated cows by

6.0-fold. Plasma insulin concentrations remained constant in control cows between d 0 and 10 (Figure 4B).

Treatment ($P < 0.05$) but not day or treatment × day interaction ($P > 0.10$) affected plasma IGF-I concentrations. Between d 5 and 10, plasma concentrations of IGF-I were lower ($P < 0.05$) in dexamethasone-treated cows than control cows (Figure 5). These differences were a result of plasma IGF-I concentrations decreasing ($P < 0.05$) by 58% between d 0 and 5 in treated cows. Plasma IGF-I concentrations remained constant in control cows between d 0 and 10 (Figure 5).

Treatment ($P < 0.01$) but not day or treatment × day interaction ($P > 0.10$) affected plasma IGF-II concentrations. Between d 5 and 10, plasma concentrations of IGF-II were lower ($P < 0.01$) in dexamethasone-treated cows than in control cows (Figure 6). These differences were a result of plasma IGF-II concentrations decreasing ($P < 0.05$) by 35% between d 1 and 5 in treated cows. Plasma IGF-II concentrations remained constant in control cows between d 0 and 10 (Figure 6).

Ligand blotting with [$^{125}$I] IGF-II revealed at least six forms of IGFBP: 34-kDa (IGFBP-2), 40- to 44-kDa (IGFBP-3), 20-kDa, 22-kDa, 26-kDa, 28-kDa and 30-kDa. Arbitrary densitometric units per 4 µl were used to express binding activities of the different IGFBPs. Treatment, day and treatment × day interaction had no effect ($P > 0.10$) on the various IGFBPs levels except for the 26 kDa IGFBP in which a trend for d effect ($P < 0.10$) was observed (Table 2). The 26 kDa IGFBP tended to decrease ($P < 0.10$) 37% in control cows and 41% in treated cows between d 0 and 10 (Table 2).

Treatment, day and treatment × day interaction had no effect ($P > 0.10$) on plasma leptin concentrations. Plasma leptin concentrations averaged 7.78 ± 1.16 ng/ ml and 7.25

± 1.27 ng/ml in dexamethasone-treated cows and control cows, respectively (data not shown).

*Plasma metabolites*

There was a treatment × day ($P < 0.001$) interaction for plasma concentrations of glucose. From d 1 through d 10 glucose concentrations were higher ($P < 0.05$) in dexamethasone-treated cows versus control cows. In treated cows, plasma glucose concentrations increased ($P < 0.05$) 72% between d 0 and 1 and then decreased ($P < 0.05$) gradually between d 1 and 10 (Figure 4A). In control cows, plasma glucose concentrations did not change between d 0 and 10 (Figure 4A).

There was a treatment × day interaction ($P < 0.05$) for plasma cholesterol concentrations. In both groups, cholesterol concentrations were lower ($P < 0.01$) on d 10 versus d 0 (Figure 7). Between d 0 and 10 plasma cholesterol concentrations decreased 46.8 % in dexamethasone-treated cows versus 19.5 % in control cows.

## Discussion

Results of the present study indicate that dexamethasone: (1) decreases plasma progesterone concentrations without affecting gonadotropin levels, (2) decreases plasma concentrations of IGF-I and – II, (3) does not affect growth rate of dominant follicles, and (4) stimulates systemic glucose and insulin concentrations.

Similar to previous studies in rats (Marfaing *et al.*, 1991; Dardevet *et al.*, 1999), humans (Aaron and Tyrrell, 1994) and cattle (Anderson and Olsson, 1984; Shpigel *et al.*,

1996), plasma insulin and glucose levels were greatly increased by dexamethasone treatment of cattle. During fasting, glucose levels in mammals is maintained by glucocorticoids, which increase glucose release by the liver, increase gluconeogenesis and glycogen deposition and consequently inducing insulin increase (Marfaing *et al.*, 1991). Glucocorticoids also increase lipolysis by contradicting the effect of insulin (Marfaing *et al.*, 1991; Ferguson and Hoenig, 1995) and inducing ketogenesis (Aaron and Tyrrell, 1994) which results in a diabetogenic state characterized by hyperinsulinemia and hyperglycemia and caused by insulin resistance and impaired glucose utilization (Marfaing *et al.*, 1991; Dardevet *et al.*, 1999). Recently, glucocorticoids were shown to protect the liver against fat accumulation in cattle via decrease in lipid concentrations in the liver and an increase in the liver glycogen concentrations (Furll and Furll, 1998). However, in cultures of adipose tissue from lactating cows dexamethasone, together with insulin, inhibits lipolysis, by maintaining the intracellular G-protein signaling system (Lanna and Bauman, 1999). Thus, the dexamethasone-induced increase in plasma glucose and insulin obtained in our study agrees with the results previously described, confirming glucocorticoid effects on metabolism.

For the first time in cattle, we report a suppressing effect of dexamethasone on plasma IGF-I concentrations, which is consistent with previous studies in rats (Ohyama *et al.*, 1997) and chickens (Leili and Scanes, 1998). Increases in hepatic IGF-I mRNA induced by GH is also inhibited by dexamethasone *in vitro* (Beauloye *et al.*, 1999). In rats, corticosteroid treatment decreased IGF-I mRNA expression by 44% and 51% in the diaphragm and gastrocnemius (Gayan-Ramirez *et al.*, 1999). GH receptor was also suppressed by dexamethasone (Beauloye *et al.*, 1999). In agreement with this, Elsasser *et*

*al.* (1997) refers to a decrease in plasma GH and IGF-I concentrations in cattle due to chronic high concentrations of glucocorticoids. In fetal sheep during late gestation, cortisol regulates the hepatic GH receptor and IGF-I gene expression, suggesting that the prepartum cortisol surge is an important maturational factor, initiating the perinatal switch from fetal to adult modes of somatotrophic regulation (Li *et al.*, 1996). Beaufrere (1999) refers that the catabolic action of glucocorticoid is counteracted by growth hormone (GH), which stimulates protein synthesis probably mediated by IGF-I. Similarly, high concentrations of GH versus low concentrations of cortisol observed in high producing dairy cows, appear to play a role in enhancing milk production (Sartin *et al.*, 1988).

For the first time in ruminants, we report a suppressing effect of dexamethasone on plasma IGF-II concentrations, which is consistent with a previous report in mice (Rooman *et al.*, 1999). In murine myogenic cells, dexamethasone enhances IGF-II gene expression and therefore stimulates myogenic differentiation (Yoshiko, *et al.*, 1998). Also IGF-II mRNA expression was decreased by corticosteroid in the gastrocnemius and diaphragm of rats (Gayan-Ramirez *et al.*, 1999).

Dexamethasone did not have an effect on plasma IGFBP levels in the present study, a finding not previously reported for cattle. Production of IGFBP-2 is enhanced by dexamethasone, while IGFBP-3 is reduced, thus inhibiting the transport of IGFs in rats (Villafuerte *et al.*, 1995) and humans (Iwashita *et al.*, 1996). These IGFBPs, with molecular weights between 17 and 43 kDa (Binoux *et al.*, 1991; Cataldo, 1997) regulate the action of IGFs, by competing with the IGF receptor for IGF binding (Bale and Conover, 1992; Wang and Chard, 1999) and thus inhibiting IGF-I activity and

bioavailability (Bale and Conover, 1992; Jones and Clemmons, 1995; Spicer and Echternkamp, 1995; Schams, *et al.*, 1999; Wang and Chard, 1999; Porestky, *et al.*, 1999). According to their molecular weights characterized for cattle (Funston *et al.*, 1996; Carr *et al.*, 1994; Simpson *et al.*, 1997), we identified IGFBP-2 (34 kDa), IGFBP-3 (40 – 44 kDa), IGFBP-5 (30 kDa), IGFBP-4 (28 kDa), and IGFBPs with molecular weights of 20 kDa and 22 kDa that may be the deglycosilated forms of IGFBP-4 (Funston *et al.*, 1996). Since the IGFBPs were not affected by dexamethasone treatment, we can conclude that dexamethasone directly reduces the amount of bioavailable plasma IGF-I and –II in cattle.

A greater decrease in plasma cholesterol concentrations between d 0 and 10 was observed in dexamethasone-treated versus control cows. In mammals, the primary source of systemic cholesterol comes from the diet or from liver synthesis. Cholesterol is the precursor of progestagens, glucocorticoids, mineralocorticoids, androgens and estrogens (Stryer, 1995; Norman and Litwack, 1997). The required cholesterol for steroidogenesis is obtained from circulating serum low- and high-density lipoproteins and through *de novo* cholesterol synthesis mediated by HMG-CoA reductase (Savion *et al.*, 1982; Veldhuis *et al.*, 1984; Baranao and Hammond, 1986; Martin *et al.*, 1997). Both processes, the synthesis of LDL and *de novo* synthesis of cholesterol, are stimulated by IGF-I (Spicer *et al.*, 1996). In men with acquired LDL cholesterol insufficiency there is a slight impairment in the initial glucocorticoid response to ACTH and in the overall production of cortisol during sustained stimulation of ACTH, which may also contribute to a decrease in testosterone in chronically ill patients (Arem *et al.*, 1997). Because plasma cholesterol was reduced 46.8% in dexamethasone-treated cows versus 19.5% in the

control cows, it might be that cholesterol synthesis was decreased due to dexamethasone effect on suppressing IGF-induced HMG-CoA reductase at the level of the liver. This greater decrease in plasma cholesterol and lower plasma IGF-I and –II levels in dexamethasone-treated versus control cows may have contributed to lower the plasma progesterone concentrations observed in treated cows and therefore to reduced steroidogenesis.

In contrast to studies conducted in humans (Larsson and Ahren, 1996; Dagogo-Jack *et al.*, 1997; Bornstein *et al.*, 1997; Janssen *et al.*, 1998; Tan *et al.*, 1998) we found no effect of dexamethasone on leptin secretion in cattle. In bovine adipose tissue culture, only high concentrations of dexamethasone (100 nM) have stimulatory effects on leptin mRNA levels (Houseckne cht *et al.*, 2000), whereas in cultured human adipocytes 50 nM of dexamethasone stimulates leptin secretion (Halleux *et al.*, 1998). Thus, bovine adipocytes may be less responsive than human adipocytes to the stimulatory effect of glucocorticoids on leptin production and the dose of dexamethasone used was probably not high enough (44 μg/kg BW) to affect plasma leptin levels.

We observed no effect of dexamethasone on FSH or LH secretion, which is in disagreement with previous *in vitro* (Padmanabhan, *et* al., 1983; Li and Wagner, 1983; Suter and Schwartz, 1985) and *in vivo* studies (Echternkamp, 1984; Thoei and Kogo, 1999; Daley *et al.*, 2000); however, most of these reports refer to the need of high doses of glucocorticoid to alter gonadotropins secretion. Using 60 ng/ml or 600 ng/ml of dexamethasone on cultured rat pituitary cells only a slight decrease on basal LH secretion (38 – 43%) was obtained (Suter and Schwartz, 1985). When comparing the addition of cortisol, dexamethasone or P4 to *in vitro* bovine pituitary cells, basal levels of LH did not

change among the different steroid concentrations used (dexamethasone concentrations varied from 1 to 10 ng/ml), although these steroids inhibited LHRH-induced LH release (Padmanabhan *et al.*, 1983). Although 10 to 20 ng/ml of systemic cortisol concentrations do not affect LH, a 10- to 20-fold increase in plasma cortisol due to intensive stress, suppresses tonic LH by mainly reducing the pulsatile releases of LH (Echternkamp, 1984). Blood samples were collected too infrequent (i. e., daily) to evaluate an effect on LH pulsatility in the present study. However, in agreement with the present study, an increase in plasma glucocorticoids had no effect on plasma LH concentrations, with samples collected every 20 min for 8 h in dairy cows (Hockett *et al.*, 2000) or every 15 min for 12 h in cows with regular estrous cycles (Vighio and Liptrap, 1990). Similarly, dexamethasone had no effect on LH secretion in ewes (Phillips and Clarke, 1990). In the rat, dexamethasone treatment (60 to 600 ng/ml) stimulates FSH synthesis and secretion *in vitro* (Suter and Schwartz, 1985) and *in vivo* (Tohei and Kogo, 1999). The stimulatory effect of glucocorticoids on basal secretion of FSH might mean a positive and specific role for glucocorticoids in reproductive function (Suter and Schwartz, 1985; Mahesh and Brann, 1998). Treatment of ACTH (3 mg, i. m.) for 14 d during the late luteal phase in cattle suppressed plasma FSH concentrations and the preovulatory surge of FSH (Kawate *et al.*, 1996). However, dexamethasone (2 mg/d) had no effect on FSH secretion in ewes (Phillips and Clarke, 1990). The secondary surge of FSH in women, which is the regulator of follicle development, is corticosteroid-dependent and this might explain the reason why glucocorticoids have been successfully used to treat ovulatory failure in women (Mahesh and Brann, 1998). Based on these studies, it seems that the

dexamethasone dosage or period of treatment used in our study was not high or long enough to alter either LH or FSH secretion.

In the present study, plasma estradiol concentrations were greater in both dexamethasone-treated and control cows on d 0 and 1. Because cortisol does not alter estradiol production by bovine granulosa cells (Spicer and Chamberlain, 1998) and the treatment difference existed before treatments were initiated, it is unlikely that the decrease in estradiol was due to treatment per se. Rather it was probably due to a slight shift in the occurrence of the ovulatory estradiol surge and subsequent start of the first follicular wave. In support of the latter statement, numbers of small follicles in control cows were still decreasing between d 0 and 5 and numbers of large follicles increasing in treated cows between d 0 and 2. In a previous study, dexamethasone treatment (16 mg/d) in diestrous cows between d 13 and 22, decreased estradiol concentrations throughout the cycle never rising above 3 pg/ml (Broussard *et al.*, 1997).

On d 2 and 4, treated cows tended to have fewer small follicles than the control cows. This was likely due to the earlier increase in the number of large follicles in treated versus control cows. A decrease in the number of small follicles and an increase in the number of large follicles is characteristic of follicular dynamics during the first follicular wave in cattle (Ginther *et al.*, 1996; Roche *et al.*, 1998). Thus, it appears that the tendency for decrease in the number of small follicles simultaneously with an increase in the number of large follicles could be associated with the deviation mechanism for the establishment of the dominant follicle, and that these events occurred a day or two earlier in dexamethasone-treated cows.

In our study we found that the dexamethasone-treated cows had a larger CL and lower systemic progesterone concentrations. A previous study using 8 mg of dexamethasone twice daily in diestrous cows, reported a significant decrease in progesterone from d 18 through 20 of treatment cycle (estrus at d 0) but not afterward, suggesting that extension of bovine estrous cycle is rather due to delayed or impairment of preovulatory follicular development than due to CL extension (Broussard *et al.*, 1997). Comparing different breeds (Brahman, Senepol and Angus) of cattle, larger luteal growth in the Brahman and Senepol cows are associated with greater amounts of IGF-I in plasma (Alvarez *et al.*, 2000), and in dairy cows decreased luteal progesterone secretion is related with a reduction in plasma IGF-I (Spicer *et al.*, 1990). Regarding its ovarian functions in livestock species, IGF-I and -II stimulate the delivery of cholesterol to the P450scc enzyme complex, the rate-limiting step in progesterone biosynthesis (Porestky *et al.*, 1999). Studies in cattle indicate that IGF-I and –II as well as GH stimulate progesterone release in luteal cells and intact tissues both *in vivo* and *in vitro* (Eisnpanier *et al.*, 1990; Sauerwein *et al.*, 1992; Liebermann *et al.*, 1996; Schams, *et al.*, 1999). Since dexamethasone suppressed plasma IGF-I and –II, but had no effect on LH concentrations, it is likely that the CL is increasing its growth in attempt to circumvent the decreasing levels of progesterone due to decreased luteotropic support. Alternatively, dexamethasone treatment may have induced hepatic enzymes involved with steroid hormone metabolism and clearance, and thus, increased progesterone clearance from the systemic circulation. Previous studies in rats have shown that dexamethasone induces cytochrome P450 3A (Wright and Payne, 1994; Lee and Lee, 1999), increasing its content in Leydig cells of 5 and 10 week old rats, but apparently dependent on their age

with older rats being more sensitive than younger rats (Agular and Vind, 1995). Further work will be needed to clarify these possibilities.

Some studies report the size of follicles and the CL as the largest diameter (Pierson and Ginther, 1987; Ginther *et al.*, 1996; Kot and Ginther, 1999) while others refer to the average between the maximum and the minimum diameters (Pierson and Ginther, 1987; Murphy, *et al.*, 1991; Alvarez, *et al.*, 2000). In order to determine any differences between the two methods, we evaluated simple Pierson correlation coefficients, between the average and the largest diameter of follicles (dominant and subordinates) and the CL, within each class of follicles and the CL. We found positive significant correlations between the average and the maximum diameter of the dominant follicle ($r = 0.97$), and of the subordinate follicle ($r = 0.95$). There was also a positive correlation between the average and the maximum diameter of the CL ($r = 0.99$). Therefore it appears either method of measurement is appropriate.

Two types of corticosteroid receptors have been identified: type I that are more related to corticosterone activity (F), and type II, which are associated with cortisol activity (B) and represent the classical glucocorticoid receptor system (Sutanto and Kloet, 1987). Studies reporting the relative binding affinities ($IC_{50}$) of cortisol and dexamethasone for type I and II receptors in the hamster found that dexamethasone is 75% and 64-fold as effective as cortisol for binding to the type II and I, respectively (Sutanto and Kloet, 1987). Another study reported that dexamethasone was 1.4-fold more potent than cortisol for reducing specifically bound [$^3$H] cortisol (Norris and Kohler, 1977). We administered on average a total of 28.8 mg of dexamethasone per day, which would have resulted in a concentration of 880 ng/ml at a maximum and 0.88 ng/ml still

present in bovine plasma at 24 h after treatment based on clearance rate of dexamethasone in humans (Cassidy *et al.*, 1998). The estimated half-life of dexamethasone (AZIUM solution) following intramuscular administration is about 3 to 4 h (Ferguson and Hoeing, 1996). Based on the reported affinities of dexamethasone for the glucocorticoid receptor (Sutanto and Kloet, 1987; Norris and Kohler, 1977), the "cortisol equivalent" dose achieved systemically in each cow would have ranged between 1 and 200 ng/ml during a 24 h period. In the female cattle, physiological levels of cortisol in the blood range between 3 and 15 ng/ml (Garverick *et al.*, 1971; Swanson *et al.*, 1972; Roussel *et al.*, 1983), and under stressful conditions cortisol concentrations vary between 10 and 100 ng/ml (Echternkamp, 1984). Therefore, the daily dose of dexamethasone used in the present study closely mimicked a physiological stress-induced release of glucocorticoids.

In conclusion, even though certain amount of cortisol is required for the normal endocrine function in the cow, abnormally high levels affect not only reproduction, but also the whole organism's physiology. This was confirmed in our study in which dexamethasone showed a direct effect on metabolism, stimulating the increase in plasma glucose, and consequently inducing hyperinsulinemia, and most probably insulin resistance. Its direct and indirect suppressing effects on the reproductive function of the dairy cow resulted in reduced luteal function, and was likely due to suppressed secretion of IGF-I and –II as well as reduced biosynthesis of cholesterol.

## Conclusions and Implications

In cattle, suppression of luteal function by short-term (10 d) glucocorticoid treatment was due to inhibition of cholesterol and IGF-I and –II secretion. This seems to indicate that suppression of luteal function is due to inhibition of the HMG-CoA reductase enzyme, which then makes cholesterol unavailable for steroidogenesis, and by limiting the conversion of pregnenolone to progesterone. Although this was the first comprehensive *in vivo* study of an exogenous glucocorticoids effects on the metabolic and reproductive functions of the dairy cow there are still questions to be addressed in future research. Studies might focus on the activity and role of the IGF-I and -II regarding the glucocorticoid effects, for which existing information comprises mainly *in vitro* studies in humans and rats. The role of glucocorticoids in leptin secretion in cattle also should be a subject of future research. The divergent actions of glucocorticoids at the HPAA, regarding the enhancement of FSH and inhibition or no effect on LH secretion need also further elucidation.

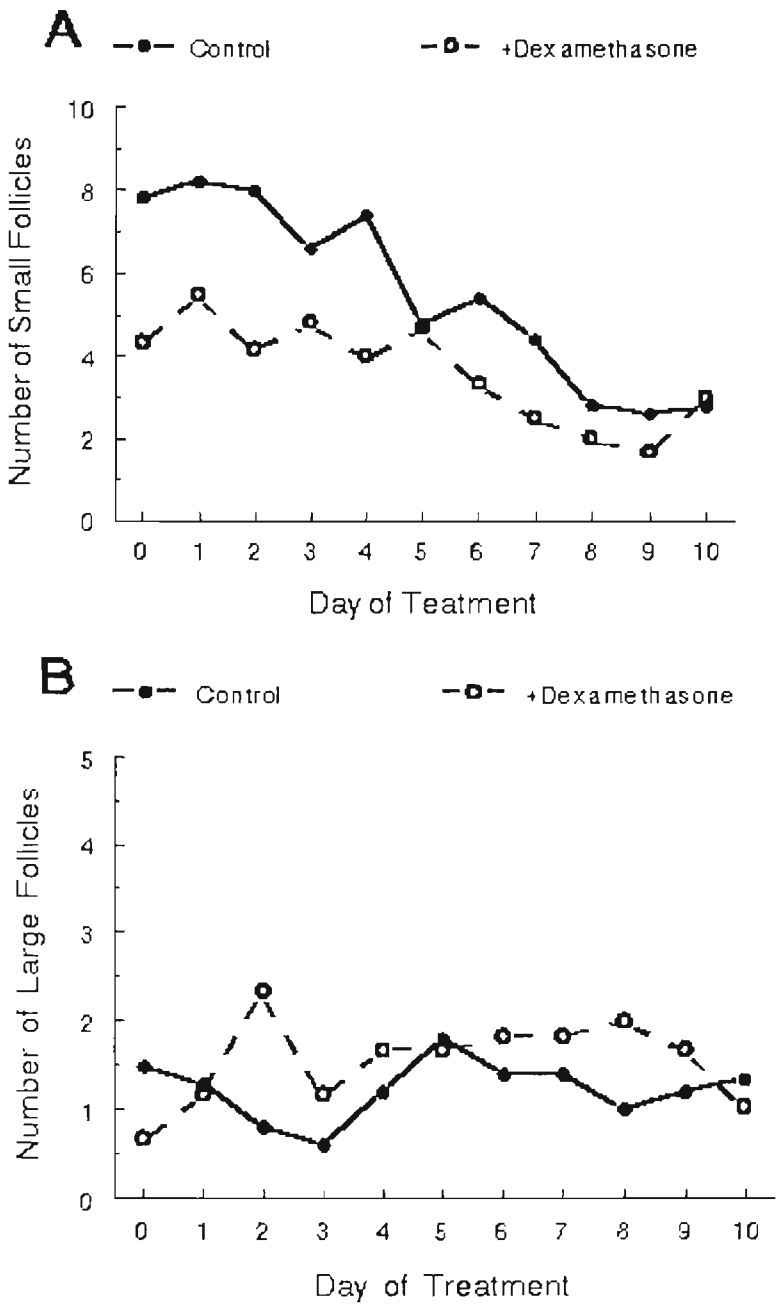
A
Control
+Dexamethasone
Number of Small Follicles
0
2
4
6
8
10
0 1 2 3 4 5 6 7 8 9 10
Day of Teatment
B
Control
+Dexamethasone
Number of Large Follicles
0
1
2
3
4
5
0 1 2 3 4 5 6 7 8 9 10
Day of Treatment

Figure 1. Changes in numbers of small follicles (≥ 5 mm and < 10 mm; Panel A) and number of large follicles (Panel B) as determined by daily rectal ultrasonography. Pooled SE for the number of small follicles averaged 0.60 and 0.66 in the treated and control cows, respectively ($P < 0.10$). Pooled SE treatment × d interaction for the number of large follicles averaged 0.32 and 0.37 on dexamethasone-treated cows and control cows, respectively ($P < 0.05$).

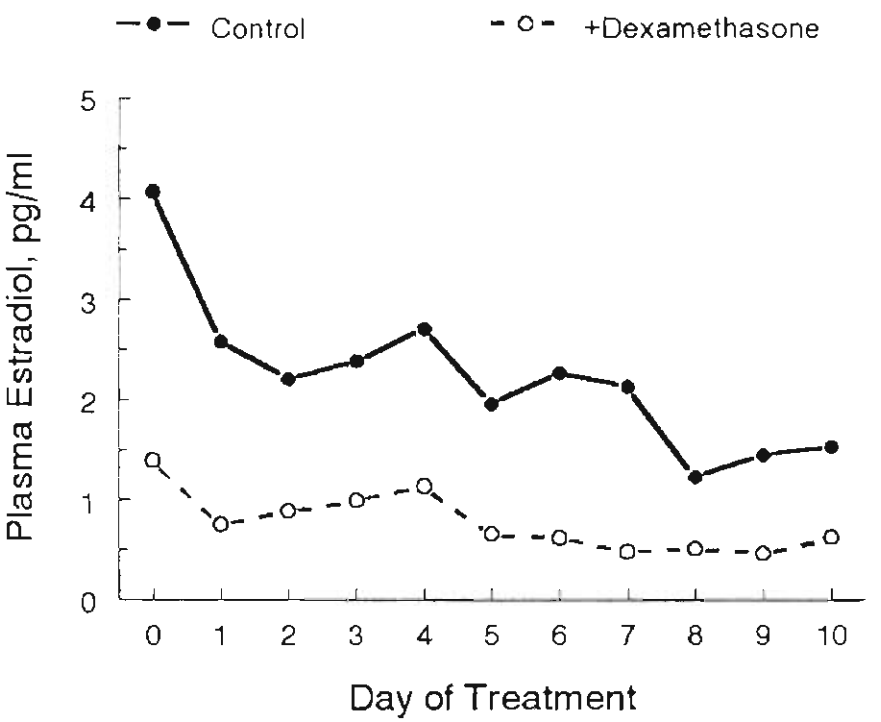


Figure 2. Changes in plasma estradiol concentrations in dexamethasone-treated and control cows. Pooled SE of estradiol concentrations averaged 0.28 pg/ml and 0.30 pg/ml in treated and control cows, respectively ($P < 0.01$).

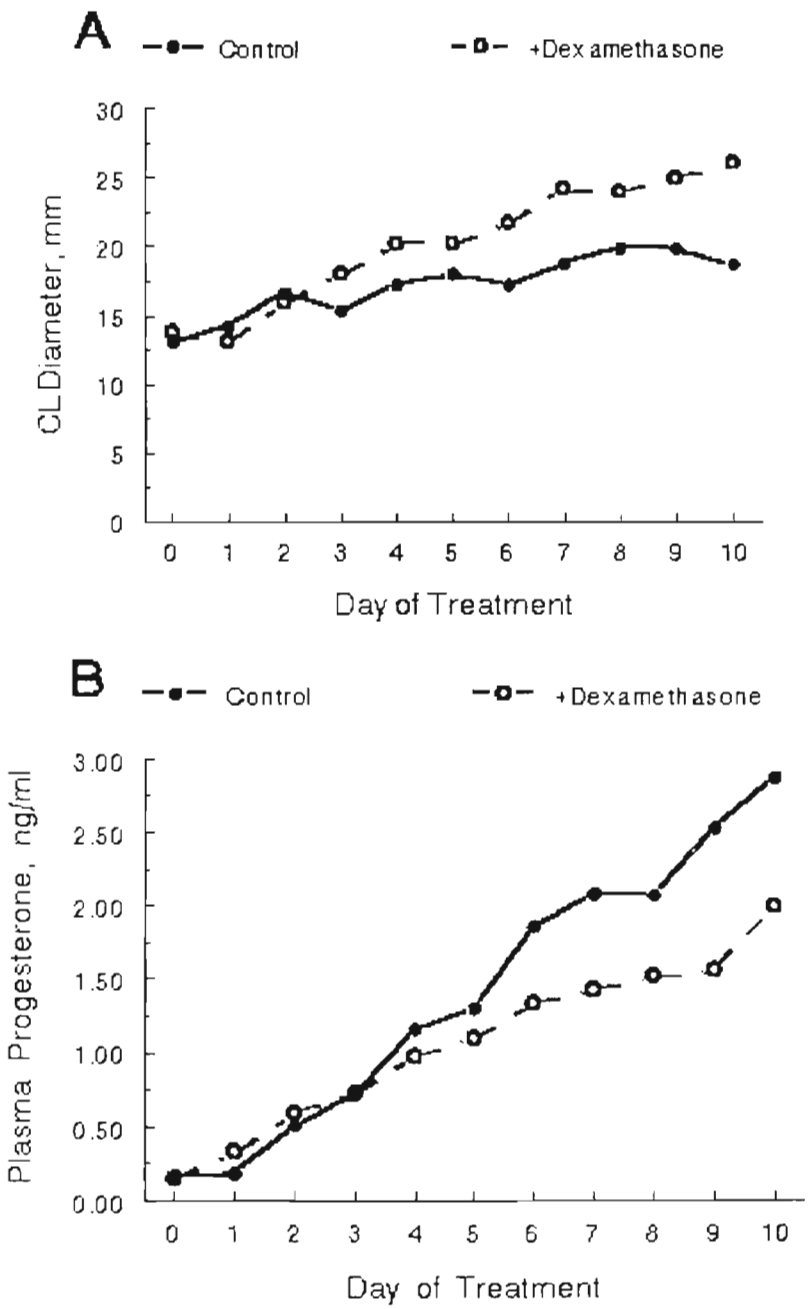
A
Control
+Dexamethasone
CL Diameter, mm
30
25
20
15
10
5
0
0 1 2 3 4 5 6 7 8 9 10
Day of Treatment
B
Control
+Dexamethasone
Plasma Progesterone, ng/ml
3.00
2.50
2.00
1.50
1.00
0.50
0.00
0 1 2 3 4 5 6 7 8 9 10
Day of Treatment

Figure 3. Changes in diameter of corpus luteum (CL; Panel A) as determined by daily rectal ultrasonography and plasma progesterone concentrations (Panel B) in dexamethasone-treated and control cows. Pooled SE of CL diameter was 0.90 mm and 1.06 mm for the treated and control cows, respectively ($P > 0.05$). Pooled SE for progesterone concentrations was 0.12 ng/ml and 0.13 ng/ml in treated and control cows, respectively ($P > 0.05$).

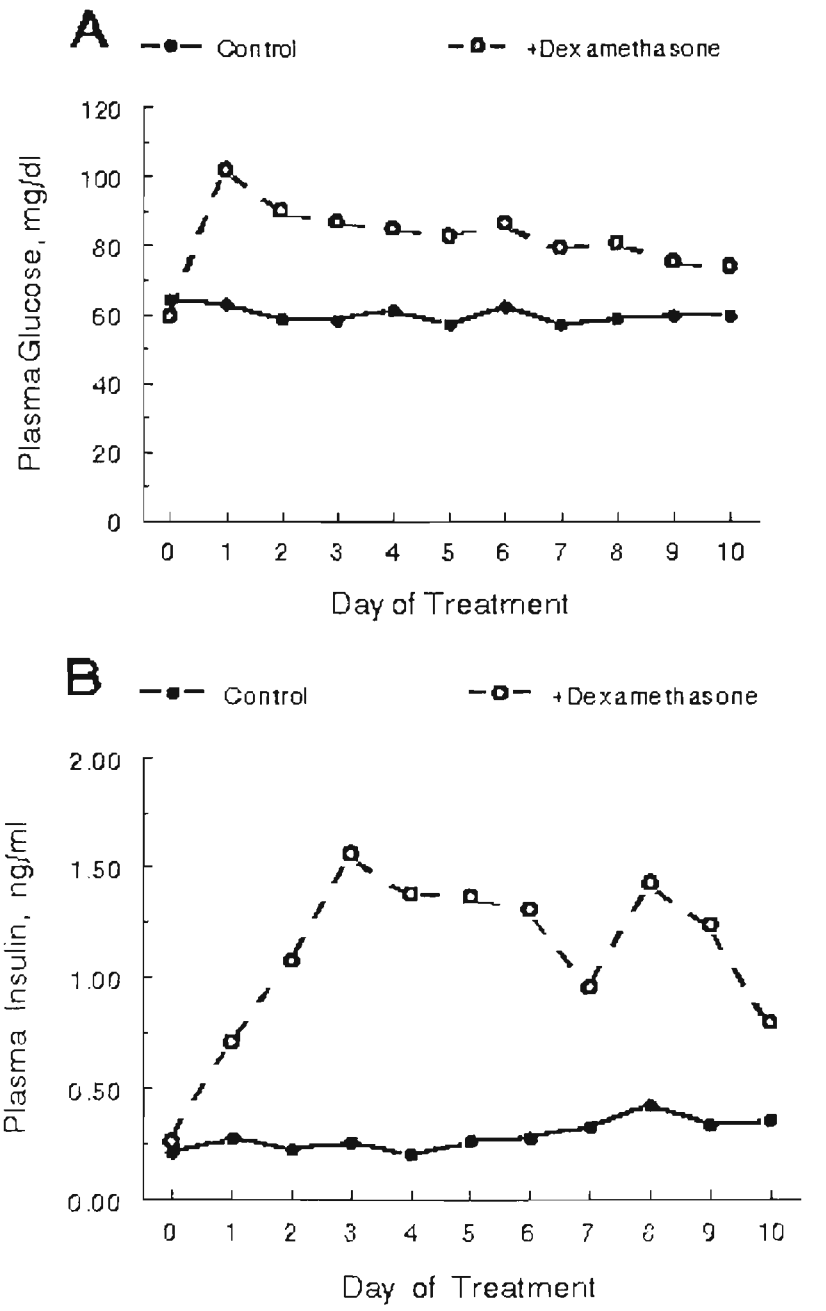
A
Control
+Dexamethasone
Plasma Glucose, mg/dl
120
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10
Day of Treatment
B
Control
+Dexamethasone
Plasma Insulin, ng/ml
2.00
1.50
1.00
0.50
0.00
0 1 2 3 4 5 6 7 8 9 10
Day of Treatment

Figure 4. Changes in daily plasma glucose concentrations as determined by enzymatic colorimetric procedure (Panel A) and plasma insulin concentrations (Panel B) in dexamethasone-treated and control cows. Pooled SE for glucose concentrations averaged 1.53 mg/dl and 1.69 mg/dl in treated and control cows, respectively ($P < 0.001$). Pooled SE for insulin concentrations averaged 0.08 mg/dl and 0.085 mg/dl in treated and control cows, respectively ($P < 0.0001$).

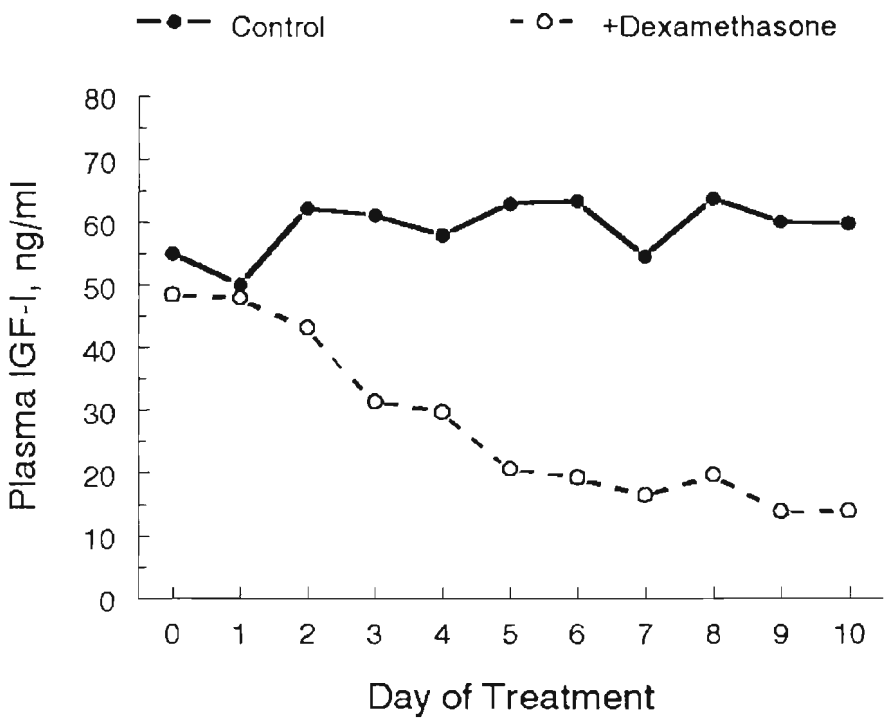


Figure 5. Changes in daily plasma concentrations of IGF-I in dexamethasone-treated and control cows. Pooled SE averaged 7.52 ng/ml and 8.24 ng/ml in treated and control cows, respectively ($P < 0.05$).

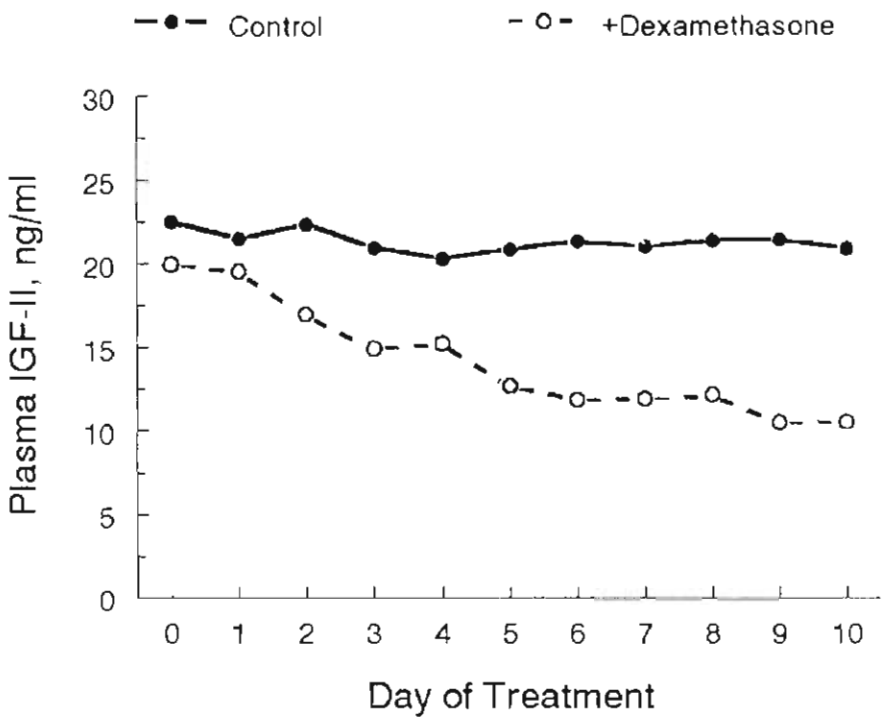


Figure 6. Changes in daily plasma concentrations of IGF-II in dexamethasone-treated cows and control cows. Pooled SE averaged 1.21 ng/ml and 1.33 ng/ml in treated and control cows, respectively ($P < 0.01$).

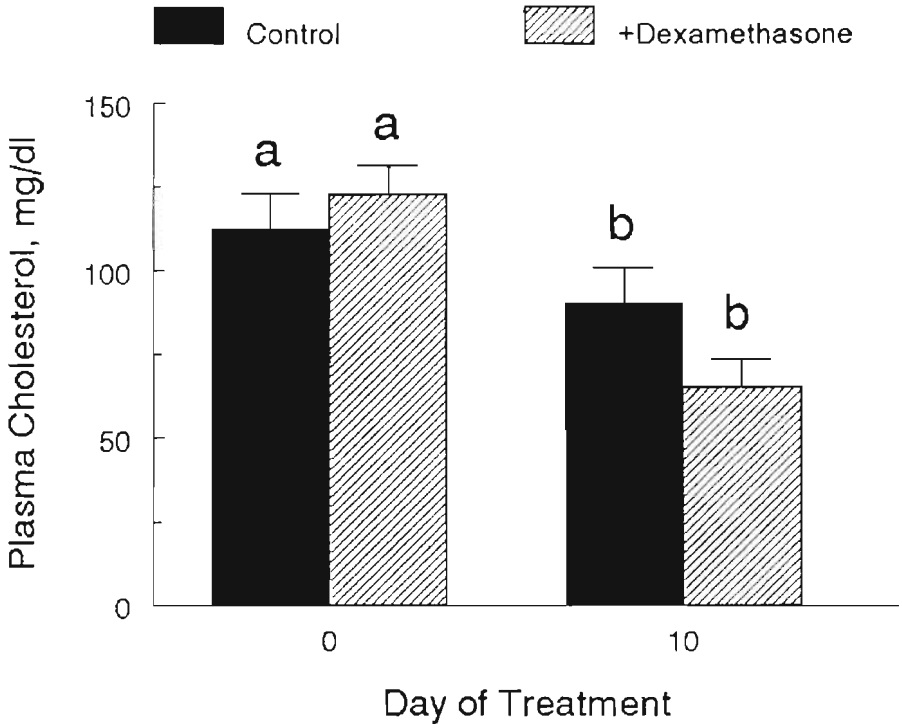


Figure 7. Changes in plasma concentrations of cholesterol in dexamethasone-treated and control cows as determined by enzymatic colorimetric procedure from samples collected on d 0 and 10. Pooled SE averaged 6.83 mg/dl and 6.51 mg/dl on d 0 and 10, respectively ($P < 0.01$). [a, b] Means without a common letter differ ($P < 0.01$).

**Table 1.** Characteristics of follicles and corpus luteum (CL) during control and dexamethasone treatment as determined by daily rectal ultrasonography.

| VARIABLE | CONTROL | TREATED |
|---|---|---|
| Dominant Follicle | | |
| Growth Rate, mm/d | 1.20 ± 0.40 | 1.71 ± 0.37 |
| Max. Diameter, mm | 15.80 ± 1.01 | 15.33 ± 0.92 |
| Subordinate Follicles | | |
| Max. diameter, mm | 7.36 ± 1.24 | 8.83 ± 1.13 |
| CL | | |
| Growth Rate, mm/d | 2.26 ± 0.43 | 2.36 ± 0.39 |
| Max. Diameter, mm | 22.9 ± 1.74 | 27.5 ± 1.59* |

*$P < 0.1$ for versus control

**Table 2.** Binding activity of IGFBP-2, IGFBP-3, 20-kDa, 22-kDa, 26-kDa, 28-kDa and 30-kDa on d 0 and 10 of treatment [a].

| | Control | | Treatment | |
|---|---|---|---|---|
| | 0 | 10 | 0 | 10 |
| **IGFBP-2** | 3.95 ± 1.39 | 3.38 ± 1.39 | 4.56 ± 1.27 | 4.09 ± 1.27 |
| **IGFBP-3** | 36.22 ± 9.38 | 36.77 ± 9.38 | 38.33 ± 8.56 | 40.77 ± 8.56 |
| **20-kDa** | 0.22 ± 0.12 | 0.21 ± 0.12 | 0.16 ± 0.11 | 0.19 ± 0.11 |
| **22-kDa** | 1.05 ± 0.34 | 0.92 ± 0.34 | 1.42 ± 0.31 | 1.38 ± 0.31 |
| **26-kDa** | 0.99 ± 0.26 | 0.63 ± 0.26 | 0.91 ± 0.24 | 0.53 ±0.24 |
| **28-kDa** | 1.60 ± 0.53 | 1.02 ± 0.53 | 1.63 ± 0.49 | 0.83 ± 0.49 |
| **30-kDa** | 1.41 ± 0.46 | 1.03 ± 0.46 | 1.46 ± 0.42 | 0.73 ± 0.42 |

[a] Arbitrary densitometric units per 4 μl of plasma

# CHAPTER IV

## SUMMARY AND CONCLUSIONS

To our knowledge, this was the first *in vivo* comprehensive study regarding the use of exogenous glucocorticoid in cattle in order to better understand their effect on ovarian and endocrine function. Cortisol, the major glucocorticoid in mammals, is the hormone that responds to stressful conditions, and without which any organism would be able to survive. It is synthesized from cholesterol, and released by ACTH into the circulation. Pituitary ACTH secretion is stimulated by CRH from the hypothalamus. Cortisol is essential for the maintenance of fluid homeostasis, blood glucose and, in general, for the maintenance of the whole physiological process/system.

High levels of glucocorticoids, in general, suppress metabolic and endocrine functions. Concerning the metabolic actions of glucocorticoids, they increase glucose secretion by enhancing gluconeogenesis, protein catabolism, lipolysis, which then finally results in insulin resistance. Consequently, there is hyperglycemia and hyperinsulinemia, which induces ketogenesis and loss in body weight. No effect of dexamethasone was seen on leptin, the secretion of which appears to be stimulated by glucocorticoid in rats and humans.

Regarding the endocrine functions, glucocorticoids inhibit steroidogenesis, by reducing cholesterol synthesis. Most likely, glucocorticoids suppress, either directly or

indirectly via decreased IGF-I and –II, HMG CoA reductase, the rate-limiting enzyme in the cholesterol synthesis. Since conversion of pregnenolone to progesterone is the rate-limiting step in steroidogenesis and is also induced by IGF-I, the suppressing effects of dexamethasone on steroidogenic hormones become obvious.

Either systemically or locally, at the ovarian level, glucocorticoids suppress the GH/IGF system, therefore inhibiting follicular development and luteal function. The lower concentrations of estradiol in dexamethasone-treated cows were most likely due to decrease in the number of small follicles induced by early advancement of the first follicular wave, rather than due to dexamethasone itself. The reduced number of small follicles, due to dexamethasone, was also associated with an increase in the numbers of large follicles.

In conclusion, dexamethasone suppressed HPAA resulting in the alteration of the endocrine and metabolic systems. In spite of major changes in metabolic hormones, follicular development was not altered dramatically. However, the changes in IGF-I and -II induced by dexamethasone likely resulted in reduced luteal function. Future studies should also be carried out in order to clarify the stimulatory effect of dexamethasone on leptin and, subsequently the effect of leptin in reproduction. Since studies regarding the effect of dexamethasone on IGFBPs are mostly referred to *in vitro* culture of adipose tissue and hepatocytes, further research is needed to clarify the role of glucocorticoid on IGFBPs secretion in cattle. In order to clarify the previously reported divergent actions of glucocorticoid on gonadotropins (i.e. enhancement of FSH and inhibition on LH), more detailed studies need to be carried out which evaluate their role on GnRH secretion in cattle.

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